



# THE UNIVERSITY *of* EDINBURGH

This thesis has been submitted in fulfilment of the requirements for a postgraduate degree (e.g. PhD, MPhil, DClinPsychol) at the University of Edinburgh. Please note the following terms and conditions of use:

This work is protected by copyright and other intellectual property rights, which are retained by the thesis author, unless otherwise stated.

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge.

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author.

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author.

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given.

**Sarcopenia and Cognitive Ageing:  
Investigating their interrelationship,  
biological correlates and the role of  
glucocorticoids**

**Dr Alixe HM Kilgour**

**MBBS MRCP(Edin)**

# Contents

|  |           |
|--|-----------|
| <b>Declaration</b> .....   | <b>i</b>  |
| <b>Acknowledgements</b> .....  | <b>ii</b> |
| <b>Abstract</b> .....  | <b>iv</b> |
| <b>Publications</b> .....  | <b>v</b>  |
| <b>Abbreviations</b> .....   | <b>vi</b> |
| <b>Chapter 1 Sarcopenia and age-related cognitive decline: diagnosis, structural change and underlying mechanistic processes</b> .....   | <b>1</b>  |
| 1.1 Introduction .....   | 1         |
| 1.2 Sarcopenia .....   | 2         |
| 1.2.1 Definition of sarcopenia .....   | 2         |
| 1.2.2 Structural changes associated with sarcopenia .....  | 5         |
| 1.2.3 Mechanistic processes causing sarcopenia .....   | 11        |
| 1.3 Age-related Cognitive Decline.....   | 26        |
| 1.3.1 Definition of age-related cognitive decline (ARCD).....  | 26        |
| 1.3.2 Cognitive ageing - a natural ageing process or a disease?.....   | 27        |
| 1.3.3 Structural changes associated with ARCD .....  | 29        |
| 1.3.4 Mechanistic processes causing ARCD .....   | 30        |
| 1.4 Conclusions .....  | 38        |
| 1.5 The research aims of this thesis .....   | 40        |
| <b>Chapter 2 A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse</b> ..... | <b>41</b> |
| 2.1 Introduction .....   | 41        |
| 2.2 Methods.....   | 42        |
| 2.2.1 Inclusion criteria .....   | 42        |
| 2.2.2 Search strategy .....  | 44        |
| 2.2.3 Study selection .....  | 44        |
| 2.2.4 Contacting authors .....   | 44        |
| 2.2.5 Quality assessment and risk of bias .....  | 44        |
| 2.2.6 Data extraction .....  | 45        |
| 2.2.7 Data analysis .....  | 45        |

|                  |  |            |
|------------------|--|------------|
| 2.3              | Results .....  | 46         |
| 2.3.1            | Association of brain structure and muscle structure .....    | 48         |
| 2.3.2            | Association of brain structure and muscle function.....      | 54         |
| 2.3.3            | Association of brain function and muscle structure.....      | 75         |
| 2.4              | Discussion .....   | 88         |
| 2.4.1            | Brain volumes and muscle mass .....                          | 88         |
| 2.4.2            | Brain structure and muscle function .....                    | 89         |
| 2.4.3            | Cognitive function and muscle mass .....                     | 94         |
| 2.4.4            | Limitations .....  | 95         |
| 2.5              | Conclusions .....  | 98         |
| <b>Chapter 3</b> | <b>Methods for the Lothian Birth Cohort 1936 study .....</b> | <b>100</b> |
| 3.1              | Introduction .....   | 100        |
| 3.2              | Background and sample .....                                  | 100        |
| 3.3              | Ethics.....  | 101        |
| 3.4              | Brain variables .....  | 102        |
| 3.4.1            | Cognitive testing .....                                      | 102        |
| 3.4.2            | G cognition, G processing speed and G memory.....            | 104        |
| 3.4.3            | Imaging Protocol.....  | 105        |
| 3.4.4            | Brain volume and WMHs .....                                  | 105        |
| 3.5              | Muscle variables.....  | 107        |
| 3.5.1            | Neck muscle CSA .....  | 107        |
| 3.5.2            | Physical function measures .....                             | 107        |
| 3.6              | Covariates from LBC 1936 used in the thesis.....             | 108        |
| 3.6.1            | Age and sex .....  | 108        |
| 3.6.2            | Height and weight .....                                      | 108        |
| 3.6.3            | Social factors.....  | 108        |
| 3.6.4            | Physical measures .....                                      | 108        |
| 3.6.5            | Comorbidity .....  | 109        |
| 3.6.6            | Blood markers .....  | 109        |
| 3.6.7            | Lifestyle factors.....                                       | 111        |

**Chapter 4 Design and Validation of a Novel Method to Measure Cross-Sectional Area of Neck Muscles Included During Routine MR Brain Volume Imaging 113**

|       |   |     |
|-------|---|-----|
| 4.1   | Introduction .....  | 113 |
| 4.2   | Methods.....  | 114 |
| 4.2.1 | Study 1: Feasibility study.....                           | 114 |
| 4.2.2 | Study 2: Study to measure inter-rater reliability .....   | 118 |
| 4.2.3 | Goal .....  | 118 |
| 4.2.4 | Study 3: Study to measure repeatability of technique..... | 119 |
| 4.2.5 | Study 4: External validity study .....                    | 121 |
| 4.3   | Results .....   | 124 |
| 4.3.1 | Study 1: Feasibility study.....                           | 124 |
| 4.3.2 | Study 2: Study to measure inter-rater reliability .....   | 124 |
| 4.3.3 | Study 3: Study to measure repeatability of technique..... | 129 |
| 4.3.4 | Study 4: External validity study .....                    | 133 |
| 4.4   | Discussion .....  | 135 |
| 4.4.1 | Summary of findings.....                                  | 135 |
| 4.4.2 | Previous research on quantifying muscle mass.....         | 136 |
| 4.4.3 | Strengths and limitations of the studies .....            | 136 |
| 4.4.4 | Implications for future research .....                    | 138 |
| 4.5   | Conclusion.....   | 138 |

**Chapter 5 Neck muscle cross-sectional area, brain volume and cognition in healthy older men; a cohort study ..... 140**

|       |  |     |
|-------|--|-----|
| 5.1   | Background .....                       | 140 |
| 5.2   | Methods.....                           | 142 |
| 5.2.1 | Participants.....                      | 142 |
| 5.2.2 | MR brain imaging .....                 | 142 |
| 5.2.3 | Brain structure measurements .....     | 142 |
| 5.2.4 | Neck muscle cross-sectional area ..... | 142 |
| 5.2.5 | Tests of cognitive function.....       | 143 |
| 5.2.6 | Statistical analysis .....             | 143 |
| 5.3   | Results .....                          | 145 |

|   |   |     |
|---|---|-----|
| 5.4   | Discussion .....  | 151 |
| 5.5   | Conclusion.....   | 153 |
| <b>Chapter 6 Interrelationships between brain and muscle structure and</b>        |   |     |
| <b>function using data from wave 2 of the Lothian Birth Cohort 1936 ..... 154</b> |   |     |
| 6.1   | Introduction.....   | 154 |
| 6.2   | Methods.....  | 155 |
| 6.2.1   | LBC 1936.....   | 155 |
| 6.2.2   | Statistics .....  | 155 |
| 6.3   | Results.....  | 156 |
| 6.3.1   | Descriptive statistics and correlations.....  | 156 |
| 6.3.2   | Relationship between muscle size and cognition.....   | 160 |
| 6.3.3   | Relationship between muscle function and cognition.....   | 161 |
| 6.3.4   | Relationship between muscle size and brain structure.....   | 164 |
| 6.3.5   | Relationship between muscle function and brain structure .....                                    | 165 |
| 6.3.6   | Relationship between muscle size and function and WML.....  | 167 |
| 6.3.7   | Interrelationships between muscle structure and function and brain<br>structure and function..... | 171 |
| 6.4   | Discussion .....  | 174 |
| 6.4.1   | Relationship between neck muscle CSA and cognition.....   | 174 |
| 6.4.2   | Relationship between physical function and cognition.....   | 174 |
| 6.4.3   | Relationship between brain structure and neck muscle CSA .....                                    | 176 |
| 6.4.4   | Relationship between brain structure and physical function.....                                   | 177 |
| 6.4.5   | Relationship between white matter lesions and neck muscle CSA .....                               | 178 |
| 6.4.6   | Relationship between white matter lesions and physical function .....                             | 178 |
| 6.4.7   | The interrelationship between brain and muscle structure and function<br>179                      |     |
| 6.4.8   | Limitations and future directions .....   | 181 |
|   | Conclusion.....   | 183 |
| <b>Chapter 7 Investigating the relationship between markers of</b>                |   |     |
| <b>immunosenescence (CMV and IL-6) and grip strength and muscle size in an</b>    |   |     |
| <b>elderly cohort study ..... 185</b>   |   |     |
| 7.1   | Introduction.....   | 185 |

|  |   |     |
|--|---|-----|
| 7.2  | Methods.....  | 188 |
| 7.2.1  | Participants – The Lothian Birth Cohort 1936 (LBC 1936) ..... | 188 |
| 7.2.2  | Neck muscle Cross-sectional Area .....                        | 188 |
| 7.2.3  | Grip strength .....   | 188 |
| 7.2.4  | CMV and IL-6 measures.....                                    | 188 |
| 7.2.5  | Childhood deprivation.....                                    | 188 |
| 7.2.6  | Statistical Analysis .....                                    | 189 |
| 7.3  | Results .....   | 190 |
| 7.4  | Discussion .....  | 195 |
| 7.5  | Conclusion.....   | 197 |
| <b>Chapter 8 Investigating the relationship between plasma cortisol, urinary glucocorticoid metabolites, GR and 11<math>\beta</math>HSD1 mRNA expression in skeletal muscle, and muscle size and strength..... 199</b> |   |     |
| 8.1  | Introduction .....  | 199 |
| 8.2  | Methods.....  | 201 |
| 8.2.1  | Participants.....   | 201 |
| 8.2.2  | Ethics Statement.....   | 201 |
| 8.2.3  | Anthropometry .....   | 201 |
| 8.2.4  | Muscle function.....  | 201 |
| 8.2.5  | Muscle size.....  | 201 |
| 8.2.6  | Plasma cortisol .....   | 202 |
| 8.2.7  | Quadriceps muscle biopsy .....                                | 202 |
| 8.2.8  | RNA Isolation .....   | 202 |
| 8.2.9  | cDNA preparation and qPCR.....                                | 202 |
| 8.2.10   | Urinary glucocorticoid metabolism.....                        | 203 |
| 8.2.11   | Statistical Analysis .....                                    | 203 |
| 8.3  | Results .....   | 205 |
| 8.4  | Discussion .....  | 208 |
| 8.5  | Conclusion.....   | 210 |
| <b>Chapter 9 A novel technique to measure 11<math>\beta</math>-hydroxysteroid dehydrogenase activity in human brain in vivo..... 211</b>   |   |     |
| 9.1  | Introduction .....  | 211 |

|   |  |            |
|---|--|------------|
| 9.2   | Materials and Methods .....                  | 215        |
| 9.2.1   | Participants .....                           | 215        |
| 9.2.2   | Reagents .....                               | 215        |
| 9.2.3   | Clinical protocol.....                       | 215        |
| 9.2.4   | Magnetic resonance scanning .....            | 216        |
| 9.2.5   | Laboratory analysis .....                    | 217        |
| 9.2.6   | Data analysis and kinetic calculations ..... | 218        |
| 9.2.7   | Statistical analysis .....                   | 218        |
| 9.3   | Results .....                                | 220        |
| 9.4   | Discussion .....                             | 223        |
| <b>Chapter 10 Assessing asymmetry and prominence of the internal jugular venous and internal carotid artery blood flow in healthy adult men .....</b> |  | <b>226</b> |
| 10.1  | Background .....                             | 226        |
| 10.2  | Methods.....                                 | 227        |
| 10.2.1  | Participants.....                            | 227        |
| 10.2.2  | MR protocol .....                            | 227        |
| 10.2.3  | Image analysis .....                         | 227        |
| 10.2.4  | Statistical analysis .....                   | 227        |
| 10.3  | Results .....                                | 228        |
| 10.4  | Discussion .....                             | 232        |
| 10.5  | Conclusion.....                              | 235        |
| <b>Chapter 11 Summary and Conclusions.....</b>  |  | <b>236</b> |
| 11.1  | Chapter order within the thesis .....        | 244        |
| 11.2  | Limitations .....                            | 244        |
| 11.3  | Summary .....                                | 245        |
| <b>References .....</b>   |  | <b>247</b> |
| <b>Appendices .....</b>   |  | <b>283</b> |
| Appendix 1: Data extraction sheet used in the systematic review .....   |  | 284        |
| Appendix 2: Publications arising from the work contained in this PhD.....   |  | 287        |



## Table of Figures

|   |     |
|---|-----|
| Figure 1-1: Pathways influencing sarcopenia .....   | 24  |
| Figure 1-2: Diagram showing the interaction between mediators which contribute to sarcopenia.....   | 25  |
| Figure 1-3: Pathways influencing ARCD .....   | 37  |
| Figure 2-1: PRISMA flow diagram showing study selection .....   | 47  |
| Figure 4-1: Figure of the posterior neck muscles and diagram demonstrating how the measurement plane was selected.....  | 116 |
| Figure 4-2: Flowchart summarizing method to measure craniad muscles cross-sectional areas. ....   | 118 |
| Figure 4-3: Diagram summarizing the methods for the four studies. ....  | 123 |
| Figure 4-4: Plot of MR slice chosen as representing the mid-point of C2 for both raters.....  | 125 |
| Figure 4-5: Bland-Altman plots for total neck muscle CSA and SCM + combined CSA measured by 2 raters. Bias of measurement between 2 different raters (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively..... | 127 |
| Figure 4-6: Bland-Altman plots for total neck muscle CSA measured on 3 different MRI scanners. Bias of measurement between different MRI scanners (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively.....    | 131 |
| Figure 5-1: Plot of whole brain volume against total neck muscle CSA.....   | 148 |
| Figure 6-1: Model representing significant interrelationships between muscle structure and function, WM volume and cognition .....  | 171 |
| Figure 6-2: Model representing significant interrelationships between muscle structure and function, GM volume and cognition .....  | 172 |
| Figure 6-3: Model representing significant interrelationships between muscle structure and function, WML volume and cognition .....   | 172 |
| Figure 9-1: Stable isotope tracers for measuring cortisol-cortisone interconversion by $11\beta$ -HSDs.....   | 214 |

|   |     |
|---|-----|
| Figure 9-2: Schematic diagram depicting set up of: subject, tracers and arteriovenous sampling with hot box.....                                    | 216 |
| Figure 9-3: Arterialized and jugular venous steroid concentrations and tracer/tracee ratios during steady state stable isotope tracer infusion..... | 221 |
| Figure 10-1: Bar chart of total IJV and total ICA blood flow split into right and left for each subject .....                                       | 231 |

# Declaration

I the undersigned declare:

- (a) that the thesis has been composed by myself, and
- (b) that the work is my own, unless stated otherwise, and
- (c) that the work has not been submitted for any other degree or professional qualification.

Dr Alixe HM Kilgour

## Acknowledgements

Firstly I would like to thank my PhD Supervisors for all their help and assistance during my PhD. John Starr has been a source of great wisdom, statistical prowess, reassurance and endless patience; Brian Walker has guided me into the wonderful world of wet lab research; and Alasdair MacLulich has been a great mentor for both this thesis and future plans.

I was fully funded during this research by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC), Economic and Social Research Council (ESRC) and Medical Research Council (MRC) is gratefully acknowledged.

The LBC 1936 studies have been funded by Age UK and the MRC. I thank A Gow, C Murray, J Corley, R Henderson and A Pattie at the Centre of Cognitive Aging and Cognitive Epidemiology, and MV Hernandez, SM Maniega, N Royle, E Sandeman, I Gerrish, of the Brain Research Imaging Centre, both of the University of Edinburgh, UK, for demographic and clinical data collection on the LBC 1936. I also thank the LBC 1936 Study, MHEM and Calibrain study participants.

I am very grateful for the help of Mrs Sheila Fiskien, University of Edinburgh, who helped design the original database searches for the systematic review.

I gratefully acknowledge the help of the study authors who replied to our request for raw data or associations not included in their original paper for the systematic review.

I am grateful to the staff of the Imaging, Nursing and Mass Spectrometry Cores of the Wellcome Trust Clinical Research Facility, Edinburgh. In particular I thank Mr Sanjaykumar Kothiya for performing the liquid chromatography–tandem mass spectrometry.

The imaging in chapters 9 and 10 was carried out at the Brain Research Imaging Centre, Neuroimaging Sciences, Edinburgh ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)), University of Edinburgh, which is part of the SINAPSE (Scottish Imaging Network - A Platform for Scientific Excellence) collaboration ([www.sinapse.ac.uk](http://www.sinapse.ac.uk)) funded by the Scottish Funding Council and the Chief Scientist Office.

I would also like to thank the following people for their advice and assistance during my PhD:

- C Gray, S Semple, T MacGillivray and staff at the Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, UK.
- D Job, the radiographers and staff at The Brain Research Imaging Centre, Edinburgh, UK.
- TWJ Moorhead, Division of Psychiatry, University of Edinburgh, UK.
- CA Greig, Department of Geriatric Medicine, University of Edinburgh, UK, who supplied the data for chapter 8 on which I performed the analyses

Finally I would like to thank my family and friends for their help and support, with a special thanks to my Mum for lots of babysitting whilst I was writing up and my husband and darling daughter for their encouragement and garden walks.

# **Abstract**

## **Background**

Sarcopenia and age-related cognitive decline (ARCD) are important age-related conditions which significantly impact upon the quality of life of older adults. ARCD is a well-established research area, whereas sarcopenia is a relatively new field. Research into the inter-relationships between them and possible common underlying mechanistic processes is lacking.

## **Methods**

Several research techniques were used: a large systematic review; the development of an image analysis technique to measure neck muscle size on volumetric MR brain scans; the subsequent use of the technique in elderly cohort studies; statistical modelling to investigate the role of glucocorticoids in sarcopenia; and an invasive clinical study to develop a novel technique to measure the activity of 11beta-hydroxysteroid dehydrogenase (11 $\beta$ HSD1) in the human brain in vivo.

## **Results**

I consistently found a relationship between: some measures of brain structure and muscle size; markers of brain structure and muscle function, mostly grip strength and gait speed; and cognition and muscle function. However, I found no relationship between current cognition and muscle size in any of the above studies. Cortisol was identified as a possible explanatory factor in the relationship between both cognition and brain volume with gait speed. I found an association between markers of immunosenescence and sarcopenia (neck muscle CSA and grip strength) and an association between expression of the cortisol amplifying enzyme 11 $\beta$ HSD1 and quadriceps strength.

I developed a technique to measure 11 $\beta$ HSD1 activity across the human brain, which found that the amount of cortisol produced within the brain was not detectable and highlighted the asymmetries within the cerebrovascular venous system.

## **Conclusions**

Further longitudinal studies looking at the association between sarcopenia and ARCD are now required to investigate these important relationships further and hopefully this will lead to improved therapeutic options.

## Publications

1. Design and validation of a novel method to measure cross-sectional area of neck muscles included during routine MR brain volume imaging.  
*Kilgour AH, Subedi D, Gray CD, Deary IJ, Lawrie SM, Wardlaw JM, Starr JM.*  
PLoS One. 2012;7(4):e34444.
2. Neck muscle cross-sectional area, brain volume and cognition in healthy older men: a cohort study.  
*Kilgour AH, Ferguson KJ, Gray CD, Deary IJ, Wardlaw JM, MacLulich AM, Starr JM.*  
BMC Geriatrics. 2013 Feb 28;13:20.
3. Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly.  
*Kilgour AH, Firth C, Harrison R, Moss P, Bastin ME, Wardlaw JM, Deary IJ, Starr JM.*  
Immunity and Ageing. 2013 Aug 13;10(1):33.
4. Increased skeletal muscle 11 $\beta$ HSD1 mRNA is associated with lower muscle strength in ageing.  
*Kilgour AH, Gallagher IJ, MacLulich AM, Andrew R, Gray CD, Hyde P, Wackerhage H, Husi H, Ross JA, Starr JM, Chapman KE, Fearon KC, Walker BR, Greig CA.*  
PLoS One. 2013 Dec 31;8(12):e84057.
5. A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse.  
*Kilgour AH, Todd OM, Starr JM.*  
BMC Geriatr. 2014 Jul 10;14:85.
6. 11beta-Hydroxysteroid dehydrogenase activity in brain does not contribute to systemic interconversion of cortisol and cortisone in healthy men.  
*Kilgour AH, Semple S, Marshall I, Andrews P, Andrew R, Walker BR.*  
Journal of Clinical Endocrinology and Metabolism. 2015 Feb;100(2):483-489.

# Abbreviations

6MWT – Six metre walk test

11 $\beta$ HSD1 – 11beta-hydroxysteroid dehydrogenase

AD - Alzheimer's disease

ALM -- appendicular lean mass

ASM - appendicular skeletal mass

BIA - bioimpedance analysis

BMI - body mass index

CC - corpus callosum

CSF - cerebrospinal fluid

CSI-D - community screening instrument of dementia

CVD - cardiovascular disease

FFM - fat free mass

GC - Glucocorticoids

GLM - General Linear Model

GM - grey matter

ICA – Intracranial area

ICV - intracranial volume

IKES - isometric knee extension strength

K-BIT - Kaufman Brief Intelligence Test

LBC 1936 – Lothian Birth Cohort Study 1936

LM - lean mass

LLMM – lower limb muscle mass

MHEM – MacLulich Healthy Elderly Men Study

MLR - multiple linear regression

MMSE – mini mental state examination



MTR - magnetisation transfer ratio

NAWM – normal appearing white matter

PVH - periventricular hyperintensities

RASM – relative appendicular skeletal mass

RCT - randomized controlled trial

ROI – region(s) of interest

SM – skeletal mass

SPPB - short physical performance battery

TBV - total brain volume

TLM - total lean mass

TMT - trail making test

VE - ventricular enlargement

WBV - whole brain volume

WM - white matter

WMH - white matter hyperintensities

WML – white matter lesions

WMSA - white matter signal abnormalities

# Chapter 1 Sarcopenia and age-related cognitive decline: diagnosis, structural change and underlying mechanistic processes

## 1.1 Introduction

As the population of the world ages there is mounting scientific interest in not just the pathologies which have a higher incidence with older age but in those conditions which are a direct and universal consequence of ageing itself (Medical Research Council, 2009, The Academy of Medical Scientists, 2009, United Nations Programme on Ageing and The International Association of Gerontology and Geriatrics, 2007). With increasing age there are declines in physiological function throughout the human body, however the biological processes underlying these changes are not fully understood.

Several hundred theories of ageing exist (Vina et al., 2007, Medvedev, 1990). Attempts to group these theories together have led to classifications such as: cellular versus organismal senescence, which groups theories together as to whether they exert a mainly cellular or tissue level effect; or damage-based (aka stochastic) and programmed theories of aging, where damage-based implies accrued injury secondary to cellular processes (eg metabolism) and programmed suggests predetermined genetic pathways occurring on a fixed time schedule. However it is being increasingly recognised that these classifications are not mutually exclusive and it is unlikely that the complex process of ageing will be underpinned by one theory. Therefore many of the identified theories are likely to play a role.

The common cause hypothesis is not an ageing theory *per se* but a construct to investigate organismal senescence. It postulates that the biological processes leading to ageing are the same in each organ and therefore each person will have an intrinsic rate of ageing affecting all their organs whilst extrinsic factors may modify that rate in specific organs (eg UV radiation and skin) (Baltes and Lindenberger, 1997, Lindenberger and Baltes, 1994, Christensen et al., 2001, Anstey and Smith, 1999, Anstey et al., 1997). Therefore if extrinsic factors played less of a role in determining the rate of senescence than intrinsic factors, then where the intrinsic rate of ageing was slow a subject's composite organs would be biologically "younger" (ie they may have less evidence of both brain and muscle ageing) than a subject who had an intrinsically faster rate of senescence of the same chronological age. If the common cause hypothesis was found to be correct, potential therapies to slow the

underlying processes could have wide therapeutic benefits by affecting multiple different organs or tissue types at once. However if extrinsic factors are more found to be more important in determining rate of senescence it may be that different organs and tissues age at very different rates depending on their relative exposures to the relevant factors (eg alcohol may be more ageing to the brain than to muscle), or if the rate of intrinsic ageing was variable between different organs (ie free radicals may cause more damage to neurons than myocytes).

Both sarcopenia and cognitive ageing are major causes of morbidity in the elderly population and research has found significant relationships between cognitive and physical ageing (Christensen et al., 2000, Deary et al., 2011). This introduction highlights current evidence for the structural changes found in sarcopenia and cognitive ageing and the mechanistic pathways underlying them, leading on to the research aims of this thesis.

## **1.2 Sarcopenia**

### **1.2.1 Definition of sarcopenia**

It has been shown that both muscle mass and function decline with age (Janssen et al., 2000, Martin et al., 2000, Overend et al., 1992a): this is termed sarcopenia. Sarcopenia is a common cause of morbidity and mortality in older persons (Cruz-Jentoft et al., 2010). However, the exact diagnostic definition for sarcopenia is still under debate with differences between all the main published criteria, and it is not yet included in the International Classification of Diseases (Cruz-Jentoft et al., 2010, Fielding et al., 2011, Muscaritoli et al., 2010). Several papers have suggested definitions based on the clinical picture or diagnostic criteria.

Most working definitions of sarcopenia state that it is a syndrome in which there is loss of both muscle mass and function (strength and/or power) during the course of normal ageing (Roubenoff, 2001, Cruz-Jentoft et al., 2010, Muscaritoli et al., 2010). The reason that both muscle mass and function must be taken into account is that the relationship between the two variables is non-linear (ie mass cannot predict strength and vice versa) and ageing muscle shows features of both decreasing mass and function (Goodpaster et al., 2006).

Several operational diagnostic criteria have been put forward. Initially these were based on DEXA measurements of appendicular muscle mass. Baumgartner et al. defined sarcopenia as being present when the ratio of appendicular skeletal muscle mass (kg) per height<sup>2</sup> (m<sup>2</sup>) falls two standard deviations below the mean of a young reference group (Baumgartner et al., 1998). This ratio is now referred to as the skeletal muscle mass index (SMI). Since then

others have attempted to modify this to account for fat infiltration of muscle with age. For example, Newman et al used a residual method to correct for fat mass and height and found it correlated with functional status better than Baumgartner's method (Newman et al., 2003).

Other diagnostic criteria have used bioimpedance analysis (BIA) as the measure of muscle mass. Janssen et al. used the ratio of total muscle mass (measured using BIA) against total weight instead of height in their analysis of the NHANES III data (Janssen et al., 2002).

Others have used the Baumgartner SMI equation but used the BIA measured value for skeletal muscle mass. Chien et al showed no statistical difference between MRI measured skeletal muscle mass and BIA estimated muscle mass, so were able to calculate a prevalence of sarcopenia using the SMI with either the MRI or BIA measurement (Chien et al., 2008).

Most studies agree that the measurement should be compared to that of a young healthy population matched for gender and ethnicity, however establishing these reference datasets for both sexes and all ethnicities is not complete, with one consensus group recommending at least 100 subjects per group (Morley et al., 2011).

Many of the original studies did not take into account that the diagnosis of sarcopenia can only be made when there is loss of both muscle mass and function. There are therefore now an increasing number of papers showing measurement techniques and cut-off values for functional measures also. These centre on handgrip strength and gait speed (Fried et al., 2001, Cesari et al., 2009, Lauretani et al., 2003), however exact cut offs are not widely agreed upon.

*Table 1-1: Proposed diagnostic criteria for sarcopenia from different consensus groups*

| <b>Group</b>  | <b>Year</b> | <b>Structural cut off</b>  | <b>Functional cut off</b>  |
|---|-------------|--|--|
| <b>European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al., 2010)</b>  | 2010        | Low muscle mass, using CT, MRI, DEXA or BIA (definition or cut off not given)  | Gait speed $\leq 0.8$ m/s or low grip strength (definition or cut off not given)   |
| <b>Specialist Interest Groups (SIG): “Cachexia-Anorexia in Chronic Wasting Diseases and Nutrition in Geriatrics” (Muscaritoli et al., 2010)</b> | 2010        | Muscle mass $\geq 2$ SD below the mean measured in young adults of the same sex and ethnic background  | Gait speed $< 0.8$ m/s in 4 metre walk test or any other well established functional test used as part of a Comprehensive Geriatric Assessment |
| <b>International Working Group on Sarcopenia (Fielding et al., 2011)</b>  | 2011        | Lean mass (measured by DEXA) $< 20^{\text{th}}$ percentile of values of healthy young adults (eg Appendicular Lean Mass/height <sup>2</sup> $\leq 7.23$ kg/m <sup>2</sup> for men and $\leq 5.67$ kg/m <sup>2</sup> for women) | Gait speed $< 1$ m/s in 4 metre walk test  |
| <b>Society of Sarcopenia, Cachexia and Wasting Disorders (Morley et al., 2011)</b>  | 2011        | Appendicular lean mass/height <sup>2</sup> $> 2$ SD below that of healthy people aged between 20-30years old of the same ethnicity   | Gait speed $< 1$ m/s in 4 metre walk test or a distance of $< 400$ metres covered during 6 minute walk test                                    |

Securing a widely accepted operational diagnosis for sarcopenia is clearly a research priority so that clinicians can make an accurate diagnosis and researchers have a standardised method with which to base future studies.

## **1.2.2 Structural changes associated with sarcopenia**

### **1.2.2.1 Decreased muscle mass and cross-sectional area**

Studies have shown that loss of muscle mass starts between 30 and 40 years of age and appears to accelerate with age (Roubenoff, 2000, Janssen et al., 2000). The rate of loss of muscle mass has been found to be between 0.5-2% per year (Frontera et al., 2000, Goodpaster et al., 2006, Mitchell et al., 2012). This widely quoted range is predominantly based on studies comparing young and old subjects however a large study looking at muscle mass within a longitudinal study found comparable figures. It followed 2300 70-79 year old men and women for 7 years and found DEXA calculated leg lean mass decreased by 0.8% per year for men and 0.7% per year for women (Koster et al., 2011). There appears to be more muscle mass lost from the lower compared to the upper limbs (Janssen et al., 2000). Even within a limb, some muscles appear to decrease in size more than others; a study looking at elderly and young women found that the soleus muscle changed the least with age, whilst the psoas major showed the most decline (Ikezoe et al., 2011). Two studies (each with  $n > 250$ ) have shown that men lose proportionately more muscle mass than women (Janssen et al., 2000, Gallagher et al., 1997).

Cross-sectional area is correspondingly seen to reduce, with studies finding a 25-35% reduction in cross-sectional area between younger (18-30s) and older (70+) subjects in the quadriceps (Young et al., 1984, Young et al., 1985, Overend et al., 1992b), the psoas major and sacrospinalis muscles (Imamura et al., 1983) and the plantar flexors (Rice et al., 1989). Cross-sectional area is important when considering muscle function as muscle strength depends on the number of sarcomeres in parallel. The number of sarcomeres in series dictates the maximum shortening velocity (ie the fibre length) which is important for muscle power.

### **1.2.2.2 Decreased strength and muscle quality**

Although decreased muscle mass appears to be the major factor in age-related loss of muscle function (strength and power), there also appears to be an impairment of muscle fibre quality which contributes to this loss to a lesser extent, which is particularly important in the very old (Welle, 2002). As mentioned briefly above, it has been shown that muscle function and mass do not decline in a parallel manner (Young et al., 1985, Skelton et al., 1994). A longitudinal study of 1880 subjects in their seventies found that strength declined at 2-4% per year whereas mass declined at about 1% per year (Goodpaster et al., 2006). Therefore there is a loss of force per cross-sectional area with age. There is also a decrease in peak power per unit

volume with age, as power (work done per unit time) and strength (maximal force) also decline with age at different rates (Cruz-Jentoft et al., 2010). In a study of 100 healthy men and women between 65 and 89, power was found to decline at 3-4% per year whilst strength was found to decline at 1-2% per year (Skelton et al., 1994).

A cross-sectional study looking at 500 Finnish men found decreases in strength between 35 and 47% between the ages of 33 and 73 for muscle groups as disparate as the truncal extensors and flexors, knee extensors, hand grip and elbow flexors (Viitasalo et al., 1985). Another cross-sectional study of 654 subjects found decreases in isometric, concentric and eccentric peak torque for knee extensors and noted that strength appears to start later and decline slower in women compared to men (Lindle et al., 1997).

Loss of strength from proximal and distal muscles may be similar and unlike muscle mass losses between the sexes also appears to be similar, on a proportional basis (Doherty, 2003). These findings are replicated in the longitudinal studies in this area (Aniansson et al., 1986, Aniansson et al., 1992, Aniansson et al., 1983, Clement, 1974).

#### **1.2.2.3 Infiltration of fat and connective tissue**

Studies have found increased amounts of interstitial fat and other non-muscle tissue in cross-sectional imaging of elderly subjects compared to younger subjects (Rice et al., 1989, Forsberg et al., 1991). One study showed a two-to-three fold difference in non-contractile muscle mass seen on cross-sectional imaging between older (>65y) and younger (<45y) men and women. This was an increase from 6% in the young group to 15% in the older group (Kent-Braun et al., 2000, Forsberg et al., 1991). Therefore the reduction in muscle cross-sectional area actually accounts for an even greater loss of contractile mass than is accounted for using the CSA of the muscle alone. Fat infiltration of muscle can itself lead to worsening sarcopenia, mediated through macrophage released inflammatory cytokines (eg  $TNF\alpha$ , IL1 and IL-6) (Goodpaster et al., 2003, Goodpaster et al., 2000).

#### **1.2.2.4 Decrease in fibre size, with type 2 fibres showing more atrophy than type 1 fibres**

The majority of studies have shown a much greater reduction in size of type 2 fibres compared to type 1 fibres. Evidence has shown that the area occupied by type 2 fibres has decreased by roughly 20-50% by old age and type 1 by 1-25% (Larsson, 1978, Andersen, 2003, Doherty, 2003). Whereas two other studies showed no decrease in type 1 fibre area and a decrease of 26-29% in type 2 surface area between ~20y and 70-80 years of age (Lexell et

al., 1988, Nilwik et al., 2013). However as one third of fibres are neither type 1 or 2 by old age (due to co-expression of myosin heavy chain 1 and 2) the differentiation between muscle fibre type is probably less important in old age (Andersen, 2003, Klitgaard et al., 1990a, Klitgaard et al., 1990b).

#### **1.2.2.5 Decrease in both type 1 & 2 fibre number**

Several studies have shown a reduction in fibre number with age. Lexell et al. examined cross-sections of the quadriceps muscle taken at autopsy from males aged from 20 to 80 years of age and found a decrease in fibre number of 39% with age which started around 30 years old (Lexell et al., 1988). This sets sarcopenia apart from disuse atrophy where there is only a decrease in fibre size, not in number (Narici and Maffulli, 2010). The main process thought to account for fibre loss is denervation. Type 1 and 2 fibres are affected to the same extent thereby maintaining the proportions of fibre types (Sato et al., 1984, Mitchell et al., 2012).

#### **1.2.2.6 Cytoskeleton changes with age**

One paper which compared muscle biopsies from young volunteers (23-37 years) with older volunteers (66-77 years) found several morphological abnormalities. These comprised: an accumulation of internal nuclei (myonuclei are usually found at the periphery of the cell), ring fibres (where myofibrils encircle the long axis of the muscle fibres), ragged red fibres, lipofuscin and nemaline rod structures and a disarrangement of myofilaments and Z-lines (Jakobsson et al., 1990).

Evidence investigating the efflux of calcium through the sarcoplasmic reticulum as triggered by the motor neuron, which causes the muscle contraction, shows mixed results. There are studies showing both an impairment of the process in older muscle and no change with age (Delbono et al., 1995).

Rate of relaxation following contraction is longer in older muscle, which may be because of the action of  $\text{Ca}^{2+}$ -ATPase. This enzyme promotes the uptake of calcium back into the sarcoplasmic reticulum from the sarcoplasm and it has been shown that there is reduced  $\text{Ca}^{2+}$ -ATPase activity in older muscle. However one study has shown that if older muscle is trained using resistance exercises the effect of age on the enzyme is reduced but with no consequent shortening of relaxation time (Hunter et al., 1999).



### **1.2.2.7 Neuromuscular junction**

The role of the neuromuscular junction (NMJ) in the development of sarcopenia is not well understood. The NMJ has a “high safety factor” which means that the chance of an action potential arriving at the NMJ and not causing an action potential in the muscle is very low (Luff, 1998). Studies have indicated that there may be a progressive decline in the NMJ with age, evidenced by increased remodelling and fragmentation (Oda, 1984, Robbins and Fahim, 1985).

One study looking at NMJs in mice found that 85% of NMJs in young mice could be considered normal, whereas only 40% of the NMJs in older mice could be. It is interesting that the lesions they found were not specific to the ageing process, but were just found more frequently (Ludatscher et al., 1985). However in view of the high safety factor associated with NMJs it appears that they can tolerate quite a degree of dysfunction before they cease to work.

*Table 1-2: Table summarizing studies on structural changes seen in ageing muscle*

| <b>Structural change seen in sarcopenia</b>      | <b>Authors</b>   | <b>Number</b> | <b>Study findings</b>  |
|--|--|---------------|--|
| <b>Decreased muscle mass/CSA &amp; strength</b>  | (Aniansson et al., 1983)<br>(Aniansson et al., 1986)<br>(Aniansson et al., 1992) | 9             | In 9 men followed up after 3, 7 & 11 years, more strength was lost from the lower than upper limbs<br><br>2.3-3.2% decrease in knee extensor strength per annum              |
|  | (Clement, 1974)  | 2000+         | Strength declines more rapidly than muscle mass with age   |
|  | (Frontera et al., 2000)  | 12            | Thigh muscle CSA decreases by 1.4%/year in 60+ year old men  |
|  | (Gallagher et al., 1997)   | 284           | Men lose more muscle mass than women in absolute terms   |
|  | (Goodpaster et al., 2006)  | 1880          | Skeletal mass decreases by approximately 1% per year in people aged 70+ years<br><br>Knee extensor strength decreases by 2.6 to 4.1% per year according to sex and ethnicity |
|  | (Janssen et al., 2000)   | 468           | Skeletal muscle lost from age 45y<br><br>Men lose proportionately more than women<br><br>Lower limbs lose more than upper limbs  |
|  | (Skelton et al., 1994)   | 100           | In men and women age 65-89 years power decreases more quickly than strength<br><br>Strength decreases at 1-2% per annum and power decreases at 3.5% per annum                |
|  | (Koster et al., 2011)  | 2300          | Leg lean mass decreases by 0.8%/year in men and 0.7%/year for women aged 70-79   |
|  | (Young et al., 1984)   | 50            | 35% reduction in CSA of quads & 35% reduction in strength in women between 20y and 70y   |
|  | (Young et al., 1985)   | 24            | 25% reduction in CSA of quads & 39% reduction in strength in men between 20y and 70y   |
| <b>Infiltration of fat and connective tissue</b> | (Forsberg et al., 1991)  | 50            | Women have more muscle fat content than men<br><br>People over 60y have more muscle fat content than people under 60y  |
|  | (Kent-Braun et al., 2000)  | 44            | Two-fold increase in non-contractile content of locomotor muscles in elderly compared to young subjects on MRI   |
|  | (Rice et al., 1989)  | 20            | Elderly subjects had significantly more non-muscle tissue within muscle than young subjects  |

|   |                           |     |   |
|---|---------------------------|-----|---|
| <b>Decrease in muscle fibre size and number</b>   | (Andersen, 2003)          | 12  | Type 1 25% decrease and type 2 57% decrease in fibre size between young (25y) and old (88y) subjects<br><br>Greater percentage of fibres co-expressing MHC 1 & 2 in older subjects  |
|   | (Klitgaard et al., 1990b) | 17  | Type 2 fibres have a greater decrease in size with age than type 1 fibres   |
|   | (Lexell et al., 1988)     | 43  | Muscle fibre loss starts at 25y and continues throughout adult life<br><br>Type 2 fibres show a bigger reduction in size than type 1 fibres   |
|   | (Sato et al., 1984)       | 200 | Type 1 fibres decreased in number and increased in size after 60y<br><br>Type 2 fibres decreased in size after 40y and in number after 60y  |
| <b>Muscle fibre cytoskeleton changes with age</b> | (Delbono et al., 1995)    | 20  | Decreased excitation-calcium release-contraction coupling in older skeletal muscle compared to younger  |
|   | (Hunter et al., 1999)     | 28  | The older group had slower relaxation rates and times, decreased sarcoplasmic reticulum calcium uptake and Ca <sup>2+</sup> -ATPase activity and a larger relative type I fibre area than the younger group                 |
|   | (Jakobsson et al., 1990)  | 28  | Type 1 fibres were the same size in young and old subjects, but type 2 fibres were smaller in the elderly group<br><br>Increased frequency of cytoskeleton micro-pathology (eg ring fibres, fibre splitting) in older group |
| <b>Neuromuscular junction</b>                     | (Oda, 1984)               | 12  | Pre and post-synaptic changes seen with increasing frequency with age (eg perijunctional acetyl-choline receptors)  |

## **1.2.3 Mechanistic processes causing sarcopenia**

### **1.2.3.1 Inflammation**

#### *1.2.3.1.1 Inflammatory Cytokines*

Increasing age is associated with chronic low grade inflammation; there are mildly elevated levels of interleukin-6 (IL-6), tissue necrosis factor-alpha (TNF $\alpha$ ) and C-reactive protein (CRP) (Bruunsgaard et al., 2001).

IL-6, interleukin-1 beta (IL-1 $\beta$ ) and TNF $\alpha$  are pro-inflammatory cytokines which are thought to be involved in the muscle catabolism seen with sepsis, trauma and cancer. It is less clear what their roles are in the pathogenesis of sarcopenia. Raised IL-6 and TNF $\alpha$  levels have been associated with reduced muscle mass and strength in both cross-sectional and longitudinal studies (Visser et al., 2002, Beyer et al., 2012). However, it remains unclear if the elevated cytokines are a causal pathway in the development of sarcopenia or a marker of incident comorbidity which in itself is a risk factor for sarcopenia.

Both TNF $\alpha$  and IL-6 are known to upregulate the proteolytic ubiquitin proteasome pathway (UPP) pathway which could be an explanatory mechanism (Skipworth et al., 2006). Another hypothesis is that during inflammation there is an increased rate of hepatic acute phase response proteins which may use amino acids that would otherwise have been used in muscle protein synthesis. However, it seems unlikely that this is a main pathway in sarcopenia as there are not large increases of acute phase response proteins with normal ageing. Another theory is that cytokines can induce muscle apoptosis by DNA fragmentation. This has been demonstrated in animal models but not humans (Skipworth et al., 2006).

#### *1.2.3.1.2 Tissue Necrosis Factor $\alpha$*

TNF $\alpha$  is the classical activator of the nuclear factor-kappa B (NF- $\kappa$ B) signalling pathway. There are 5 known NF- $\kappa$ B transcription factors, all of which are expressed in skeletal muscle. Their functions include roles in apoptosis, inflammation and tissue differentiation. NF- $\kappa$ B can upregulate proteolytic pathways and inhibit muscle differentiation and function through suppression of MyoD mRNA and protein. Tissue expression of IL-1 $\beta$  and TNF $\alpha$  is induced by NF- $\kappa$ B activation and therefore this cycle is self-perpetuating and may explain the chronic progressive atrophy seen in sarcopenia. Interestingly NF- $\kappa$ B has also been shown to be activated via non-classical pathways (eg in disuse atrophy) therefore TNF $\alpha$  would not have to be raised for NF- $\kappa$ B to be contributing to sarcopenia (Skipworth et al., 2006).

TNF $\alpha$  seems to play a role in cancer cachexia by inducing catabolic genes including the E3 ubiquitin ligases; atrogin-1 and MuRF-1. It had been postulated that this could be mirrored in ageing muscle, however human data has shown the mRNA levels for these two genes are unchanged in ageing muscle (Leger et al., 2008).

### **1.2.3.1.3 Interleukin-6**

Higher IL-6 and CRP levels have been found to be associated with loss of muscle strength in a large longitudinal study (Schaap et al., 2006), and muscle mass in the Health ABC study (Visser et al., 2002). Levels of IL-6 appear to increase with age, particularly following the menopause in women (Hager et al., 1994, Ershler and Keller, 2000). IL-6 is known to be suppressed by dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) therefore it could be that the lower levels of DHEA and DHEAS seen with increasing age contribute to raised IL-6 levels (Straub et al., 1998).

There are several other hypotheses as to how raised IL-6 levels may contribute to sarcopenia. Plasma IL-6 levels are known to increase proteolysis within muscle however it is not known if the degree of increase seen with ageing is enough to cause atrophy and it is thought that proteolysis is not a major factor in normal ageing muscle (Tsujinaka et al., 1996). However IL-6 may exert its effect through less direct routes. It is known that raised IL-6 causes anorexia, which would lead to decreased protein substrate. Also, IL-6 can both activate cortisol secretion and induce 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD1) expression, so it may exert its effect via a glucocorticoid pathway (Weber et al., 1997, Tomlinson et al., 2001). IL-6 is known to stimulate the release of corticotrophin-releasing factor from the hypothalamus which will lead to increased levels of circulating corticosteroids, however glucocorticoids are also known to inhibit IL-6 production from a variety of tissues (Ershler and Keller, 2000). Therefore a negative feedback loop may exist.

We do not know how local cytokine production within muscle is affected by age. Research to date has used circulating values of IL-6 therefore this area merits further study.

### **1.2.3.2 Cellular senescence**

#### **1.2.3.2.1 Mitochondrial ageing**

Older muscle shows reduced activity of mitochondrial enzymes, reduced mitochondrial volume and an increased proportion of abnormal mitochondria (Trounce et al., 1989, Conley et al., 2000, Rifai et al., 1995, Marzetti et al., 2013). As mentioned above, it has been shown

that the number of ragged red fibres increases with normal ageing. These are clumps of diseased mitochondria which accumulate in the subsarcolemmal region of the muscle fibre and may reflect an age-related decline in muscle mitochondrial oxidative metabolism (Rifai et al., 1995). However it is unclear if the degree of mitochondrial dysfunction found in normal ageing muscle is sufficient to cause the corresponding decrease in muscle function (Welle, 2002).

#### *1.2.3.2.2 Oxidative damage*

Markers of damage caused by oxidative stress are found to accumulate with age. Both lipofuscin, which is thought to result from the oxidative polymerization of lipids, and the antioxidant enzyme glutathione are found in higher levels in old rats (Weindruch, 1995).

There are several possible hypotheses as to how oxidative damage may contribute to sarcopenia. Cellular metabolism generates free radical particles which include reactive oxygen and nitrogen particles and reactive aldehydes. These particles can cause damage to proteins, DNA and lipids. Mechanisms to repair the damage caused by these particles are not perfect therefore over time damage is accrued. Damage to both somatic and mitochondrial DNA occurs, although it is difficult to categorise exactly what the functional outcome of this damage is. Despite the accruing damage to mitochondrial DNA most copies are in fact normal (Welle, 2002).

Interleukins 4, 10, 13 and 15 are anti-inflammatory cytokines. IL-15 in particular has shown direct inhibition of proteolysis and lipid oxidation in skeletal muscle (Magee et al., 2008), and there is evidence that IL-15 decreases with age in rats (Marzetti et al., 2009).

A study investigating transgenic mice that lacked the enzyme superoxide dismutase showed increased mitochondrial dysfunction leading to neuromuscular junction dysfunction and accelerated sarcopenia (Jang et al., 2010). This supports the theory that impairment in dealing with oxidative stress contributes to sarcopenia.

#### *1.2.3.2.3 Satellite cells*

Satellite cells are myogenic precursor cells, which are found between the sarcolemma (muscle cell wall) and the basal lamina of muscle fibres. They are activated following muscle injury whereupon they proliferate and differentiate into myoblasts. These myoblasts then fuse with other myoblasts to form myotubes which can then form a new muscle fibre or fuse with an existing fibre as a repair mechanism. However the muscle fibre loss which occurs with

ageing does not appear to trigger these cells (Welle, 2002). It appears that the trans-membrane receptor notch is responsible for activating the satellite cells and that activation of notch appears to decline with age (Carlson et al., 2009). Insulin-like growth factor 1 (IGF-1) can trigger activation of satellite cell division also, and this can be triggered by exercise (Adams and Haddad, 1996, Adams and McCue, 1998). This represents a pathway through which the decreased levels of IGF-1 seen with age can contribute to the sarcopenic process.

Satellite cells are crucial for maintaining muscle mass, but their role in the pathogenesis of sarcopenia is still unclear; however satellite cell activation upon exercise is a potentially effective countermeasure for sarcopenia (Thornell, 2011).

#### *1.2.3.2.4 Denervation*

Multiple studies have shown a significant loss of motor units with age which appears to occur predominantly after the age of 60 years old (Lexell et al., 1983, Campbell et al., 1973). A motor unit consists of a motor neuron and the muscle fibres it innervates. Therefore, if there is a reduction in the number of motor neurons there will be a reduction in the number of motor units. There is a 70% reduction in the number of motor neurones in the spinal cord by the age of 90 compared to young, healthy controls (Vandervoort, 2002). Electrophysiological tests have shown there is a greater decrease in motor unit number in distal rather than proximal muscles (Galea, 1996). Number of motor units recruited and firing frequency of motor nerves have both been found to be reduced with increasing age (Kamen et al., 1995).

As motor neurons are lost with age the remaining motor fibres are reinnervated by neighbouring motor neurons through axonal sprouting and it has been found that the size of motor units increases with age, ie the number of fibres innervated by each motor neuron. These findings have been replicated in areas as diverse as the lumbosacral spinal cord to the biceps brachii to the extensor digitorum brevis in the leg (Tomlinson and Irving, 1977, Campbell et al., 1973, Brown et al., 1988). Fibre type is dependent in part on innervation and this period of change in innervation may account for the changes in fibre types seen with age (Welle, 2002). It has been shown that larger motor units can show more fatigability and this may contribute to the declining strength seen with age (Welle, 2002).

It is interesting to note that diseases of alpha motor neurons ( $\alpha$ mn) in which there are rapid neuronal loss do not show similar signs to sarcopenia (ie decreased fibre number, size) until there has been a loss of 80-90% of the number of  $\alpha$ mns. An explanation may be that there is

impairment in reinnervation of the fibres seen with age which would lead to muscle fibre loss, although there is evidence which both refutes and supports this hypothesis (Carlson and Faulkner, 1996, Carlson and Faulkner, 1998, Kanda and Hashizume, 1991).

One possible factor contributing to the denervation seen with age is the damage that free radicals may cause to motor neuron DNA. As this damage is cumulative and as each motor neuron has only one nucleus, unlike myocytes, damage to a few key genes could lead to significant malfunction of the cell or even cell death.

#### *1.2.3.2.5 Protein Turnover*

The proteins within muscle are in constant turnover, being synthesised and broken down at an average rate of 1-2% per day (Fearon et al., 1988). Protein turnover is in equilibrium when protein synthesis is equal to protein half-life, a proxy for protein breakdown.

The rate of muscle protein and mitochondrial protein synthesis has been shown to be slightly slower in older adults in samples of vastus lateralis (Yarasheski et al., 1993). This appears to be due to reduced translational efficiency, as total RNA and mRNA concentrations are stable between young and old subjects (Welle et al., 2000). Another study found that the rate of myosin heavy chain synthesis declines with age and that the rate was correlated with IGF1, DHEA and free testosterone in men (Volpi et al., 2001). However, these decreases in muscle synthesis rate are small. Older muscle does not appear able to increase rates of muscle protein synthesis following anabolic stimuli (eg amino acid feed or acute exercise) as successfully as young muscle can (Cuthbertson et al., 2006).

Results from studies looking at rates of muscle proteolysis are conflicting but overall it seems that an increased rate of proteolysis is not a main pathway of sarcopenia. It appears that proteolysis is either unaffected or only very slightly increased with age (Volpi et al., 2001, Volpi and Rasmussen, 2000, Welle, 1995, Balagopal et al., 1997).

#### *1.2.3.2.6 Gene expression*

There have been multiple studies looking at differences in gene expression between young and old muscle, with many of the studies using mice. However, it appears that mice are unlikely to be a good model for the effect of ageing on gene expression in human muscle. Most of the genes found to be over or under-expressed in older human muscle were not so in mice and genes that were found to be over-expressed in aged mice were not so in human tissue (Welle et al., 2001).



Of the articles looking at human muscle, one review article surmised that fewer than 2% of genes expressed in muscle are consistently over- or under-expressed in old compared to young muscle (Welle, 2002). Welle et al found that older muscle has reduced levels of several mRNAs which encode: proteins involved in mitochondrial electron transport and ATP synthesis; and enzymes involved in glucose and glycogen metabolism (Welle et al., 2000). However, Coggan et al. found no change in the level of enzymes involved in the glycolytic pathway (Coggan et al., 1993). Roth et al identified around 50 genes as differentially expressed in relation to age from a microarray representing around 4000 human genes and using a cut-off of >1.7-fold; these represented structural, metabolic, and regulatory gene classes (Roth et al., 2002). With new and improved techniques developing in the field of molecular biology, hopefully future studies will deliver more reproducible findings.

### **1.2.3.3 Hormones and Growth Factors**

#### *1.2.3.3.1 Testosterone and DHEA*

Testosterone is associated with muscle strength and testosterone levels are known to decrease with age. Several studies have looked at testosterone supplementation and muscle mass and strength. The results have been mixed with some studies documenting improvements in lean mass and muscle strength and a decline in fat mass, whilst others showed no change (Kim, 1999, Wang et al., 2000, Maggio et al., 2013). It may be that the degree of hypogonadism of the subjects prior to commencing therapy affects the outcome. There are frequent side effects with supplementation also including a raised haematocrit and raised PSA.

DHEA is a precursor of testosterone secreted mainly from the adrenal glands which contributes significantly to testosterone production in women. However, DHEA levels have been found to be correlated with lean body mass in men but not women (Abbasi et al., 1998). A study of DHEA replacement therapy in women resulted in no increase in muscle mass or strength and in men only slightly improved strength with no increase in mass (Morales et al., 1998). Therefore it appears that DHEA will not be a useful treatment in sarcopenia.

Testosterone and IGF1 levels have been associated with muscle strength and this generates a hypothesis for how lowered levels of testosterone could lead to sarcopenia (Ashton et al., 1995). Raised levels of testosterone increase levels of IGF1 and its binding protein, therefore it would follow that lowered levels could cause the converse.

#### *1.2.3.3.2 Oestrogen and Progesterone*

It is not clear what role oestrogen and progesterone have to play in the aetiology of sarcopenia. Most studies looking at HRT and sarcopenia have found no improvement in muscle strength or mass with HRT (Haarbo et al., 1991, Baumgartner et al., 1999). However matters are complicated by the fact that oral oestrogens decrease both IGF1 levels and the free fraction of testosterone (by increasing sex hormone binding globulin levels). One study on HRT did however find an increase in lean body mass and a decrease in total fat mass; however it only included 16 subjects (Sorensen et al., 2001).

#### *1.2.3.3.3 IGF1 and Growth Hormone (GH)*

IGF1 has several anabolic effects on muscle, including; increased protein synthesis, enhanced satellite cell proliferation and improved reinnervation of muscle fibres following denervation (Barton-Davis et al., 1999).

Growth hormone levels fall with increasing age and this leads to decreased circulating levels of IGF1. Patients with GH deficiency have been shown to have more adipose tissue and less lean tissue than age-matched controls (Binnerts et al., 1992). The decline in GH and IGF-1 levels seen with aging is attributed to changes in the effect of the hypothalamic factors (somatostatin (SRIF) and growth hormone-releasing hormone (GHRH)) on the pituitary gland. As people age there is a reduction in the response to GH to GHRH and a simultaneous increase in the inhibitory SRIF tone (Kamel, 2003). Low protein diet in rats has been found to decrease levels of IGF1, suggesting another possible mechanism for the levels seen in older humans (Miura et al., 1992).

Trials of treatment with growth hormone, to increase IGF1 levels, have shown increased muscle mass but no improvement in strength (Welle et al., 1996, Papadakis et al., 1996). There also appears to be a high incidence of side effects which would largely prohibit any long-term use of GH as a treatment for sarcopenia (eg glucose intolerance, raised blood pressure). Therefore, alternative treatments which would increase levels of IGF1/GH without incurring the side-effects have been investigated. One potential method is intravenous or subcutaneous GHRH which appears to raise levels of IGF1 and GH whilst maintaining the major counter regulatory mechanisms which attenuate the adverse effects of overtreatment; however, this approach requires further human studies (Iovino et al., 1989, Corpas et al., 1992). Interestingly a study investigating a GHRH antagonist in mice showed some anti-

ageing effects including improved longevity but no improvement in muscle strength (Banks et al., 2010).

GH may affect muscle mass through the glucocorticoid system. GH replacement therapy has been shown to decrease the conversion of cortisone to cortisol, possibly through decreased activity of 11 $\beta$ HSD1 (Sigurjonsdottir et al., 2009). In human hypopituitary patients (Weaver et al., 1994) and in rats, treatment with either GH or IGF1 or both has been shown to prevent steroid induced myopathy (Matsushita et al., 1996).

IGF1 and insulin are known to activate the anabolic phosphatidylinositol 3-kinase pathway (PI3K) which leads to an activation of the Akt pathway which subsequently activates the mammalian target of rapamycin (mTOR) kinase which leads to increased protein synthesis. It is known that this pathway is deactivated in muscle wasting (Sacheck et al., 2004). This pathway (ie IGF1/PI3K/Akt) inhibits the forkhead box class O (FOXO) transcription factors. The FOXO transcription factors regulate expression of parts of the proteolytic ubiquitin-proteasome pathway (UPP), therefore if the IGF1/PI3K/Akt pathway is deactivated, the FOXO pathway is not inhibited and the UPP pathway is upregulated causing proteolysis (Stitt et al., 2004). However, a review by Murton et al has highlighted that the pathway seems to only be upregulated in inflammatory muscle wasting (eg cancer cachexia, COPD) (Murton et al., 2008). Also, as proteolysis has been shown not to be a major factor in sarcopenia, it seems unlikely that the UPP pathway plays a major role.

Increased inflammatory cytokines may also contribute to growth factor inhibition with age. There are 6 IGF binding proteins and each can either positively or negatively affect the bioavailability of IGF1 and both GH and TNF $\alpha$  appear to modulate the levels of the IGF binding proteins (Skipworth et al., 2006, Lang et al., 2006). TNF $\alpha$ , IL-1 $\beta$  and other inflammatory cytokines all cause growth factor inhibition, mainly IGF1 (Broussard et al., 2004).

#### *1.2.3.3.4 Insulin*

Insulin is an anabolic hormone which promotes muscle protein production. Insulin resistance is increasingly prevalent with increasing age and therefore may present a possible pathway for the development of sarcopenia, either through decreased anabolism or increased glycation which, is thought to impair muscle strength (Hook et al., 2001, Haus et al., 2007). This process seems self-perpetuating as it is known that sarcopenia causes insulin resistance,

secondary to increasing fat deposition within muscles and other metabolic pathways, which leads to decreased anabolism which will worsen sarcopenia (Janssen and Ross, 2005).

#### *1.2.3.3.5 Ghrelin*

Ghrelin is a peptide which appears to have opposing effects to leptin. It stimulates GH secretion and increases appetite. No human studies have been performed as yet looking at ghrelin as a treatment for sarcopenia, although one study has found a negative association between age and both ghrelin and IGF1 (Akamizu and Kangawa, 2007).

#### *1.2.3.3.6 Glucocorticoids*

It is well known that glucocorticoids at pharmacological levels or within the context of pathology (eg Cushing's disease) can cause muscle atrophy (Schakman et al., 2013). However, the role of glucocorticoids (GC) in the context of normal ageing and their effect on musculature is much less well understood. It is thought that the relatively small increases seen with normal ageing are not enough to contribute in any significant way to the process of sarcopenia (Welle, 2002). However, two hypotheses could explain how glucocorticoids contribute to sarcopenia at normal circulating levels.

Firstly it may be that 11 $\beta$ HSD1 is upregulated in muscle in old age. 11 $\beta$ HSD1 is an intra-cellular enzyme found in muscle and other tissues which plays a pivotal role in increasing the amount of intra-cellularly active glucocorticoid (Cooper et al., 2002). There is some evidence that 11 $\beta$ HSD1 expression increases with age in bone but it is not known if this is also the case in muscle (Cooper et al., 2002).

Secondly, muscle's ability to repair and renew itself following bouts of acute illness lessens with increasing age (eg satellite cells are less active with increasing age). Therefore recurrent bouts of ill-health across the lifecourse with subsequent acute rises in cortisol could have an additive effect on muscle mass and structure even if basal levels of cortisol were not elevated.

There are several potential pathways by which glucocorticoids may contribute to sarcopenia. Firstly, they upregulate protein degradation pathways and secondly they cause an increase in glutamine synthetase activity (to increase gluconeogenesis rates) which decreases the amount of amino acids available for muscle protein synthesis. As noted below they also upregulate myostatin in a dose dependent manner (Ma et al., 2003).

It is interesting to note that hypercortisolaemia as seen in Cushing's disease and medical treatment, causes muscle atrophy of the proximal limb muscles (a different distribution from sarcopenia), and causes preferential wasting of the fast-twitch (type 2) fibres (the same as sarcopenia) (Schakman et al., 2013, Gupta and Gupta, 2013).

#### *1.2.3.3.7 Myostatin*

Myostatin inhibits muscle differentiation and growth. It has a major role in maintaining satellite cells in their quiescent state and it also inhibits MyoD, a protein which promotes myogenesis (Doherty, 2003). It has been found that glucocorticoids upregulate myostatin expression (Ma et al., 2003). Serum myostatin levels negatively correlate with fat free mass and muscle mass, and therefore are found to increase with age (Yarasheski et al., 2002). One potential issue with suppressing myostatin as a sarcopenia treatment strategy is that it may lead to depletion of muscle stem cells.

#### *1.2.3.3.8 Angiotensin II*

Angiotensin II upregulates the UPP pathway, inhibits protein synthesis and modulates the IGF1 pathway (Skipworth et al., 2006). Plasma angiotensinogen levels are increased by corticosteroids, indicating another potential mechanism for the effect raised GC levels can have on muscle. The use of ACE-inhibitors as a potential therapeutic treatment has therefore been explored. However, studies have found conflicting results on whether they provide a functional benefit (Witham et al., 2008, Witham et al., 2014, Sumukadas et al., 2014).

#### *1.2.3.3.9 Ciliary neurotrophic factor*

Another growth factor called ciliary neurotrophic factor (CNTF) has been shown to promote motor neuron survival in rodents and increase muscle mass, but its expression in human muscle has not been investigated (Ma et al., 2003, Morley, 2012).

### **1.2.3.4 Inherited factors**

Between 30 and 85% of adult muscular strength and 45–90% of adult muscle mass is hereditary (Rolland et al., 2008, Tan et al., 2012). Low birth weight has been associated with sarcopenia in older men and women when corrected for weight and height (Aihie-Sayer et al., 2004, Yliharsila et al., 2007). This indicates that early life-exposures and epigenetics play an important role in the development of sarcopenia.

The number of studies investigating potential candidate genes for individual differences in muscle strength and mass has rapidly expanded over the past few years. Candidate genes

have been tested and variants in the following have been associated with sarcopenia: PI3-K/Akt/mTOR pathway, serum response factor, peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 $\alpha$ , ubiquitin-proteasome system, autophagy-dependent signalling, myostatin, TNF- $\alpha$  and NF- $\kappa$ B and IGF-I signalling (Tan et al., 2012, Sakuma et al., 2014).

Genetic linkage analysis of the myostatin pathway found polymorphic markers which were linked to lower extremity muscle strength around both the myostatin and IGF1 gene (Huygens et al., 2004, Huygens et al., 2005). An IGF2 variant has also been associated with arm and leg strength (Schrager et al., 2004). Mutations in the ciliary neurotrophic factor A allele have been found to be associated with lower quadriceps strength (Roth et al., 2001). Polymorphisms of the angiotensin converting enzyme gene have been shown to influence peak knee extensor power in response to resistance training (Giaccaglia et al., 2008). Polymorphisms in the vitamin D receptor have been associated with muscle mass and strength in elderly men and women (Roth et al., 2004, Geusens et al., 1997). Also, variants in the thyrotrophin-releasing hormone receptor (TRHR) have been found to be associated with clinically relevant differences in muscle mass in older women (Lunardi et al., 2013). Clearly further research in this important area is required, with particular emphasis on the possible location of a potential drug target for improving muscle mass or more importantly muscle strength in older aged adults.

### **1.2.3.5 Lifestyle**

#### *1.2.3.5.1 Physical Activity*

Reduced levels of physical activity appear to be a universal phenomenon of ageing, found in animals ranging from fruit flies to mice to humans (Sallis, 2000). Inactivity at any age leads to muscle atrophy and even amongst the physically active elderly, rates of physical activity are roughly 20% lower than they were in younger age groups (Morse et al., 2004).

What is less clear is whether this is cause or effect, ie whether people have lower levels of physical activity because they have a greater degree of sarcopenia or whether lower levels of activity cause sarcopenia. Although increased physical activity can delay the disability caused by sarcopenia it cannot halt the underlying process and there will continue to be a relative decline in mass and function (Liu and Latham, 2009). Studies looking at athletes have found that whilst exercise does slow down losses in muscle mass and strength, there is still a continued decline (Faulkner et al., 2007). Therefore it is hypothesised that whilst exercise can

hypertrophy the remaining fibres it cannot prevent the neuropathic processes which contribute to muscle apoptosis.

Two things have been found to predict muscle bulk in older age; the peak muscle bulk achieved in young adulthood and the rate of decline thereafter (Roubenoff and Hughes, 2000). This would support the lifecourse approach study of ageing, where not only factors which affect rate of decline are investigated but also factors which promote achieving a good peak muscle mass, which would include physical activity in young and mid-adulthood. Resistance exercise has been shown to increase muscle mass and muscle strength even in the very elderly (Fiatarone et al., 1990, Fiatarone et al., 1994, Liu and Latham, 2009). One possible mechanism is that resistance exercise has been shown to decrease skeletal muscle TNF $\alpha$  expression even in the very elderly (80y+) and TNF $\alpha$  has been shown to be inversely correlated with skeletal muscle protein synthesis (Greiwe et al., 2001). Resistance and endurance exercise have also been shown to increase circulating IGF1 levels in the young, however these findings were not replicated in the older population. One study has shown increased IGF1 immunoreactivity in older muscle following resistance exercise, showing that IGF1 may play an important autocrine/paracrine role in older human muscle rather than as a circulating hormone (Singh et al., 1999).

Physical activity does not solely affect the muscle fibre changes seen within sarcopenia. Doubling the rate of physical activity can also half the amount of non-contractile tissue (Kent-Braun et al., 2000).

#### *1.2.3.5.2 Nutrition*

There are many reasons for the decreased food intake seen with older age. These include: poor dentition, decreased sensations of taste and smell, psychological factors (eg depression), early satiety secondary to decreased relaxation of the fundus and increased CCK and leptin levels (Morley et al., 2001). It is postulated that decreased nutritional intake contributes to the aetiology of sarcopenia, by both a subsequent weight loss, including muscle mass, unless energy expenditure is concurrently reduced, and failure to meet nutritional requirements within a smaller diet (eg inadequate protein intake).

Evidence for whether nutritional supplements or high-protein diets increased muscle mass, strength or protein synthesis have found contradictory evidence, with some studies finding a positive effect and others not (Fiatarone et al., 1994, Campbell, 1995, Welle and Thornton,

1998, Borsheim et al., 2008). One study suggests this may be because the supplements contained carbohydrate which appears to impair the anabolic response to ingested protein and that unless energy expenditure also increased the subjects decreased their nutritional intake to match previous levels; therefore their overall nutritional intake remained the same. They also found that supplementation with only essential amino acids improved muscle protein synthesis in elderly subjects (Volpi et al., 1998). A study looking at the effects of nutritional supplementation and exercise on muscle strength and mass found no additional benefits of taking the supplements in either those exercising or not (Fiatarone et al., 1994). Conflicting results in these studies may partially be representative of how protein deficient the subjects' diets were prior to the intervention.

Other possible nutritional effects have been found. Eicosapentaenoic acid is an omega-3 fatty acid which may have anti-inflammatory effects which could attenuate sarcopenia (Magee et al., 2008). Both low vitamin D levels and high parathyroid hormone levels have been found to increase the risk of muscle wasting in old age (Visser et al., 2003). The effect of improved nutrition on sarcopenia requires further research.

#### *1.2.3.5.3 Smoking and alcohol*

Perhaps surprisingly, smoking and alcohol were found to have no strong association with muscle mass in a large cross-sectional study of older British men (Atkins et al., 2014) or in a cohort of in-patients, where sarcopenia was diagnosed as the combination of decreased muscle mass and function (Gariballa and Alessa, 2013). In a community study in Brazil, where sarcopenia was again defined using the EWGSOP criteria (requiring decreased muscle size and function) smoking status was found to be associated with sarcopenia. Alcohol consumption was not associated with sarcopenia status in women, or in men at moderate levels, but high daily intake of alcohol was associated with an increased odds ratio of having sarcopenia in men (Alexandre Tda et al., 2014). It may be that smoking and alcohol intake do not have a linear relationship with sarcopenia, where low or moderate intake has no negative effect, but that excessive consumption has deleterious consequences.



Figure 1-1: Pathways influencing sarcopenia

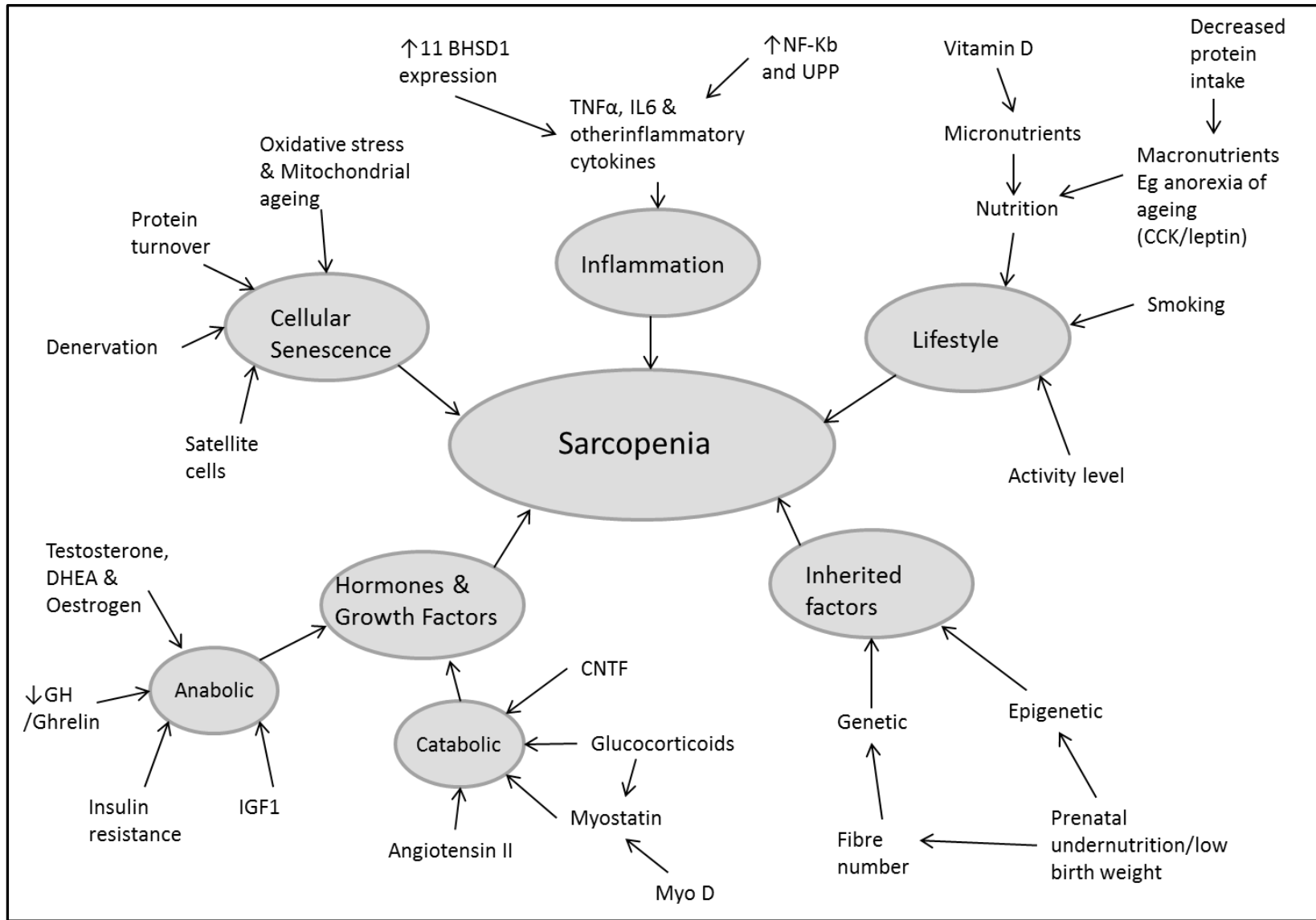
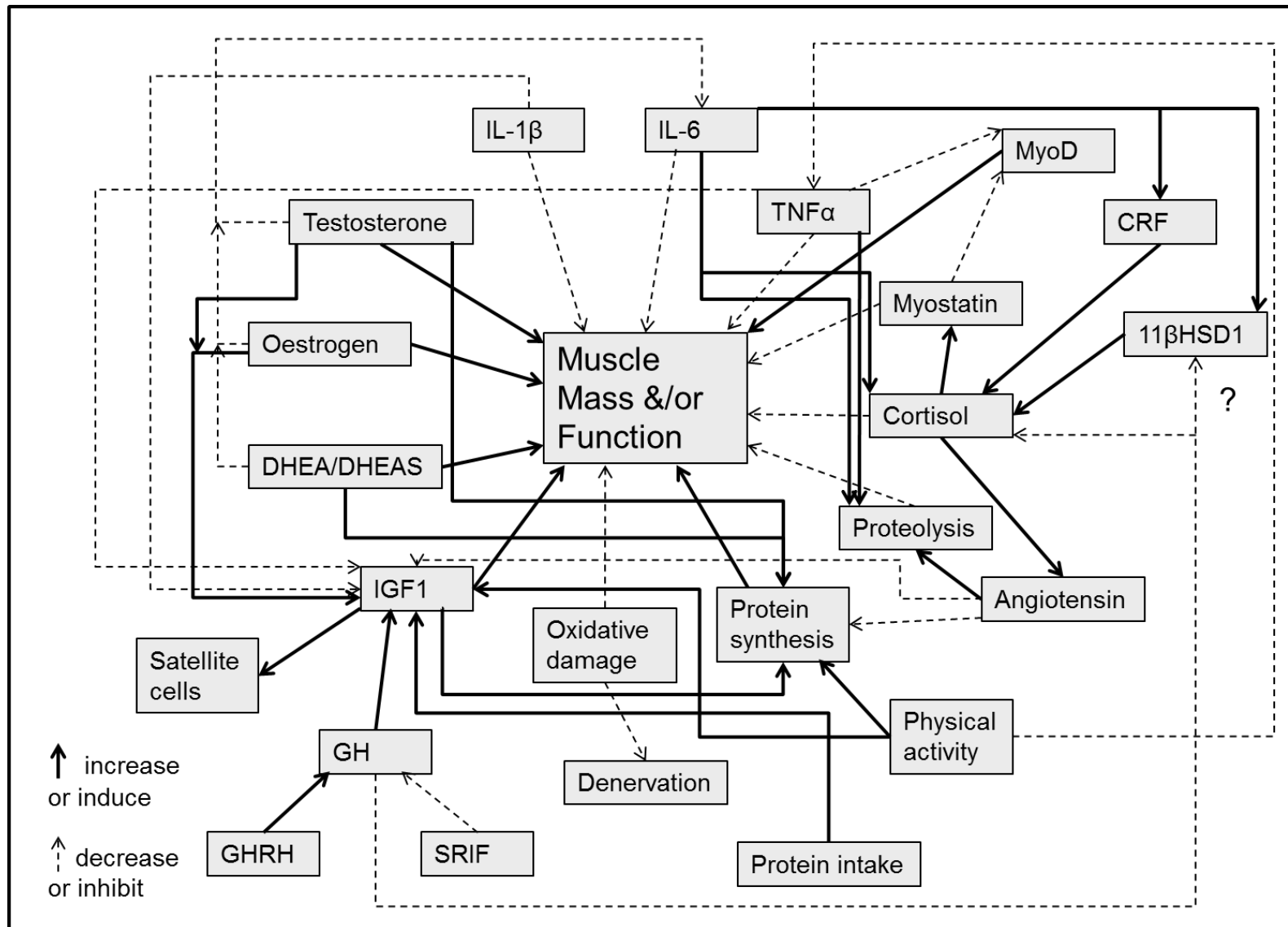


Figure 1-2: Diagram showing the interaction between mediators which contribute to sarcopenia



## **1.3 Age-related Cognitive Decline**

### **1.3.1 Definition of age-related cognitive decline (ARCD)**

In 1962 Kral suggested a distinction between benign and malignant senescent forgetfulness (Kral, 1962). He described the benign form as following a relatively static course, whereas the malignant form frequently progressed to dementia and consequently early death. This early paper documenting distinct patterns of cognitive decline has led to much scientific investigation (Petersen et al., 2009). Although scientific understanding of the cognitive impairments seen with ageing is continuing to evolve, there are some central tenets which are widely agreed upon.

There are deteriorations in some parts of cognition seen with age. This is now termed “age-related cognitive decline” and is in the ICD-10 (code R 41) as such. Other commonly used terms include: age-associated cognitive decline (AACD), normal cognitive ageing, cognitively impaired not demented (CIND) and age-associated memory impairment.

Unfortunately their use has not been consistent, being used for various populations within the normal/pathological cognitive ageing spectrum in different articles, which has led to some confusion in the literature.

Not all cognitive processes decline with age. When describing which areas of cognition appear to be affected by the ageing process it is typical to divide cognitive processes into fluid and crystallised abilities. It has been shown that whilst crystallised abilities remain largely resistant to changes with age, fluid abilities do show evidence of decline. Crystallised abilities include: autobiographical memory, semantic knowledge, emotional processing and some language skills. Fluid abilities include: speed of processing, working memory, inhibitory function and long-term memory (Hedden and Gabrieli, 2004, Park and Reuter-Lorenz, 2009). Studies have shown that the decline seen in these processes begins in early adulthood, with some deterioration seen by the early 20s (Salthouse, 2009).

It is also apparent that the rate of decline varies greatly between individuals. Although some people show sharp cognitive decline, others can have a more benign picture whilst others can even show improvement in some cognitive domains with age (Wilson et al., 2004). However, the underlying mechanisms which account for these differences are not fully understood. This is further complicated by the fact that the neuropathology found in Alzheimer’s disease (amyloid plaques and neurofibrillary tangles) can also be found in ARCD (Keller, 2006).

A further entity exists where subjects have a degree of cognitive decline which is considered pathological, but which is not severe enough to be termed dementia. The term now used for this entity is mild cognitive impairment (MCI) and it is under the umbrella of mild neurocognitive disorder in DSM-5. MCI has a variety of definitions but typically is described as non-normative cognitive ageing with no effect on social functioning (eg ability to perform activities of daily living), whereas a diagnosis of dementia must include an effect on social functioning. However diagnostic cut offs or criteria for MCI are less well agreed upon and clinical judgement appears to remain paramount. In fact in several large studies investigating MCI, a team of specialists were required to distinguish between ARCD, MCI and dementia (Manly et al., 2008, Fischer et al., 2007), which is indicative of the degree of overlap between them.

However, whilst ARCD, MCI and dementia do show a degree of overlap, it is unlikely that they all lie on the same pathological continuum for two reasons. Firstly, the pattern of which cognitive areas are affected by dementia and normal cognitive ageing are different and secondly, there are behavioural and psychological symptoms which accompany dementia which are not seen to any degree with normal cognitive ageing (Petersen, 2004).

It is widely quoted that the rate of conversion from MCI to dementia is around 10-15% per year (Petersen et al., 1999). However, these figures come from studies looking at selective populations (eg referrals to a memory clinic). Prospective studies show lower rates, between 6-10%. Both these figures are much higher than the conversion rate from ARCD to dementia which is 1-2% per year (Petersen et al., 1999).

The prevalence of MCI varies around the world and according to diagnostic criteria but most studies show the prevalence to be around 14-18% in people over 70 y (Petersen et al., 2009). The prevalence of dementia in the UK is around 7% of people over 65 y.

### **1.3.2 Cognitive ageing - a natural ageing process or a disease?**

A fundamental question when considering the changes seen in cognition with age is whether they are due to intrinsic ageing processes, as sarcopenia appears to be, or whether there are pathological processes involved. Conditions which appear as part of the natural ageing process should be universal, should not be reliant on specific risk factors and can vary widely in rate of progress and timing of incidence between individuals. Studies have shown evidence for both sides of this argument with regard to cognitive ageing.

Small argues that if cognitive decline was an inevitable part of normal ageing then the mean scores for relevant memory tests should simply move to the left and retain a similar variance, however animal and human studies have demonstrated that the variance increases with age and some studies have found bimodal distributions (Small, 2001, Rapp and Amaral, 1992). However, the human studies did look solely at cross-sectional studies and no longitudinal studies were examined. Another paper which looked at the relation between incident disease and decreases in MMSE found that in healthy subjects who were not diagnosed with any disease process within a set time period, MMSE only decreased by 0.1 points per year. They concluded that significant cognitive change cannot be attributed to age alone. However the MMSE is not a particularly sensitive test therefore a further study is required to repeat this process using more sensitive cognitive tests (Starr et al., 1997). Another large study (n=1360) found that co-morbidities only explained a small part of the individual differences seen in normal cognitive ageing: additional variance added for each specific disease ranged from 1.5% to 3.5% (van Boxtel et al., 1998).

Furthermore, variances in childhood IQ have been associated with disease states in later life including psychiatric illness, hypertension, lung cancer and ischaemic heart disease, and childhood IQ is known to predict later life IQ (Zammit et al., 2004, Starr et al., 2004, Hart et al., 2003, Deary et al., 2004b). Therefore it may be that low childhood IQ contributes to the risk of developing the diseases which increases risk of cognitive decline, so that childhood IQ is acting as a confounding factor.

It is therefore extremely complicated to pick apart what is due to the intrinsic ageing process and what is due to pathological processes. Some of the risk factors for developing cognitive decline (eg level of physical fitness) increase with age but it is difficult to fully appreciate if this is due to the natural ageing process or to accumulating pathology (eg osteoarthritis). Many diseases increase in prevalence and incidence with age further complicating the picture.

Currently there is evidence for both sides of the argument. Therefore until we have a large enough study with sensitive enough tests of fluid ability, we will have to accept that cognitive decline with age could indeed represent part of the normal ageing process but may be due to age accumulated pathology, the extent of which has not yet been fully delineated.

### **1.3.3 Structural changes associated with ARCD**

#### **1.3.3.1 Brain size**

The human brain shrinks with age but not in a uniform pattern. Certain areas shrink throughout adult life (the tertiary association cortices, the neostriatum, and the cerebellum); some stay relatively stable in size (sensory cortices and the pons); and some show accelerated decline in older age (subcortical white matter and the hippocampus, which is accelerated by hypertension) (Raz and Rodrigue, 2006). Changes in cortical thickness and subcortical volume can be mapped using MRI, with annual reductions of between 0.5% and 1.0% seen in most brain areas (Fjell and Walhovd, 2010). As the brain shrinks there is a corresponding increase in the size of the ventricles and the volume of CSF. Most studies so far have not demonstrated any strong association between structural brain differences and cognitive ability in old age (Deary et al., 2009). This is perhaps surprising, but may be because as functional areas of the brain atrophy, the brain recruits other areas to perform its tasks. One review did find that neuroanatomical change accounted for a substantial degree of the reductions in specific cognitive abilities. They found that between 25% and 100% of the differences seen in selected cognitive functions with age could be explained by differences in structural brain characteristics (Fjell and Walhovd, 2010). Large longitudinal studies with careful covariate selection are required in this area.

#### **1.3.3.2 Neuronal and white matter structural changes**

Although there is evidence of limited neuronal loss from the hippocampus with age, loss of neurons with age is otherwise not a major feature contributing to the volume loss seen in the ageing brain (Keller, 2006). Shrinkage of individual neuron size, reduction of synaptic spines and a lower numbers of synapses probably accounts for the reduction in grey matter volume (Fjell and Walhovd, 2010).

White matter structural change is also an important feature of normative brain ageing; studies demonstrate that the presence of white matter hyperintensities (evidenced by both T2-weighted and diffusion tensor imaging (DTI)) is linked to cognition in older age (Bendlin et al., 2010, Shenkin et al., 2005), and that the length of myelinated axons is reduced by up to 50% in healthy ageing (Fjell and Walhovd, 2010).

### 1.3.4 Mechanistic processes causing ARCD

#### 1.3.4.1 Inherited factors

##### 1.3.4.1.1 Genetics

Heritability studies have estimated that roughly 50% of general cognitive *ability* is inherited and this proportion appears to be lowest in childhood and increases throughout life to old age, perhaps dropping again in the very old (Haworth et al., 2010, Deary et al., 2006b). Measures of cognition with age reflect peak cognitive ability and subsequent rate of decline. Therefore genetic influences will affect both these factors in different individuals to different extents.

The most studied gene with regard to cognitive ageing is *apolipoprotein E (APOE)*. The *APOE* gene has three common alleles: epsilon 2, 3 and 4. In a caucasian population the E3 allele is the most prevalent with an occurrence rate of 79% and there is no increased or decreased risk of cognitive decline with this allele. The E4 allele has been associated with increased risk of developing Alzheimer's disease (AD) (occurrence rate 14%, 2-3 times increased risk of AD) and the E2 allele with increased longevity and survival and it is thought to be protective against Alzheimer's disease (occurrence rate 8%) (Wisdom et al., 2011). A large meta-analysis of the effects of the *APOE* alleles on cognitive functioning in a healthy elderly population found that carriers of the E4 allele performed significantly worse on tests of episodic memory, executive functioning, and overall global cognitive ability. Carriers of the E2 allele had higher scores on episodic memory (Wisdom et al., 2011). However the effect sizes are small, probably only accounting for 1-2% of the variance seen in cognitive decline (Deary et al., 2009).

Other candidate gene studies have been more successful. A polymorphism in brain-derived neurotrophic factor (BDNF) has been found to be associated with individual differences in memory function and variation in DISC1 genotype has been associated with cognition in old age (Egan et al., 2003, Thomson et al., 2005). Variations in the *KLOTHO* gene (which is known to play a part in oxidative stress) have been shown to affect cognition in childhood and may also play a role in cognitive ageing, but further studies are needed in this area (Deary et al., 2005). A polymorphism in the prion protein gene (PRNP), part of the antioxidant defence genes, has been found to be associated with improved cognition age 79 y (Kachiwala et al., 2005). However associations do not necessarily imply underlying mechanism. Also, gene-gene and gene-environment effects are thought to play a role in the

differences seen with cognitive ageing but further investigation is needed to map out these interactions further.

Several other candidate genes have been investigated and found to have no relation to individual differences in cognitive ageing. These include polymorphisms in the angiotensin-1 converting enzyme (which plays a role in regulation of blood pressure) and methylenetetrahydrofolate reductase (MTHFR) (which affects homocysteine levels) both of which failed to show any association (Visscher et al., 2003).

Unfortunately studies of candidate gene in this field so far have lacked consensus (Payton, 2009). This might be because they have missed the large to moderate effect polymorphisms or that small effect sizes and interactions will prove to be more important. They have largely been under-powered considering the small effect size the genes appear to have and they have looked at differing populations and age-ranges when it is likely that different genes may be important in different populations and age groups. They are also reliant on selection by researchers from within previously decided candidate areas therefore genome wide association studies (GWAS) may bypass some of these issues.

GWAS has identified SNPs in the APOE region and the TOMM40 region (translocase of the outer mitochondrial membrane 40 homolog) as having genome wide significance in their association with ARCD (Davies et al., 2014). Further replicative GWAS studies in this area would now be useful.

#### *1.3.4.1.2 Lifecourse influences*

The association between childhood IQ and cognitive decline has already been discussed, but there are other pieces of evidence which support a lifecourse approach to studying the aetiology of cognitive ageing. A large cohort study has found that birthweight and postnatal growth are independently associated with cognition (Richards et al., 2002) and a cross-sectional study has found that parental socioeconomic status and cognitive ability in late adulthood are associated even after adjustment for education (Turrell et al., 2002).

Furthermore placental weight and birth weight have been found to be associated with markers of white matter disease in elderly subjects (Shenkin et al., 2009). As all age related decline represents the combination of peak function and subsequent decline, determinants of peak function will always prove important when trying to find ways to modify the ageing process.



### **1.3.4.2 Hormones and neurotransmitters**

#### *1.3.4.2.1 GH/IGF1/Insulin*

The effect on the brain of the falling levels of growth hormone and IGF-1 seen with age is a controversial topic at present. There are observational studies showing both correlations of IGF-1 levels with cognitive function and no correlation (Sonntag et al., 2005). However, most of these studies are quite small in size. Animal studies looking at treatment with GHRH and IGF-1 show improved cognition, however human studies have shown mixed results (Sonntag et al., 2005). One study of elderly women treated with IGF-1 for 1 year showed IGF-1 levels comparable to younger women but no benefit to cognition and a study of elderly men treated with GH for 6 months showed no overall improvement in cognition (Friedlander et al., 2001, Papadakis et al., 1996). Whereas a randomised controlled trial of growth hormone-releasing hormone was found to improve cognition in both healthy older adults and those with MCI (Baker et al., 2012).

#### *1.3.4.2.2 Glucocorticoids*

Chronically elevated cortisol has been shown to impair memory and cognition, whereas acutely elevated cortisol may enhance memory in the short term (Het et al., 2005). There is evidence that chronically elevated glucocorticoids (GC) contribute to ARCD (Meaney et al., 1995). High levels of glucocorticoids (eg during chronic stress) have been shown to decrease neuronal survival following hypoxic ischaemia and to inhibit neurogenesis (Sapolsky and Pulsinelli, 1985) and GCs regulate pathways involved in inflammation, the immune response, cellular proliferation, cell death, mood and memory (Chapman and Seckl, 2008). Therefore potential pathways which would explain their negative effects are numerous.

Another potential pathway is via the intracellular enzyme, 11 $\beta$ HSD1. 11 $\beta$ HSD1-deficient mice show increased learning ability in old age than wild type mice and treatment with an 11 $\beta$ HSD1 inhibitor has shown an improvement in cognition within 10 days in wild type mice (Sooy et al., 2010). Human studies in elderly men and diabetic subjects also found 11 $\beta$ HSD1 inhibition to improve cognition even in a short time frame (Sandeep et al., 2004).

Interestingly, a study investigating polymorphisms affecting the 11 $\beta$ HSD1 gene found no association with variances in cognitive ageing (Deary et al., 2006a).

#### *1.3.4.2.3 Testosterone, DHEA and Oestrogen*

Testosterone levels are associated with cognitive function in old age (ie hypogonadal men have poorer cognitive function) but whether testosterone replacement improves cognition is

less clear with some studies showing a minor benefit and some no benefit (Sih et al., 1997, Kenny et al., 2002). DHEA and DHEAS have been shown to decrease markedly with age and correlations have been found between levels and degree of cognitive function, however studies looking at the benefit of replacement on cognition have been disappointing (Sorwell and Urbanski, 2010). There is evidence that oestrogen replacement is beneficial for cognitive ageing and memory but it must be started straight after the menopause as replacement commenced later than this does not confer the same benefit (Sherwin, 2006).

#### **1.3.4.2.4 Neurotransmitters**

Dopamine is a catecholamine neurotransmitter which has many functions, including being involved in cognition and memory, and cerebral dopamine receptor density decreases with age starting in the 40s (Volkow et al., 1996). A polymorphism of the COMT gene, which plays a role in dopamine metabolism, has been associated with verbal declarative memory age 79, which may highlight a role for this pathway in normal cognitive ageing (Harris et al., 2005).

Levels of serotonin have also been found to decrease with age and a polymorphism within the serotonin transporter gene has been found to be associated with increased rates of cognitive decline in a non-demented population (Payton et al., 2005).

#### **1.3.4.3 Inflammation**

Current studies investigating markers of inflammation and cognitive function and decline have thus far found some conflicting results. However, even when positive these studies typically show a small effect size.

Evidence that inflammation has a role in the development of ARCD is plentiful.

Homocysteine was found to be negatively associated with cognitive function and cognitive decline and this association is stronger in those with high IL-6 or CRP (van den Kommer et al., 2010). Increased circulating levels of CRP, fibrinogen, and elevated plasma viscosity predicted poorer subsequent cognitive ability and were associated with age-related cognitive decline in several domains, including general ability (Marioni et al., 2009). One study with a 31 year follow up period demonstrated an association between CRP and degree of cognitive decline (Laurin et al., 2009) and a follow up study of elderly women showed that high serum CRP predicts poorer memory 12 years later (Komulainen et al., 2007). Another study demonstrated that raised levels of IL-6 and CRP in midlife are moderately associated with

lower cognitive status, but there was only a small association with cognitive decline over roughly 10 years (Gimeno et al., 2008). Genetic variation in the interleukin-1 beta-converting enzyme gene is associated with better cognitive function and lower IL-1B production levels (Trompet et al., 2008). Schram et al found systemic markers of inflammation to be only moderately associated with cognitive function and decline, but they found stronger associations in carriers of the APOE epsilon4 allele (Schram et al., 2007).

In a study of 500 healthy middle aged adults an inverse relationship between circulating levels of IL-6 and performance was found on clusters of tests assessing auditory recognition memory, attention/working memory and executive function, in contrast there was no association between IL-6 and performance on tests of general memory (Marsland et al., 2006). It may be that inflammation mediates the negative effect that the metabolic syndrome plays on cognition. A large longitudinal study (n=2600) showed an increased relative risk of cognitive decline when comparing those with metabolic syndrome and those without. However, this risk was completely attenuated after stratifying for inflammation. Relative risk for cognitive impairment in the high inflammation group was 1.66 (1.19-2.32) and in the low risk group was 1.08 (0.89-1.30) (Yaffe et al., 2004).

IL-6 and CRP were found to be prospectively associated with cognitive decline in well-functioning elders but TNF $\alpha$  was not in the Health, Aging, and Body Composition Study (Yaffe et al., 2003). The MacArthur Study of Successful Aging found an association between IL-6 levels and subsequent cognitive decline in 70-79 year olds (Weaver et al., 2002).

However, there is also strong evidence which shows no association between inflammation and cognitive ageing. A population-based study (n=1200) showed that markers of inflammation (CRP, IL-6, and albumin) are not associated with cognitive decline in older persons (Dik et al., 2005). Also, the Women's Health Study (n=4200) found no association between level of CRP and poorer cognitive performance (Weuve et al., 2006).

One further point is that age 11 IQ scores have predicted age 70 CRP levels, therefore the argument of reverse causation again comes to the fore, whereby lower IQ in childhood leads to greater levels of inflammation in later life (Luciano et al., 2009). This may be because low childhood IQ is a marker of "system integrity" (ie how well the body as a whole is put together) or because it is associated with cardiovascular risk factors in later life.

### **1.3.4.4 Lifestyle**

#### *1.3.4.4.1 Diet*

Low levels of B vitamins (mainly B6, folate (B9) and B12) have been shown to be associated with high homocysteine levels and predict cognitive decline (Tucker et al., 2005).

Supplementation with folic acid has been shown to improve cognition and decrease plasma homocysteine levels whether the subjects are deficient in folate or not (Durga et al., 2007).

Studies looking at dietary antioxidant intake and cognitive decline have had mixed results, with some showing a degree of benefit and others showing no benefit at all (Dai et al., 2006, Engelhart et al., 2002, Morris et al., 2002, Morris et al., 2005). A clinical trial investigating antioxidant supplements (vitamin C and E) has shown improved cognitive function when both are taken but no improvement when taken separately (Grodstein et al., 2003).

Polyphenols have been found to improve longevity, decrease tauopathy and improve memory in animal studies (Cellerino, 2009, Pflieger et al., 2010, Richetti et al., 2011). They have antioxidant properties and are thought to improve the cellular senescence of neurons. Direct evidence in humans is lacking.

Omega-3 fatty acids have been found to decrease risk of cognitive decline possibly by upregulating genes that are important for maintaining synaptic function and plasticity (McCann and Ames, 2005). They have been shown to be anti-inflammatory and anti-oxidant (Deary et al., 2009). Interestingly one study found that only those who were not carriers of the APOE E4 allele benefitted from the positive effects of omega-3 fatty acids (Whalley et al., 2008). Saturated fats have been found to reduce molecular substrates that support cognitive processing and therefore increase the risk of neurological dysfunction (Greenwood and Winocur, 2005).

An important addendum to the above evidence is that it appears that it is not the exact intake of the nutrients themselves that mediates the beneficial effect on cognition but the diet as a whole. Therefore whilst each nutrient may play a role, some of the effect may be due to a combination of micronutrients being ingested together. Dietary patterns are influenced by wealth, geography, culture and multiple other factors and perhaps investigating successful dietary patterns rather than specific nutrients may be more fruitful.

#### *1.3.4.4.2 Smoking and alcohol*

Drinking moderate amounts of alcohol, particularly red wine, may be beneficial for cognition in old age (Truelsen et al., 2002, Stampfer et al., 2005). The skin of red grapes contains a polyphenol which may be a mechanistic explanation. However prior intelligence and socioeconomic status may mediate this relationship as these have both been found to be associated with amount of alcohol and type consumed (Corley et al., 2011).

Smoking is a major risk factor for cognitive decline and dementia (Anstey et al., 2007b). This may be due to increased risk of atherosclerosis or increased inflammation.

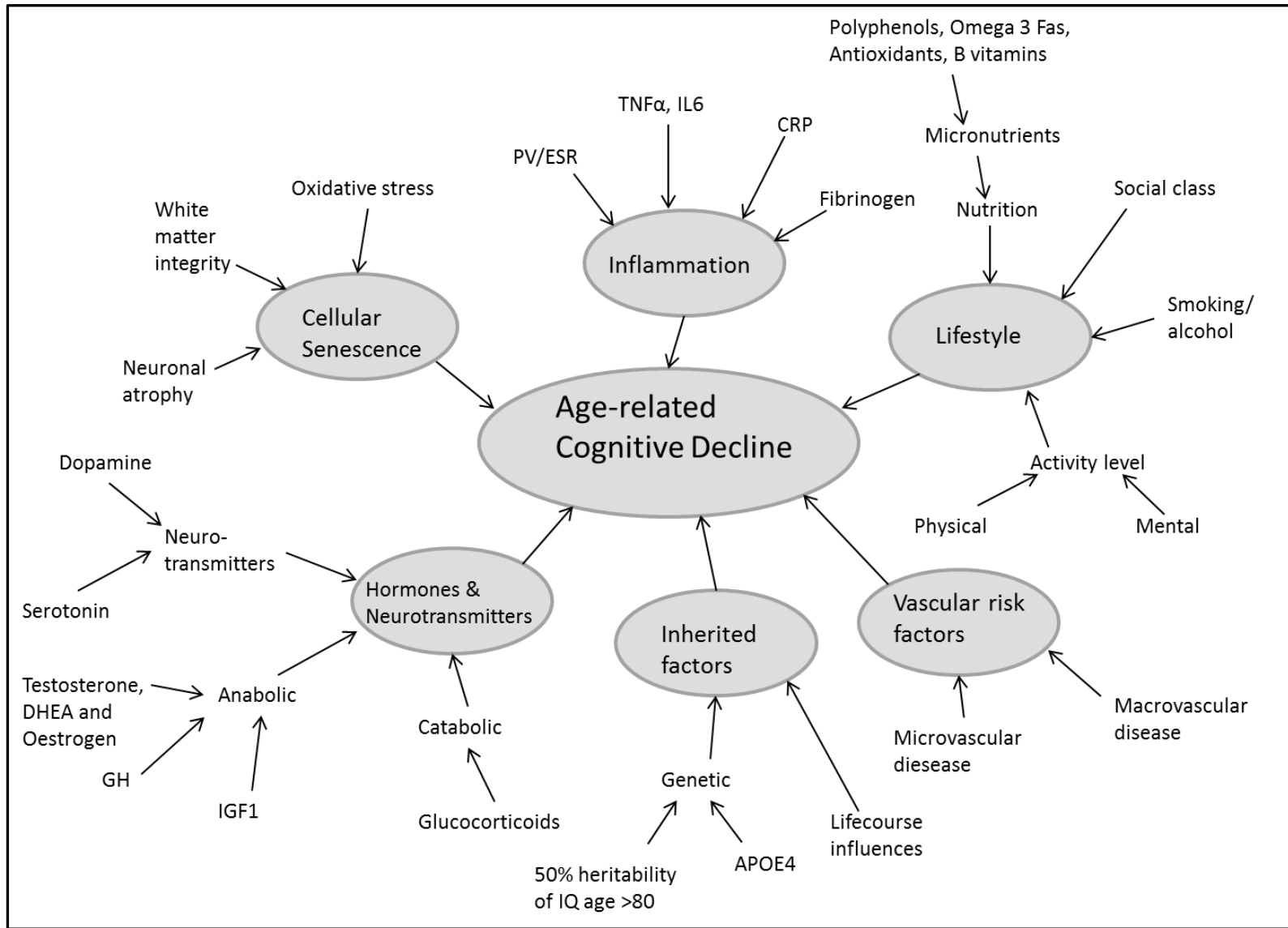
#### *1.3.4.4.3 Physical activity*

Several large studies have shown a decreased risk in cognitive decline with increased levels of physical activity (Rovio et al., 2005, Yaffe et al., 2001) and a Cochrane review found that physical exercise interventions improved some domains of cognitive function (Angevaren et al., 2008). One study has shown that this effect may be stronger in those carrying the APOE E4 allele (Rovio et al., 2005). This effect could be mediated via decreased risk of the metabolic syndrome or atherosclerotic disease and cardiovascular fitness.

#### *1.3.4.4.4 Mental activity*

Studies appear to have determined a “use it or lose it” effect. That is that people who remain mentally engaged appear to show less cognitive decline. The effects need to be corrected for prior cognitive ability or else they may just be demonstrating that people with higher cognitive ability stay more mentally engaged and are already known to have lower rates of cognitive impairment. That would be an example of the cognitive reserve hypothesis in action, which is that people with higher cognitive abilities have to experience more cognitive decline before any impairment becomes apparent. However evidence in this area is not consistent and further studies are required (Salthouse, 2006).

Figure 1-3: Pathways influencing ARCD



## 1.4 Conclusions

This literature review summarises the evidence that there is deterioration in muscle structure and function, and brain structure and function seen as part of normal ageing within humans. Whilst there is evidence for relationships (although non-linear) between muscle size and function, and brain size and cognition, the interrelationships between brain and muscle structure and function are less well understood. It will be important to investigate whether those who are subject to steeper declines in cognitive function with normal ageing also have a steeper decline in muscle mass and, more importantly, muscle function with age. If this is found to be true, it would support the theory that there are common mechanisms underlying both brain and muscle ageing, which if addressed could simultaneously improve deterioration in both.

This review also highlights that many of the potential processes underlying the development of these age-related conditions may be shared and therefore that future treatments to slow the progress of one may have positive effects on the other. Possible common underlying factors include: glucocorticoids, immunosenescence and inflammation, genetics, and environmental and lifestyle factors.

Pathologically raised levels of glucocorticoids are known to affect both muscle and brain, however much less is understood about why the small elevation seen with ageing is associated with such profound effects. Potential hypotheses which might explain this include; the effect of recurrent elevations of glucocorticoids (eg with acute illness) as opposed to mean levels, or raised  $11\beta$ HSD1 activity in muscle and brain with age.

Immunosenescence and inflammation appear to play a role in the development of sarcopenia and ARCD. However the mechanisms underlying the relationships are not clear and current evidence remains contradictory.

The role of genetics in the rate of development of age-related conditions such as sarcopenia and ARCD is still not clear with candidate gene studies thus far showing conflicting results. *APOE E4* is known to have a role in several ageing conditions including cognitive ageing but it is not known whether it is associated with sarcopenia.

The role of environmental factors (eg maternal nutrition, socioeconomic status) and lifestyle choices (eg smoking, levels of physical activity) also require further study as possible common aetiological factors.



## 1.5 The research aims of this thesis

1. To undertake a systematic review of the literature linking brain and muscle structure and/or function
2. To develop a novel method for measuring neck muscle cross-sectional area on magnetic resonance (MR) brain scans to be used as a measure of muscle size within healthy ageing studies
3. To examine the relationship between muscle size and function with brain volume and cognition in healthy ageing studies and to examine the role of potential covariates in these relationships
4. To examine the role of immunosenescence in sarcopenia and explore the role of important covariates within a healthy ageing study
5. To investigate the relationship between glucocorticoids and muscle structure and function in younger and older healthy adults
6. To develop a novel method to quantify 11 $\beta$ HSD1 activity in the human brain in vivo
7. To assess methodological aspects of human brain cerebrovascular studies which may impact on future studies using these techniques

# **Chapter 2 A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse**

## **2.1 Introduction**

Maintaining good levels of brain and muscle function across the lifespan is crucial to achieving a good quality of life (Salthouse, 2012, Johansson et al., 2012, Sayer et al., 2006). There is substantial evidence showing an association between cognition and muscle function (Abellan van Kan et al., 2009, Atkinson et al., 2007, Scherder et al., 2007, Drago et al., 2011, Moran et al., 2013), however the role of muscle and brain structure within this association is less well understood. A greater understanding of this role will help to improve current knowledge of the mechanisms linking brain and muscle function over the lifecourse.

Several theories have been proposed as to why relationships between brain and muscle structure and function may exist. The common cause hypothesis postulates that there are core common underlying processes which drive ageing throughout the human body. The construct was originally described in a paper by Lindenberger and Baltes in 1994 who noted that measures of visual and auditory acuity accounted for variance in intelligence in old age (Lindenberger and Baltes, 1994). Since then experiments in caloric restriction have demonstrated that the ageing process can be slowed down in multiple systems throughout the body by one intervention (Speakman and Mitchell, 2011, Park and Prolla, 2005). However, environmental factors also impact on how tissues change across the lifecourse and another theory by Mitnitski et al proposes that the number of environmental stressors experienced (eg disease, smoking) and the ability to recover from them, vary the level of deficit accumulation experienced in multiple organ systems, and hence how tissues like brain and muscle change with age (Mitnitski et al., 2013).

Potential underlying mechanisms include: pro-inflammatory cytokines (eg TNF-alpha and IL-6); the role of glucocorticoids and their intracellular amplifier 11beta-hydroxysteroid dehydrogenase type 1 (Meaney et al., 1995, Cooper et al., 2002, Het et al., 2005); the role of vitamin D (Roth et al., 2004, Geusens et al., 1997); exercise as a way to improve cardiovascular fitness in addition to its beneficial effect through hormones and cytokines

(Adams and Haddad, 1996, Adams and McCue, 1998, Davis et al., 2011); and cellular senescence (eg through oxidative stress) (Deary et al., 2005, Jang et al., 2010).

In view of these theories, there should be a correlation between the structure and function of brain and muscle throughout our lifetime in the absence of significant pathology. This systematic review will search for studies that test the hypotheses that brain structure is related to muscle structure and/or function and that muscle structure is related to brain function in healthy children and adults. Previous studies and reviews have looked at evidence relating brain function (eg MMSE score) to muscle function (eg walking speed) therefore this separate but closely related field of literature will not be included in this review (Duff et al., 2008, Atkinson et al., 2007, Alfaro-Acha et al., 2006, Angevaren et al., 2008).

## **2.2 Methods**

The study protocol was published online in December 2011 at:

<http://www.ccace.ed.ac.uk/sites/default/files/Kilgouretal.pdf>.

### **2.2.1 Inclusion criteria**

#### **2.2.1.1 Population**

All human subjects regardless of age were included in the study; from newborn babies to the oldest old, including post-mortem studies. This study is examining the relationship between brain and muscle in health, not within the effects of pathology therefore studies looking at how a disease affects brain or muscle were excluded. However studies which included a healthy control group, where the data from these subjects can be or was analysed separately were included. As morbidity increases in frequency with age it would be very restrictive to include solely those studies which include only participants who are free from any disease, therefore studies will be included provided the subjects have been recruited in a way that did not predispose to morbidity being more prevalent than in the general population (eg from a diabetes clinic).

It was planned that subgroup analysis would be undertaken where possible and would include data being extracted to investigate the effects of gender, age, socioeconomic status and ethnicity.

#### **2.2.1.2 Interventions/Comparators**

Not applicable as the study is investigating normal physiology.

### **2.2.1.3 Outcomes**

#### *Brain structure*

- Whole brain volume (WBV) or total brain volume (TBV)
- Volume or cross sectional area of regions within the brain (eg hippocampus, frontal lobes)
- White matter integrity (eg White matter hyperintensities (WMH) or white matter signal abnormalities (WMSA))
- Histological findings about brain structure on autopsy

#### *Brain function*

- Any recognised measure of cognitive function including: memory, attention, executive function, language and processing speed
- Reaction time will not be used as this is dependent on aspects of brain and muscle structure and function

#### *Muscle structure*

- Muscle cross sectional area on CT, MRI or USS
- Muscle volume (using CT or MRI)
- Whole body lean tissue mass using DEXA, giving: total lean mass (TLM) or appendicular lean mass (ALM)
- Bioimpedance analysis (BIA)
- Histological findings on muscle biopsy or on autopsy

#### *Muscle function*

- Any recognised test of muscle strength, including isometric, isotonic, isokinetic tests
- Any recognised test of muscle power
- Functional tests of muscle function (eg usual or maximum gait speed)

### **2.2.1.4 Study Design**

As this review is studying a physiological relationship, intervention studies were not included, unless they contained either a control arm with extractable data with no placebo treatment or baseline data prior to the intervention. Observational studies including cohort studies and cross sectional studies were included and the control arm of case control studies. Case reports were excluded as these would not contain evidence of normal physiological relationships out with pathology. The only other limiter used was “human” in Medline, Embase and PsycINFO but not Cinahl as it appears to screen out human studies erroneously.

### **2.2.2 Search strategy**

Database searches of Medline, Embase, CINAHL and PsycINFO were undertaken. All languages were included in the search. The Medline search strategy can be found in Appendix 1. The searches were all performed on 6<sup>th</sup> March 2014. A grey literature search was performed using Google and Google Scholar. Hand searching through citations and references of relevant articles was also undertaken.

### **2.2.3 Study selection**

The search was undertaken by two independent researchers. Titles +/- abstracts found using our search strategy were independently screened for relevance. The full text of the selected studies was reviewed against the inclusion criteria, and reasons for exclusion at this stage were recorded. At this point the two researchers met to discuss shortlists and discuss any articles which only one researcher had selected to decide if they should be included or not. Disagreements were resolved by consensus or adjudication by a third party (a Professor in Geriatric Medicine).

### **2.2.4 Contacting authors**

Of the 84 studies found through our search, I wrote to 79 to request data or associations which were not given in the text. Five of the studies had given all the associations for the variables listed in the text. A letter was sent by email to either the corresponding author (after checking they were still working at the study location) or the last author (after the same checking process). Only one author was written to from each study (eg all articles arising from the Kansas Brain Aging Project, were grouped together when requesting extra data/associations). After the initial email a further email was sent around 2 weeks later to act as a reminder. Studies were given a minimum of around 1 month to reply.

Out of the 79 studies I wrote to: 25 studies (32%) sent either the requested data or associations; 22 (28%) replied stating they would try and send the data or associations to us but then never did; 12 studies (15%) replied stating they either no longer had access to the data or did not want to send either the requested data or associations to us; and 20 (25%) never replied to either of the emails.

### **2.2.5 Quality assessment and risk of bias**

All papers included in the study had their inclusion and exclusion criteria reviewed to check for possible bias in the study selection. The topic of the review is not looking at an

intervention, therefore the risk of reporting bias for an individual paper is small. Also, in most of the papers, the relationship between muscle and brain was not the primary topic of the paper, further decreasing the risk of reporting bias. However when contacting the authors, asking for either the data or the associations, it was considered that the studies which replied may show some bias. The authors may look at their data and only reply if an association was found, or if they found a strong relationship they may not want this to be initially reported within a systematic review, but rather in a paper in its own right. All summary measures were included (eg odds ratio, beta).

We were unable to choose a single quality assessment tool to use for our systematic review as we had decided to include any type of study which contained the required data, and the major quality assessment tools are usually focussed on one type of study design (eg interventional or observational). I therefore reviewed the most well-known of the quality assessment tools, identified using the EQUATOR website and relevant internet searches, and used these to compile the data extraction sheet (eg STROBE and PRISMA guidelines) which was used to assess methodological quality and bias. Furthermore as we were using control group data from several of the studies we were unable to find any pre-existing quality assessment tool which specifically dealt with this scenario.

### **2.2.6 Data extraction**

I performed the data extraction using a data extraction sheet which I had written, which had been approved by the two co-authors (OT, JS) (appendix 1).

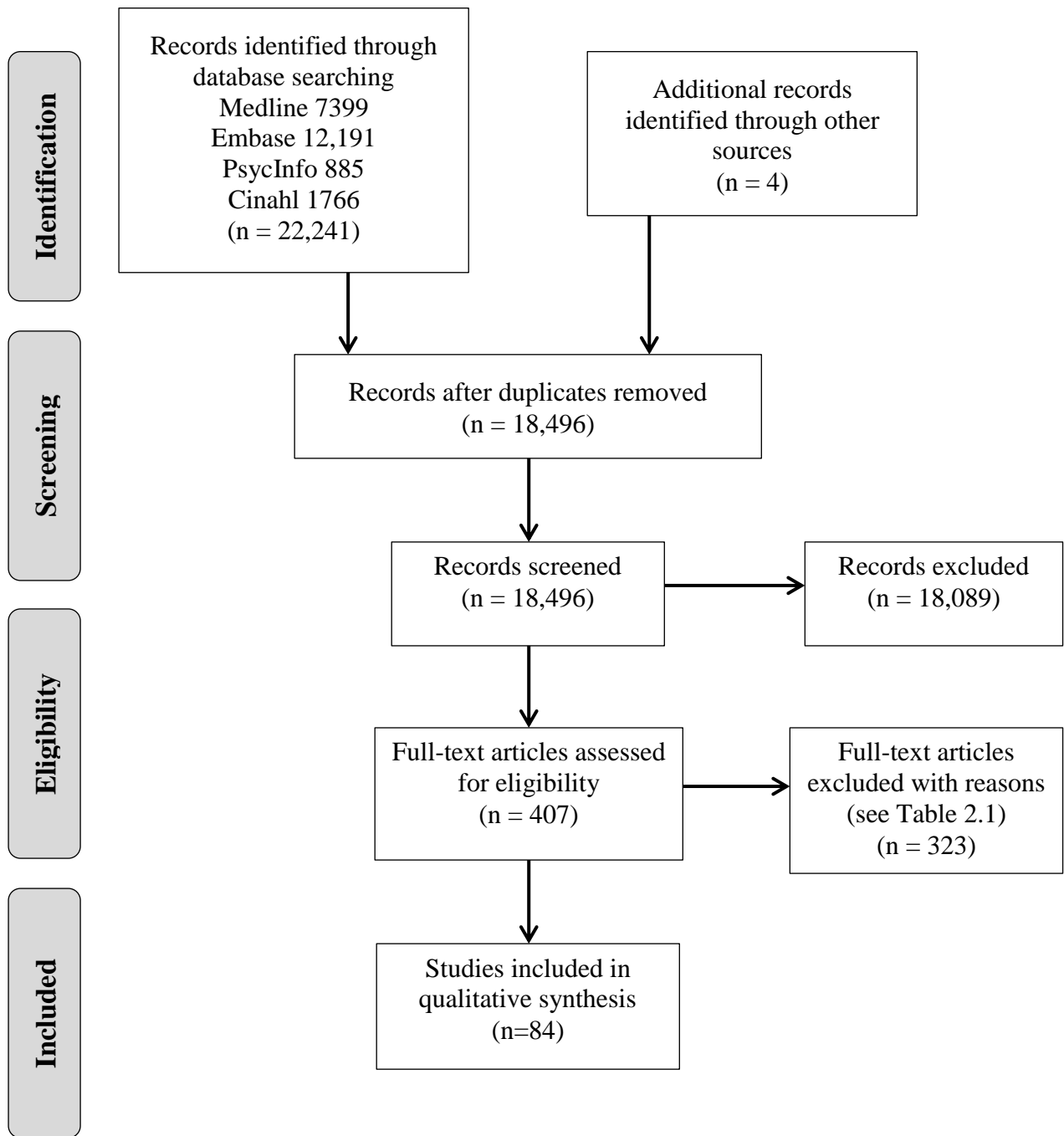
### **2.2.7 Data analysis**

A narrative synthesis was completed. It was thought unlikely that the data would be comparable enough to allow meta-analysis (ie different measures of cognition, different muscle groups studied using different machines) and this proved correct. It was hoped that sub-group analysis would be undertaken, either in the form of a meta-analysis or more likely as a narrative synthesis for the reasons mentioned in the above paragraph.

## 2.3 Results

The search results are presented in the PRISMA flow diagram in figure 2.1. Reasons for exclusion of articles after reviewing the full text are reported in table 2.1. After applying the inclusion and exclusion criteria 84 articles were identified; 53 articles either reported the appropriate associations or sent us the data or associations requested (table 2.2, 2.3 & 2.4), and 31 articles contained the required data but did not report the association between them and did not supply either the data or associations requested (table 2.5). Out of the 53 articles which could be included in the review; 6 contained data on brain structure and muscle structure (table 2.2); 33 contained data on brain structure and muscle function (table 2.3); and 14 contained data on brain function and muscle structure (table 2.4).

Figure 2-1: PRISMA flow diagram showing study selection





*Table 2-1: Full-text articles excluded, with reasons*

| <b>Reason</b>  | <b>Number</b> |
|--|---------------|
| <b>Selected subjects (eg all had hip fracture, all had dementia etc)</b> | <b>73</b>     |
| <b>No measure brain or muscle structure</b>                              | <b>57</b>     |
| <b>Review article, no relevant references</b>                            | <b>56</b>     |
| <b>No measure muscle structure or function</b>                           | <b>47</b>     |
| <b>Abstract, no published results within timeframe or irrelevant</b>     | <b>34</b>     |
| <b>No measure brain structure or function</b>                            | <b>19</b>     |
| <b>Protocol paper, no published results within timeframe</b>             | <b>12</b>     |
| <b>Anthropometry only measure of structure</b>                           | <b>10</b>     |
| <b>Letter or editorial, no results</b>                                   | <b>7</b>      |
| <b>Technique or theory paper</b>   | <b>5</b>      |
| <b>Case Report</b>   | <b>2</b>      |
| <b>No full text available</b>  | <b>1</b>      |
| <b>Total</b>   | <b>323</b>    |

### **2.3.1 Association of brain structure and muscle structure**

Of the six articles which looked at the relationship between brain structure and muscle structure, three were from the Kansas Brain Aging Project (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011), and the others were from Germany, UK and USA, Phoenix (Heymsfield et al., 2012, Kilgour et al., 2013, Weise et al., 2013) (table 2.2). There were no longitudinal data available for this section as the studies identified were either cross-sectional in nature or only supplied data from a single wave of a longitudinal study.

The Kansas Brain Aging Project was set up to determine the effects of exercise and cardiorespiratory fitness on age-related brain changes. Only one of the papers from this project reported the relationship between brain and muscle structure (Burns et al., 2010): Burns et al reported a positive relationship between WBV and TLM (beta 0.20,  $p < 0.001$ ) when control and subjects with Alzheimer’s disease (AD) were grouped together, adjusting for age sex and intracranial volume (ICV), and they note that this was driven by WM volume (Burns et al., 2010). They state that this relationship persists in just the control group but do not give any statistics for this relationship. A General Linear Model (GLM) was performed on the data from the non-demented group supplied to us by the study authors from the Kansas

Brain Aging Project (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011). WBV, grey matter (GM) volume and hippocampal volume were not predicted by TLM adjusting for age, sex and ICV +/- education. White matter (WM) volume was predicted by TLM ( $t$  3.12,  $p=0.003$ , partial eta squared 14%) adjusting for age, sex and ICV. Adjusting for total years of formal education only slightly attenuated the results ( $t$  2.99,  $p=0.004$ , partial eta squared 13%). The Kansas Brain Aging Project states that it recruited its subjects using media advertising; however, this may lead to a skewed sample as it may be that the controls identified were more likely to have a relative with dementia and therefore be interested in the topic. Also the exclusion criteria included diseases very common in older age (eg diabetes mellitus, any cardiovascular disease in previous 2 years etc) meaning that the “normal” controls are likely to be healthier than a random sample of subjects within this age group. This means that the associations we are looking at may be present to a lesser or greater extent in this population than the population as a whole.

Kilgour et al also looked at older subjects however they used neck muscle CSA as a measure of muscle bulk (Kilgour et al., 2013). They found that total neck muscle CSA predicted 17% of the variance in whole brain volume ( $p=0.01$ ), but they found no significant association between total neck muscle CSA and ventricular, hippocampal or cerebellar volumes ( $p > 0.05$ ), adjusting for age, sex, ICV and NART (a measure of childhood intelligence). This study only looked at older men and again the exclusion criteria may mean the sample represented a “supra-normal” population in terms of health in this age group.

The other two studies looked at younger subjects. Heymsfield et al specifically set out to investigate the relationship between brain mass and body composition (Heymsfield et al., 2012). They performed multiple linear regression and found that after adjusting for age and fat mass, FFM predicted brain mass in men (beta 0.023,  $R^2$  5%,  $p=0.01$ ) and women (beta 0.003,  $R^2$  6%,  $p<0.0001$ ). Fat mass or bone mineral content did not significantly predict brain mass in either sex. So they conclude that it is FFM that drives the relationship between body size and brain size not bone or fat mass. This study only included non-smokers and recruited its subjects from very specific sites (eg university students and staff and local businesses) which will affect the generalisability of the results.

Weise et al investigated the associations between regional grey matter volume and fat free mass index ( $FFMI = FFM/height^2$ ) (Weise et al., 2013). They found several areas of grey matter volume that were significantly associated with FFMI ( $p<0.01$ , see table 2.2), however

after adjusting for percentage body fat or fat mass only two areas remained significant (the right temporal pole and bilateral ventromedial prefrontal cortex). No regression coefficients were shown in the paper, meaning that although the results were significant the effect size was not known. Again this study excluded all smokers and anyone with a “significant” medical history affecting the generalisability of the results.

**Table 2-2: Studies identified with brain structure (+/- brain function) and muscle structure**

| <b>Authors</b>                      | <b>Country and dataset</b>              | <b>n</b> | <b>Study design</b>  | <b>Mean age (sd)</b>            | <b>Male (%)</b> | <b>Brain Structure/ Function</b>   | <b>Muscle Structure</b>                         | <b>Associations*</b>   |
|-------------------------------------|---|----------|--|---------------------------------|-----------------|--|---|--|
| <b>1. (Heymsfield et al., 2012)</b> | Germany                                 | 260      | Cross-sectional study  | M 45.1 (14.9),<br>F 38.6 (13.7) | 43.1            | Structure: Brain volume transformed into mass using 1.036g/cm <sup>3</sup><br><br>Function: not measured   | DEXA for FFM                                    | Study: Linear regression models found that after adjusting for age and fat mass, FFM predicted brain mass in men (beta 0.023, R <sup>2</sup> 5%, p=0.01) and women (beta 0.003, R <sup>2</sup> 6%, p<0.0001).  |
| <b>2. (Kilgour et al., 2013)</b>    | UK, MacLulich Healthy Elderly Men Study | 51       | Longitudinal ageing study (data from wave 2 of the study)  | 73.8 (1.5)                      | 100             | Structure: Whole brain, hippocampal, ventricular, cerebellar volumes and ICV<br><br>Function: 9 tests of cognitive function reduced to 2 factors (cognitive processing factor and memory factor)             | Neck muscle CSA on MR head scan                 | Study: Total neck muscle CSA was found to predict 17% of the variance in whole brain volume (t = 2.86, p = 0.01). However, total neck muscle CSA did not significantly predict the variance in ventricular, hippocampal or cerebellar volumes (p > 0.05). Total neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor (p > 0.05), however, it did predict 10% of the variance in the NART score (t = -2.12, p < 0.05). Adjusting for age, sex, ICV and NART where appropriate. |
| <b>3. (Wetmore et al., 2011)</b>    | USA, Kansas, Brain Aging Project        | 60       | 2 year observational case-control study (Alzheimer's dementia vs. non-dementia) (baseline data analysed) | 73.0 (7.2)                      | 43.4            | Structure: MRI for WM, GM, CSF, WBV and ICV<br><br>Function: Logical Memory I & II, Free & Cued Selective Reminding Task, Boston naming test, Verbal fluency, Digit span forward and backward, Letter-number | DEXA for lean mass and ASM (just arms and legs) | Study: none<br><br>Calculated: Non-demented group only. WBV, GM volume and hippocampal volume not predicted by TLM adjusting for age, sex and ICV +/- education. WM volume was predicted by TLM (t 3.12, p=0.003, partial eta squared 14%) adjusting for age, sex and ICV. TLM did not significantly predict global cognitive score or MMSE, adjusting for age and sex.  |

| Authors                        | Country and dataset              | n                 | Study design   | Mean age (sd) | Male (%) | Brain Structure/ Function  | Muscle Structure         | Associations*  |
|--------------------------------|----------------------------------|-------------------|--|---------------|----------|--|--------------------------|--|
|                                |                                  |                   |  |               |          | sequencing, Trail making A & B, Stroop color-word test and Block design, MMSE  |                          | Adjusting for height and education did not affect this.  |
| <b>4. (Burns et al., 2010)</b> | USA, Kansas, Brain Aging Project | 70                | Cross-sectional case-control study (Alzheimer's dementia (AD) vs. non-dementia) (baseline data analysed) | 73.3 (7.3)    | 42.9     | Structure: MRI for WM, GM, CSF, WBV and ICV<br><br>Function: Logical Memory I & II, Free & Cued Selective Reminding Task, Boston naming test, Verbal fluency, Digit span forward and backward, Letter-number sequencing, Trail making A & B, Stroop color-word test and Block design, MMSE | DEXA for total lean mass | Study: Positive relationship between WBV and TLM when control and AD subjects grouped together (beta=0.20, p<0.001), adjusting for ICV, age and sex. This appears to be driven by WM (beta 0.19, p<0.001) rather than GM (beta 0.06, p=0.27) States this persists in just the control group but doesn't give any statistics for this. Positive relationship between MMSE and global cognitive score (composite of the cognitive tests) and lean mass when grouping AD and control subjects together. States that controlling for dementia status attenuates these results, but no specific statistics given.<br><br>Calculated: See Wetmore et al (2011) for Kansas Brain Aging Project data analysis. |
| <b>5. (Honea et al., 2009)</b> | USA, Kansas, Brain Aging Project | 56 health control | Cross-sectional case-control study (Alzheimer's dementia vs. non-dementia)                               | 73.3 (6.2)    | 41.1     | Structure: MRI for GM, WM, CSF, WBV, hippocampal and parahippocampal volumes<br><br>Function: MMSE   | DEXA for total lean mass | Study: none<br><br>Calculated: See Wetmore et al (2011) for Kansas Brain Aging Project data analysis.  |
| <b>6. (Weise et al., 2013)</b> | USA, Phoenix                     | 76                | Cross-sectional  | 32.1 (8.8)    | 31.6     | Structure: MRI brain volumes (GM, WM,  | DEXA, FFMI (FFM/heig     | Study: Fat-free mass index (FFMI) was negatively associated with GMV of the bilateral temporal lobes, ventromedial   |

| <b>Authors</b> | <b>Country and dataset</b> | <b>n</b> | <b>Study design</b> | <b>Mean age (sd)</b> | <b>Male (%)</b> | <b>Brain Structure/ Function</b>             | <b>Muscle Structure</b> | <b>Associations*</b>  |
|----------------|----------------------------|----------|---------------------|----------------------|-----------------|--|-------------------------|---|
|                |                            |          | study               |                      |                 | CSF, regional GMV)<br>Function: not measured | ht <sup>2</sup> )       | prefrontal cortex (vmPFC) (mainly subgenual portion of the ACC) and caudolateral orbitofrontal cortex and unilaterally with the left insular cortex (all p<0.01). After adjusting for percentage body fat and fat mass, negative associations of FFM with GMV of the right temporal pole and bilateral vmPFC remained. All models adjusted for age, sex and handedness. |

### **2.3.2 Association of brain structure and muscle function**

Thirty three studies which included measures of brain structure and muscle function were identified (table 2.3). The muscle function variables most commonly studied were grip strength and gait speed. Only one study was identified which used a different measure of muscle function and that was maximal isometric knee extension strength (IKES) (Rosano et al., 2010). The brain structure variables include: corpus callosum area, and volumes for total and regional GM and WM, cerebrospinal fluid (CSF), cerebellum, hippocampus, basal ganglia and whole brain volume and measures of prevalence of WMH, either volume or scoring systems (eg Fazekas).

#### **2.3.2.1 Brain structure and grip strength**

The PATH through life project (Anstey et al., 2007a, Sachdev et al., 2005, Sachdev et al., 2006, Sachdev et al., 2009), the Cardiovascular Health Study (Barnes et al., 2009, Rosano et al., 2005, Rosano et al., 2006, Rosano et al., 2008, Rosano et al., 2011, Longstreth et al., 1996), the Lothian Birth Cohort 1936 study (Aribisala et al., 2013), a study from Japan (Doi et al., 2012) and a study from Philadelphia (Hardan et al., 2003) all looked at the relationship between grip strength and brain structure. All the identified papers looked at the relationship in cross-sectional analyses except for the Lothian Birth Cohort 1936 study which looked at the longitudinal relationship.

There are four papers identified by our search strategy from the PATH through life project, which was set up to track and define the lifespan course of depression, anxiety, substance use and cognitive ability. In one paper from this project, Anstey et al (2007) studied the relationship between the area of the corpus callosum (CC) (measured in three sections: anterior, midbody and posterior; and total area) and grip strength (Anstey et al., 2007a). They used the grip strength from the hand the subject wrote with and adjusted for age, sex and ICV. They found no significant relationship between total, anterior or posterior CC area and grip strength however they found a positive relationship between midbody CC area and grip strength (beta -0.09,  $p < 0.05$ ). They conclude that this is due to the association between midbody CC and the motor cortices. Another paper from the PATH through life project studied the association between grip strength and the percentage of WM occupied by WMH in different brain areas (Sachdev et al., 2005). They found that a larger percentage of WMH per WM volume is associated with decreased grip strength for both the total brain and several brain areas (frontal, temporal, parietal, anterior horn and periventricular body (all  $p < 0.01$ )).

However, the amount of WMH in the occipital lobe, the cerebellum and the posterior horn was not associated with grip strength. The 2009 paper from this study further investigated the relationship between WMH and grip strength (Sachdev et al., 2009). This time they looked at the relationship in men and women separately. They found that larger amounts of WMH was associated with reduced grip strength, adjusting for age, depression severity and brain atrophy index, in men ( $p < 0.05$ ) but not in women (n/s). However they comment that they feel that the relationship between WMH volume and motor function is likely to be the same in both sexes and that their finding may be due to the difference in WMH amount between men and women in their study population. Sachdev's 2006 paper from this study did not look at the relationship between motor function and brain structure and the authors did not respond to our data request (Sachdev et al., 2006). The recruitment method appears sound in this study using electoral rolls and a good response rate of 58.3% response rate, however only 18.6% of the subjects had a MRI brain and the studies are not clear as to how they selected who had a scan and who didn't.

The Cardiovascular Health Study (CHS) is a large, longitudinal, observational study of risk factors for cardiovascular disease in adults 65 years or older, which commenced in 1989. The CHS measured grip strength and gait speed and WMSA, however only one paper from this study looked at the relationship between grip strength and WMSA (Longstreth et al., 1996). In this paper Longstreth et al (1996) performed a partial correlation which found no significant association between grade of WMSA (graded on a scale of 0-9) and grip strength in either the dominant or non-dominant hand ( $p > 0.05$ ) after adjusting for age, sex and presence of clinically silent stroke on MRI. Since this study was performed, measurement of WMH has improved greatly and now most studies would use WMH volume and mention site of lesions as opposed to the 10 point scale used in this study. Recruitment was performed through medicare lists, meaning only certain sections of society would be included (ie not those who have never paid into the scheme or those fully covered by private health insurance).

The paper by Doi et al used multiple linear regression to show that grip strength is not related to brain atrophy (beta -0.082 (SE 0.005)  $p = 0.54$ ) (Doi et al., 2012). They measured brain atrophy by mapping the MR brain scans from their subjects to those from healthy controls. Most studies used an index to intracranial volume to calculate degree of brain atrophy. No associations with the other measured brain volumes were included in the paper. The MLR



included adjustment for age, gender, BMI, education, MMSE, Tokyo Metropolitan Institute of Gerontology Index of Competence, geriatric depression scale and change in walking speed whilst dual tasking, which may represent over adjustment considering the size of the study (n=110).

The paper by Hardan et al looked at the association between caudate volume and grip strength in both hands in children and young adults (Hardan et al., 2003). They found non-significant statistical trends using Pearson's correlation between total caudate volume and mean grip strength in the right ( $r=-0.303$ ,  $p=0.05$ ) and left ( $r=-0.28$ ,  $p=0.07$ ) hands. The relationships are negative, therefore there is a trend that those with larger caudate nuclei were found to have lower grip strength in both hands. The study does not state the gender of the subjects and the correlation was not adjusted for age, which considering the rapid changes in body size that occur throughout this age period (mean age 18.6y (sd 8.6)) seems a major flaw.

The Lothian Birth Cohort 1936 study measured grip strength at baseline and 3 years later at which point brain volumes were also measured (Aribisala et al., 2013). It is the only study to look at longitudinal changes in muscle strength and brain structure. Grip strength at wave 1 predicted ventricular volume at wave 2 (standardized beta -0.10), however there was no significant association with other brain volumes, and grip strength at wave 2 predicted ventricular volume (-0.11) and NAWM (0.08). Therefore, stronger grip strength was associated with less brain atrophy in this wave. However, decreased grip strength (ie change in grip strength) over 3 years was not significantly associated with any brain volume measure. Therefore longitudinal change in grip strength in this study was not associated with brain structure. This may be because the actual change in grip strength between the two waves in this cohort is very small and a longer follow up period may be required to demonstrate longitudinal changes.

### **2.3.2.2 Brain structure and gait speed**

The Sydney Older Person's Study (Piguet et al., 2006), the TASCOG study (Callisaya et al., 2013, Srikanth et al., 2010, Srikanth et al., 2009), the Three-City Study (Elbaz et al., 2013, Dumurgier et al., 2012, Dumurgier et al., 2010, Soumare et al., 2009), the AGES-Reykjavik study (Rosano et al., 2010), ABC1921 study (Starr et al., 2003), WML and mobility study (Wolfson et al., 2005, Guttmann et al., 2000), further studies from Boston (Manor et al., 2012, Hajjar et al., 2010, Novak et al., 2009, Moscufo et al., 2012, Moscufo et al., 2011), the Cardiovascular Health Study (Rosano et al., 2012, Barnes et al., 2009, Rosano et al., 2006,

Rosano et al., 2005, Rosano et al., 2011, Rosano et al., 2008, Longstreth et al., 1996), the Oregon Brain Aging Study (Silbert et al., 2008, Marquis et al., 2002), the LBC 1936 study (Aribisala et al., 2013) all looked at the relationship between structural brain measures and gait speed. There were 27 papers, comprising 12 studies, identified to include in this section, making it the most researched association in our review. Six of the studies only looked at cross-sectional analyses and the other six studies also included longitudinal analyses. Cross-sectional and longitudinal analyses will be reported separately below. The measurement of gait speed varied considerably, with studies variously using maximum speed or usual pace, and some studies requiring a turn halfway through the measurement and others not. The distance used for the measurement also varied from 2.5 to 75 metres, however the most commonly used measure was usual pace over 6 metres.

#### *2.3.2.2.1 Cross-sectional analysis of the relationship between brain structure and gait speed*

The following studies either only looked at the relationship between brain structure and gait speed in cross-sectional analyses or looked at cross-sectional and longitudinal analyses. Here the cross-sectional results are discussed. The Sydney Older Person's Study was set up to investigate the environmental, biological and social determinants of healthy ageing. Within it Piguet et al looked at the relationship between timed walk over 5 meters, adjusted for lower limb arthritis, and cerebellar vermis area (broken down into V1, V2, V3 and total), and total cerebellar volume. None of the measures of cerebellar size/volume significantly predicted the timed walk (Piguet et al., 2006). Around 50% of the subjects in this study were war veterans and war widows selected from a specific register which may cause issues with the generalisability of results. Also, the analyses were not adjusted for gender which is unusual as both brain structure and gait speed are frequently found to be predicted by gender, however as detailed above the results were not significant anyway.

The Three-City study is a longitudinal study of the relation between vascular diseases and dementia in persons aged 65 years and older in France, which includes measures of WM volume and maximum walking speed over 6 metres and a repeat walking speed test at the fourth follow up assessment (ie roughly 7 years after the first). This was a well-designed study with a large n, random sampling from the electoral rolls and minimal exclusion criteria. There were four papers identified from this study which contained reference to these variables.

Soumare et al looked at the association between WMH volume and baseline walking speed (Soumare et al., 2009). They adjusted for age, gender, education and brain white matter volume. They found a significantly lower mean walking speed in those with a total WMH volume above the 75<sup>th</sup> percentile compare to those below the 25<sup>th</sup>. They found similar relationships for both deep WMH and periventricular hyperintensities (PVH), however further analyses revealed that PVH may have more of an effect on walking speed than deep WMH. Elbaz et looked at this association further and found that large WMH volumes were not associated with slow walking speed among highly educated participants (OR = 0.72), but were associated with a 2-fold-increased risk of slow walking speed among those with low education (OR = 3.19/1.61 = 1.99) (p interaction=0.026) (Elbaz et al., 2013). Results remained unchanged after adjustment for height, BMI, and MMSE score.

Dumurgier et al looked at GM volumes and gait speed in the same cohort and found that only basal ganglia volume (beta 0.075 (SE 0.025) p=0.003) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.114 (SE 0.024) p<0.001) (Dumurgier et al., 2012). All other regional GM volumes were not significantly associated with walking speed.

The authors from the Three-City study provided further associations between the variables of interest on written request (Callisaya et al., 2013, Srikanth et al., 2010, Srikanth et al., 2009). They looked at the relationship between WM volume and maximal walking speed at baseline using a multiple linear regression (MLR) and found no significant association.

The AGES-Reykjavik study includes an MRI brain and usual walking pace over 6 metres (Rosano et al., 2010). There was good study design for an observational cohort study, with no exclusion criteria on the grounds of health and a large randomly sampled population. The MR brain imaging included a magnetization transfer imaging sequence, which can be used to calculate the magnetisation transfer ratio (MTR), which can detect normal and diseased brain tissue by looking at the homogeneity of the brain tissue being studied. They found that in men usual walking speed was predicted by WMH volume (beta 0.13, p=0.02) but not by degree of brain atrophy or peak MTR height (both p>0.05) (adjusted for age and brain size) (Rosano et al., 2010). However in women slower walking speed was associated with: lower MTR height (ie indicating abnormal brain tissue) (beta -0.14 (p=0.01); increased WMH (beta 0.12, p=0.003); and greater brain atrophy (beta 0.15, p=0.01) (Rosano et al., 2010). Additionally they comment that isometric knee extension strength was found to positively

correlate with peak height MTR ( $p < 0.005$ ) however they do not give the strength of the correlation or say what it was adjusted for.

The Aberdeen Birth Cohort 1921 study is a longitudinal study which includes a measure of gait speed (self-paced walk time over 6 metres) and a MR brain scan, which was assessed for WMH. Lower gait speed was significantly associated with increased WMH in the brainstem ( $p = 0.009$ , partial eta squared 7%), but not in the cerebral white matter or with PVHs (Starr et al., 2003). It is not clear when looking at the analyses in this paper what they were adjusted for.

Seven papers were identified which met the inclusion criteria from the Boston area in the United States. These include two papers from the WML and mobility observational follow up study (Wolfson et al., 2005, Guttmann et al., 2000), two papers looking at mobility, brain changes and cardiovascular risk factors at baseline (Moscufo et al., 2011) and follow up at 2 years (Moscufo et al., 2012), two papers conducted at the Beth Israel Deaconess Medical Centre, where it seems there may be overlap between the study volunteers (Hajjar et al., 2010, Novak et al., 2009) and a case-control study about diabetic peripheral neuropathy (Manor et al., 2012). The baseline paper from the WML and mobility study comments that gait velocity was not significantly predicted by WMSA corrected for ICV, however does not give any specific figure for this analysis (Guttmann et al., 2000).

Moscufo et al recruited 99 subjects to a longitudinal study about mobility, brain changes and cardiovascular risk factors (Moscufo et al., 2011, Moscufo et al., 2012). Here, the baseline associations are discussed. Gait speed was measured using time to walk 2.5 metres as part of the Short Physical Performance Battery (SPPB). This is a considerably shorter distance than most other measures of gait speed used. The authors supplied Spearman partial correlations between the brain volumetric variables and gait speed, which were not described in the paper. Greater WMH burden ( $\rho = -0.365$ ,  $p = 0.0002$ ) and CSF volumes ( $\rho = -0.284$ ,  $p = 0.004$ ) are associated with slower gait speed. White matter was not found to significantly predict gait speed, however larger GM volume did predict faster gait speed ( $\rho = 0.232$ ,  $p = 0.020$ ) (Moscufo et al., 2011).

An analysis was made in the baseline paper, to investigate whether location of WMH affected gait speed (Moscufo et al., 2011). They selected 10 regions of interest (ROI), which were neural pathways involved in sensory input or motor response and performed a Spearman's

correlation with a corrected significance threshold of  $\leq 0.005$  (calculated using the Bonferroni method to adjust for multiple comparisons). All 10 ROI were found to significantly correlate with the walking speed score at  $p < 0.005$  (rho values between 0.279 and 0.426), except in the superior longitudinal fasciculus ( $p = 0.035$ ) (Moscufo et al., 2011). This study included a large list of exclusion criteria which may affect the generalisability of the results. Also, it was not clear what was adjusted for in the analyses in the paper however the author responded to this query and showed that adjustment for age and gender did not affect their results.

Two papers carried out their studies at the Beth Israel Deaconess Medical Centre. One paper looked at healthy volunteers (Novak et al., 2009) and the other looked at stroke patients in comparison to healthy volunteers (Hajjar et al., 2010). It does not explicitly state the healthy volunteers are the same for each study, but the exclusion criteria, time period and author list would indicate this. The first study measured gait speed over 12 minutes at normal walking speed. MR brain images were analysed for WMH burden and brain volumes corrected for ICV. They found that gait speed was correlated to frontal WM volume ( $r = 0.4$ ,  $p = 0.003$ ) and frontal grey matter volume ( $r = 0.3$ ,  $p = 0.01$ ) (Novak et al., 2009). However total WMH burden was not associated with gait speed. It is not exactly clear why they looked at frontal brain volumes and gait speed and no other regions of the brain or total brain volume. The second paper also measured gait speed over 12 minutes and used MR brain images. In the non-stroke group, white matter volume was found to predict gait speed ( $B = 1.30$ ,  $p = 0.03$ ) but not grey matter ( $p > 0.05$ ). They comment that greater brain atrophy is associated with slower gait speed, but this is for the whole group, so includes stroke patients. The volunteers were recruited from a health centre patient list, but it doesn't say why they were attending the health centre or whether this is where they receive their primary care, more details would have been useful to assess how generalisable the results are.

The final study from Boston was by Manor et al and quoted results from the control group (Manor et al., 2012). They found no association between total GM volume or regional GM volumes and walking speed over 75 metres ( $p > 0.005$ , Bonferroni adjusted). They were being compared to subjects with diabetic peripheral neuropathy in this study. No results for WM or CSF were reported in the study. As this study used 75 metres for their gait speed it may be that this represents a test of cardiorespiratory function more than shorter distances and therefore this measure may be less reliant on muscle function.

Seven studies from the Cardiovascular Health Study (CHS) met our criteria for inclusion. However three of the studies did not contain any associations between the variables of interest and the study authors did not supply the raw data or correlations (Barnes et al., 2009, Rosano et al., 2008, Rosano et al., 2011). As above, the participants were all selected from Medicare Lists possibly affecting the generalisability of results, and the papers used a 10 point scale for WMH grade and ventricular enlargement, whereas now this would usually be measured in exact volumetric terms which may improve errors secondary to subjective judgement. The first study used gait speed measured over 15 feet, and MR brain images which were used to measure ventricular enlargement (VE) and WMH both of which were recorded on a 10 point scale (0-9). Both greater ventricular enlargement ( $p < 0.001$ ) and greater WMH burden ( $p = 0.003$ ) were associated with slower baseline gait speed (Rosano et al., 2005). The model included adjustment for age, sex, race and education.

The next study looked at a subset of the CHS who had undergone two MR brain scans, separated by roughly 5 years, and a MMSE and had undergone assessment on the GaitMat, a 4 metre long instrumented walking surface (Rosano et al., 2006). THE MR brain scans were classified as above, but given binary cut-offs for the analysis of WMH grade  $\geq 3$  or  $< 3$  and VE  $> 4$  or  $< 4$  for some of the analyses. Gait speed was correlated with WMH grade ( $r = -0.18$ ,  $p < 0.0001$ ) and with WMH in the brainstem ( $r = -0.18$ ,  $p < 0.01$ ). Logistic regression was used to analyse the relationship further and gait speed was separated into quartiles. This showed that those in the lowest two quartiles of gait speed (ie  $< 1.02$  m/s) had double the likelihood of having WMH graded 3 or above ( $p = 0.03$ ). VE graded  $> 4$  was not found to be significantly predicted by gait speed, however VE graded  $> 5$  was significantly predicted by gait speed (OR = 2.91 for 1<sup>st</sup> vs. 4<sup>th</sup> quartile, OR 3.82 for 2<sup>nd</sup> vs 4<sup>th</sup> quartile) (Rosano et al., 2006).

Longstreth et al is mentioned in the above section on grip strength and brain structure, as this was also studied in this paper (Longstreth et al., 1996). Gait speed was again measured over 15 feet and WMH burden was scored 0-9 on MR brains scans. Time to walk 15 feet was found to correlate with WMH grade (partial correlation coefficient 0.153,  $p < 0.001$ , adjusting for age, sex and presence of clinical silent stroke on MR brain). The population in this study and the study by Rosano et al (Rosano et al., 2005) overlap considerably and only appear to differ in the time they were still in the study and the particular inclusion and exclusion criteria for that part of the study.

In a separate paper, Rosano et al found that prefrontal area volume significantly predicted time to walk in a stepwise forward model (beta -0.15,  $p=0.02$ ) (Rosano et al., 2012). This relationship was attenuated when adjustment was made for DSST score, which is a measure of processing speed. They conclude that smaller prefrontal area volume may contribute to slower gait speed through slower information processing.

The final two studies identified are both from the Oregon Brain Aging Study (OBAS) (Marquis et al., 2002, Silbert et al., 2008). OBAS I is a prospective study commenced in 1989 of healthy older adults age 65 years or older at the initial assessment, a second arm was added in 2004, OBAS II, with subjects 85y or older at the start of the study. The exclusion criteria were extensive, with no known comorbidities being allowed at the time of recruitment to the study. In a population of 65 year olds, this will lead to results not really applicable to the general population. Marquis et al (2002), looked at the correlation of timed walk, measured at self-selected pace over 9 metres, against brain volumes. Hippocampal volume was found to negatively correlate with timed walk (partial  $r=-0.12$ ), however no significance value was given and it did not explicitly state what was adjusted for in the correlation (Marquis et al., 2002). The correlation between TBV and timed walk was  $<0.1$ .

Further data from the study was requested, which was kindly provided. Baseline data from all subjects from OBAS I and II who had had a MRI brain scan and a timed walk at baseline was used to perform our analysis ( $n=176$ ). GLMs were performed to investigate the relationship between brain structures and gait speed, calculated in metres per second for the analysis. In an unadjusted model, gait speed was predicted by TBV, hippocampal volume and WMH volume, all  $p<0.001$ . Upon adjusting for age, sex, ICV and height, TBV ( $t\ 3.61$ ,  $p=0.004$ , partial eta squared 4.3%) and WMH ( $t\ -2.80$ ,  $p=0.006$ , partial eta squared 4.4%) significantly predicted gait speed, but hippocampal volume did not ( $p>0.05$ ).

#### *2.3.2.2.2 Longitudinal analysis of the relationship between brain structure and gait speed*

The following studies reported longitudinal relationships between brain structure and gait speed. The Tasmanian Study of Cognition and Gait was set up to examine the role of age-related brain changes in causing problems with walking, balance and cognitive abilities in the general community. It measured brain volumes and usual walking speed over 4.6 metres at baseline and 31 months (Callisaya et al., 2013). They found that a greater decline in gait speed over this period was associated with more WM atrophy and hippocampal atrophy and

greater accumulation of WML ( $p < 0.05$ ). There was a non-significant trend with GM atrophy and decline in gait speed ( $p = 0.06$ ). Subjects recruited to the TASCOG study were selected from electoral rolls and only minimal exclusion criteria were applied. The response rate was also good (56%).

The following papers looked at the longitudinal relationship within the three-city study as described above. Soumare et al looked at the association between WMH volume at baseline and decline in walking speed over the 7 year follow up period (Soumare et al., 2009). They found that having a WMH volume greater than the 90<sup>th</sup> percentile, more than doubled the risk of decline in walking speed compared with subjects with lower volumes of WMH. This finding was replicated when looking at PVH but not for deep WMH volume.

The authors from the Three-City study provided further associations between the variables of interest on written request (Callisaya et al., 2013, Srikanth et al., 2010, Srikanth et al., 2009). They looked at the relationship between WM and walking speed decline over 31 months using a multiple linear regression (MLR) and found no significant association. Finally they performed a logistic regression between a one standard deviation increase in WM volume and the risk of having the highest walking speed decline, which was again not significant. Although the walking speed was measured longitudinally, the brain structure measurements were only recorded at baseline in the study.

The second paper from the WML and mobility study recorded variables after a period of follow up (19-22 months) (Wolfson et al., 2005), the baseline data is described above in the cross-sectional analysis section (Guttmann et al., 2000). The follow up paper found a change in gait speed between visit 1 and 2 did not predict WMSA volume at follow up ( $p = 0.07$ ). They also state they found a significant negative relationship between change in gait speed between visits and CSF volume at follow up ( $r = 0.733$ ,  $p < 0.005$ ) and a positive relationship between change in gait speed and WM volume at follow up ( $r = 0.558$ ,  $p < 0.05$ ) (Wolfson et al., 2005). However both the quoted correlations are positive. In this study gait speed was measured at both visits, but the brain structure measurements were only performed at the second visit.

The follow up paper from the Moscufo study was performed after 2 years and found that change in WMH burden, either total or in any of the 7 regional areas, over 2 years was not associated with a decline in usual walking speed ( $p > 0.1$ ) (Moscufo et al., 2012). However



decline in walking speed was entered as a binary variable for this analysis (ie decline or no decline in walking speed over 2 years), which may have missed a relationship between greater WMH burden and greater declines in walking speed.

The CHS papers included some longitudinal analyses. Rosano et al found that both greater ventricular enlargement (VE) ( $p < 0.001$ ) and greater WMH burden ( $p = 0.003$ ) were associated with greater decline in gait speed over the 4 year follow up period (Rosano et al., 2005). Indeed, after adjusting for baseline performance, those with severe VE were found to have 2.5x the decline in gait speed compared to those with minimal VE at baseline. The model included adjustment for age, sex, race and education. Again, in this study gait speed was measured longitudinally whereas the brain structure measurements were only performed at the initial time point.

Silbert et al (2008) used longitudinal data from the OBAS study and found that a higher baseline total WMH volume was associated with a greater increase in timed walk over follow up ( $R^2 = 0.08$ ,  $p = 0.0052$ ), the average follow up was 9.1 years (Silbert et al., 2008). They then looked at whether location of WMH mattered and found that whilst periventricular (PV) WMH volume was associated with a greater change in timed walk over follow up ( $R^2 = 0.12$ ,  $p = 0.0039$ ), a higher subcortical WMH volume was not. These analyses were adjusted for age and ICV. They next looked at change in WMH volume with time and found that a higher rate of accumulation of PV WMH was associated with a greater increase in timed walk ( $R^2 = 0.15$ ,  $p = 0.0453$ ). However there was no relationship described between subcortical or total WMH accrual and change in timed walk, and it is not clear if these analyses were not performed or possibly more likely, that there was no significant association found. This is probably an example of reporting bias for negative findings.

**Table 2-3: Studies identified with brain structure and muscle function**

| Author                                   | Country and dataset                  | n   | Study design               | Mean age (sd)                    | Male (%) | Brain Structure#  | Muscle Function               | Associations*   |
|--|--------------------------------------|-----|----------------------------|----------------------------------|----------|---|-------------------------------|---|
| <b>Brain structure and grip strength</b> |                                      |     |                            |                                  |          |   |                               |   |
| <b>1. (Sachdev et al., 2009)</b>         | Australia, PATH through life project | 432 | Observational cohort study | M 62.61 (1.42)<br>F 62.62 (1.44) | 52.8     | Volumes of GM, WM and CSF, ICV and TBV (GM plus WM). Brain atrophy and subcortical atrophy, WMH | Grip strength in writing hand | Study: Total brain WMH volume predicted grip strength in men (beta -0.140, delta R <sup>2</sup> 0.019, p<0.05) but not in women (beta -0.140, delta R <sup>2</sup> 0.018, p>0.05).  |
| <b>2. (Anstey et al., 2007a)</b>         | Australia, PATH through life project | 432 | Observational cohort study | 62.63 (1.43)                     | 51.6     | Total, anterior, midbody and posterior corpus callosum (CC) area                                | Grip strength in writing hand | Study: Grip strength adjusted for sex and ICV was found to correlate with CC midbody area (r=0.103, p<0.05), however CC total area and anterior and posterior CC areas did not significantly correlate with grip strength (p>0.05).   |
| <b>3. (Sachdev et al., 2006)</b>         | Australia, PATH through life project | 469 | Observational cohort study | M 62.56 (1.44)<br>F 62.53 (1.47) | 51.8     | Volumes of GM, WM and CSF, ICV and TBV (GM plus WM). Brain atrophy and subcortical atrophy, WMH | Grip strength in writing hand | Study: None, see other articles from the PATH through life project for analysis using this dataset.   |
| <b>4. (Sachdev et al., 2005)</b>         | Australia, PATH through life project | 478 | Observational cohort study | M 62.56 (1.44)<br>F 62.54 (1.47) | 52.3     | WMH, ICV  | Grip strength in writing hand | Study: Total brain WMH significantly predicted grip strength (beta -0.09, p=0.002) adjusted for age, sex and depression. Correcting for comorbidity, cognition and brain atrophy did not attenuate the results (beta -0.13, p=0.001). |
| <b>5. (Doi et al., 2012)</b>             | Japan                                | 110 | Cross-sectional study      | 75.4 (7.1)                       | 50       | GM, WM, CSF, brain atrophy (measured using healthy  | Grip strength                 | Study: A MLR model found that grip strength is not related to brain atrophy (beta -0.082 (SE 0.005) p=0.54). Adjusting for age, gender, BMI, education, MMSE, Tokyo Metropolitan Institute of Gerontology                             |

|                                       |   |     |   |                                  |           |   |  |   |
|---------------------------------------|---|-----|---|----------------------------------|-----------|---|--|---|
|                                       |   |     |   |                                  |           | volunteers)   |  | Index of Competence, geriatric depression scale and change in walking whilst dual tasking. No other associations given.   |
| <b>6. (Hardan et al., 2003)</b>       | USA, Philadelphia   | 41  | Case-control study  | 18.6 (8.6)                       | Not given | Caudate, putamen and total brain volume   | Grip strength  | Study: Non-significant trends showed a negative correlation between right grip strength and total caudate volume ( $r=-0.303$ , $p=0.05$ ) and left grip strength ( $r=-0.28$ , $p=0.07$ ) in the control group. Not corrected for age or sex. No relationships given for other measures.   |
| <b>Brain structure and gait speed</b> |   |     |   |                                  |           |   |  |   |
| <b>7. (Piguet et al., 2006)</b>       | Australia, Sydney Older Person's Study                    | 111 | Longitudinal observational cohort study (year 6 f/u data used in paper) | M 85.29 (2.89)<br>F 85.72 (3.41) | 54.5      | Cerebellar vermis area, (V1, V2 and V3 and total), Cerebellar volume, cerebral volume and ICV | Timed walk over 5m, adjusted for lower limb arthritis    | Study: None of the brain size measures (cerebellar vermis area, cerebellar volume or cerebral volume) significantly predicted timed walk ( $p>0.05$ ) after adjustment for age (but not sex, as was not deemed to be a significant contributor after univariate analyses).  |
| <b>8. (Callisaya et al., 2013)</b>    | Australia, Tasmanian Study of Cognition and Gait (TASCOG) | 225 | Longitudinal cohort study   | 71.4 (6.8)                       | 56.4      | ICV, GM, WM-lesion free, hippocampal volume, WML  | 4.6metre GaitRite computerized walkway (preferred speed) | Study: MLR were performed to investigate the relationship of longitudinal change in brain volumes and gait speed. They found that white matter atrophy ( $\beta$ 0.25 (CI 0.09-0.40) $p=0.001$ ), greater WML progression ( $\beta$ -0.89 (CI -1.75- -0.02) $p=0.045$ ), grey matter atrophy ( $\beta$ 0.25 (CI 0.00-0.19) $p=0.06$ ) and hippocampal atrophy ( $\beta$ 0.01 (CI 0.00-0.02) $p=0.006$ ) were all associated with a greater decline in gait speed. |
| <b>9. (Srikant h et al., 2010)</b>    | Australia, TASCOG   | 385 | Longitudinal cohort study   | 72.2 (7.1)                       | 56        | WMLV, TBV   | Gait speed using 4.2m GAITRite system                    | Study: none, see Callisaya et al (2013) for analysis using the TASCOG dataset.  |
| <b>10. (Srikant h et al., 2009)</b>   | Australia, TASCOG   | 294 | Longitudinal cohort study   | 72.3(7.0)                        | 55.4      | WMLV, TBV   | Gait speed using 4.2m GAITRite                           | Study: none, see Callisaya et al (2013) for analysis using the TASCOG dataset.  |

|                                    |                          |      |              |            |      |   | system                                 |  |
|------------------------------------|--------------------------|------|--------------|------------|------|---|--|--|
| <b>11. (Elbaz et al., 2013)</b>    | France, Three-city study | 4010 | Cohort study | 73.4 (4.6) | 38.4 | WML volumes   | 6 metre walk speed (usual and maximum) | <p>Study: Logistic regression stratified by education found that high WML volumes were not associated with slow walking speed among highly educated participants (OR = 0.72), but were associated with a 2-fold-increased risk of slow walking speed among those with low education (OR = 3.19/1.61 = 1.99) (p interaction=0.026), adjusted for sex, age and total WM volume. Results remained unchanged after adjustment for height, BMI, and MMSE score.</p> <p>Given: WM volume did not predict walking speed at baseline, adjusted for age, gender and ICV in a MLR (p&gt;0.05, n=1510), or decline in walking speed over 7 years, adjusted for age, gender, ICV and baseline walking speed, (p&gt;0.05, n=928). A logistic regression found that WM volume was not significantly associated with an increased risk of being in the quartile with the highest walking speed decline (p&gt;0.05).</p>                                 |
| <b>12. (Dumurger et al., 2012)</b> | France, Three-city study | 1623 | Cohort study | 73.3 (4.1) | 39.5 | Regional grey matter volumes (sensorimotor cortex; frontal, parietal, temporal, occipital, and limbic lobes; insula; cerebellum; thalamus; basal ganglia nuclei, including the caudate nucleus, putamen and pallidum) and | Maximum walking speed over 6 metres    | <p>Study: A linear regression found that only basal ganglia volume (beta 0.075 (SE 0.025) p=0.003) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.114 (SE 0.024) p&lt;0.001). All other regional GM volumes were not significantly associated with walking speed. A semi-bayes model found again only the basal ganglia volume (beta 0.061 (SE 0.028) p=0.03) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.050 (se 0.019) p=0.007). There was found to be a linear relationship between quartiles of caudate nucleus volume and faster walking speed (p for linear trend (0.001). These relationships were attenuated slightly for total basal ganglia volume by adjusting for MMSE and comorbidity plus smoking but not for caudate nucleus volume. All models adjusted for; age, sex, BMI, education level, ICV, volume of WMLs and silent</p> |

|                                    |                          |                               |  |                                    |                             | WMLs   |  | infarcts.   |
|------------------------------------|--------------------------|-------------------------------|--|------------------------------------|-----------------------------|--|--|---|
|                                    |                          |                               |  |                                    |                             |  |  | Given: See Elbaz et al (2013) for Three-City Study data analysis.   |
| <b>13. (Dumurger et al., 2010)</b> | France, Three-city study | Baseline 3604, f/u at 4y 1774 | Cohort study                                   | Baseline 73.4 (4.6) f/u 71.5 (3.6) | Baseline 38.1 %, f/u 38.4 % | WMH volume   | Maximum walking speed over 6 metres, 1st and 4th follow up, mean 7 years | Study: none<br>Given: See Elbaz et al (2013) for Three-City Study data analysis.  |
| <b>14. (Soumaré et al., 2009)</b>  | France, Three-city study | 1702                          | Cohort study                                   | 72.4 (4.1)                         | 39.4                        | PVH, deep WMH and total WMH and total WM and ICV           | Maximum walking speed over 6 metres, 1st and 4th follow up, mean 7 years | Study: A significantly lower mean walking speed was found in those with a total WMH volume above the 75 <sup>th</sup> percentile compared to those below the 25 <sup>th</sup> (Beta -0.026, p=0.0003). A similar relationship was found for both deep WMH and PVH. A WMH volume greater than the 90th percentile more than doubled the risk of decline in walking speed compared with subjects with lower volumes of WMH (OR 2.6 (1.5-4.5), p=0.001). This finding was replicated when looking at PVH but not for deep WMH volume.<br><br>Given: See Elbaz et al (2013) for Three-City Study data analysis. |
| <b>15. (Starr et al., 2003)</b>    | UK, ABC1921 cohort study | 97                            | Longitudinal cohort study (baseline data used) | 78-79years                         | 59.8                        | WMH in deep/subcortical, PVH and brain stem, Fazekas score | Self-paced time to walk 6metres  | Study: A slower 6metre walk test was associated with increased brain stem lesions (F 7.11, p=0.009, partial eta <sup>2</sup> 0.070), but not with WMH (deep) (F 3.33, p=0.071) or PVH (F 2.47, p=0.12). Doesn't state if age and sex are adjusted for in these models. If HADS score and Raven's score are adjusted for, brainstem lesions are no longer significantly associated with walking time.  |
| <b>16. (Manor et al., 2012)</b>    | USA, Boston,             | 89 in control                 | Case-control                                   | 65.3 (8.2)                         | 48.3                        | GM, WM, CSF, regional GM volumes;                          | 75 metre walk test at preferred  | Study: Within linear regression models, global GM volume and all of the regional GM volumes were not associated with walking speed in the control group   |

|                                   | group   | study               |                                  |            |      | precentral and postcentral gyri, basal ganglia, cerebellum, and dorsolateral prefrontal cortex       | pace   | (p>0.005, Bonferroni adjusted). Adjusted for age, sex and body mass.  |
|-----------------------------------|---|---------------------|----------------------------------|------------|------|--|--|---|
| <b>17. (Hajjar et al., 2010)</b>  | USA, Boston, BP in stroke study (?overlap with Novak et al) | Non-stroke group 43 | Case-control observational study | 68 (1)     | 44   | WM, GM (global and regional), CSF normalized for ICV   | Gait speed over 12mins at usual pace   | Study: Gait speed was not significantly associated with GM volume (p=0.85), but was significantly associated with WM volume (B=1.30, p=0.03) adjusting for age, gender, BMI and antihypertensive use.   |
| <b>18. (Novak et al., 2009)</b>   | USA, Boston (?overlap with Hajjar et al)                    | 76                  | Observational study              | 64.7 (7.2) | 47.4 | GM, WM, CSF, WMH all as % brain tissue volume. WMH using Wahlund scale                               | Gait speed over 12mins at normal walking pace  | Study: Gait speed was significantly associated with frontal WM normalized for brain tissue volume (R=0.4, p=.003). Gait speed was significantly associated with frontal GM normalized for brain tissue volume (R=0.3, p=.01). Adjusted for age and BMI (but not gender). Doesn't say about other regional brain volumes, ie temporal etc. WMH volumes and PVH and punctuate scores were not associated with gait speed (p>0.05).  |
| <b>19. (Moscufo et al., 2012)</b> | USA, Boston, Moscufo study – 2 year f/u                     | 77                  | Longitudinal cohort study        | 84 (3.9)   | 40   | WMH volume as % of ICV and regional WMH burden expressed as % of ROI volume. At baseline and 2y f/u. | Gait speed over 2.5 metres, maximum velocity and usual walking speed At baseline and 2y f/u. | Study: Total WMH burden was significantly associated with usual walking speed at baseline but not at follow-up, and maximum walking speed was not associated with total WMH at baseline or follow up. At baseline, regional WMH burden in the splenium of corpus callosum and anterior and superior corona radiata, was significantly associated with both walking measures (p<0.05) and in addition the body of the corpus callosum was also associated with usual walking speed (p<0.05). At follow-up, WMH burden in the splenium was significantly associated with both walking measures (p<0.05) and in the body with maximum walking speed. Change in WMH burden, |

|                                   |                                       |                                 |  |   |      |   |  |   |
|-----------------------------------|---------------------------------------|---------------------------------|--|---|------|---|--|---|
|                                   |                                       |                                 |  |   |      |   |  | <p>either total or in any of the 7 regional areas, over 2 years was not associated with a decline in usual walking speed (<math>p&gt;0.1</math>).</p> <p>Given: WMH burden is significantly associated with lower gait speed after adjustment for age, sex and BMI (<math>\rho=-0.327</math>, <math>p=0.0008</math>). WM/ICV is not significantly associated with gait speed with or without adjustment (<math>p&gt;0.05</math>). GM/ICV is significantly associated with gait speed with adjustment for age, gender and BMI (<math>\rho=0.232</math>, <math>p&lt;0.05</math>). CSF/ICV is significantly associated with gait speed with adjustment for age, sex, BMI (<math>\rho=-0.285</math>, <math>p=0.004</math>).</p> |
| <b>20. (Moscufo et al., 2011)</b> | USA, Boston, Moscufo study - baseline | 99                              | Cross-sectional observational study          | 83(4)   | 42.4 | WM, GM, WMH and CSF volumes all corrected for ICC. Brain atrophy. Regional WMH burden expressed as % of ROI volume. | Gait speed over 2.5metres (done as part of SPPB) | <p>Study: Total WMH burden (ie % of ICV) correlates with gait speed (<math>\rho=-0.288</math>, <math>p=0.004</math>). Also all 9x regional burden measurements correlate with gait speed score too except sup. longitudinal fasciculus. No adjustment.</p> <p>Given: See Moscufo et al (2012) for analysis using this dataset.</p>  |
| <b>21. (Wolfson et al., 2005)</b> | USA, Boston, WML and mobility         | 28 at baseline, 14 at follow up | Prospective longitudinal observational study | SPPB 11or12 mean 81(1.7), SPPB<8 mean 84(3.4) | 64.3 | GM, WM, WMSA, CSF, ICCV volumes   | Gait velocity over 8metres                       | <p>Study: Slower baseline gait velocity predicted more WMSA at visit 1 (<math>p&lt;0.05</math>), but not change in WMSA volume between visit 1 and 2 (<math>p&lt;0.07</math>). Significant negative relationship of between-visit change in gait velocity to CSF volume (<math>r=0.733</math>, <math>p&lt;0.005</math>) and a positive relationship of between-visit change in gait velocity to WM volume (<math>r=0.558</math>, <math>p&lt;0.05</math>). Betas not given. Brain volumes normalized for ICCV according to image processing section.</p>   |
| <b>22. (Guttman et al., 2000)</b> | USA, Boston, WML and mobility         | 28 (12 with SPPB score >10)     | Observational cross-sectional study          | SPPB>10 79(5) SPPB<9 83(6)                    | 42.9 | WM, WMSA, GM, CSF (normalized for ICCV)   | Gait velocity over 8metres                       | <p>Study: Gait velocity was not significantly predicted by age nor WMSA volume (no figures given or p value) adjusted with and without MMSE score.</p>  |

and 16  
<9)

|                                  |  |      |  |             |      |   |   |   |
|----------------------------------|--|------|--|-------------|------|---|---|---|
| <b>23. (Rosano et al., 2012)</b> | USA, Cardiovascular health study   | 214  | Longitudinal observational study<br><br>(cross-sectional data used, 1997-99)                 | 72.3 (3.8)  | 35.5 | Brain volumes (GM, WMH, Prefrontal area, WM, CSF) | Timed 15ft walk at usual pace                           | Study: Prefrontal area volume significantly predicted time to walk in a stepwise forward model (beta -0.15, p=0.02).  |
| <b>24. (Barnes et al., 2009)</b> | USA, Cardiovascular Health Cognition Study, nested within the CVS Health Study | 3375 | Prospective, population-based, longitudinal study  | 75 (no sd)  | 41   | White matter disease and ventricular enlargement  | Gait speed over 15ft                                    | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset   |
| <b>25. (Rosano et al., 2006)</b> | USA, Cardiovascular health study   | 321  | Longitudinal observational study mean f/u 4years<br><br>(cross-sectional data used, 1997-99) | 78.3(no sd) | 39.3 | WMAs, ventricular enlargement                     | Gait speed at usual pace over 4 metres using GaitMat II | Study: Gait speed was significantly correlated to total WMAs (r=-.18, p<0.0001) and white matter lesions in the brainstem (r=-.18, p=0.01). After adjusting for age, slower gait speed was still significantly associated with white matter grade (p=0.02). Logistic regression found that those in the lowest two quartiles of gait speed (ie<1.02m/s) had double the likelihood of having WMH graded 3 or above (p=0.03), after adjustment for age, race, gender, and prevalent clinical CVD. VE graded >4 was not found to be significantly predicted by gait speed, however VE graded>5 was, independent of age, gender, race and presence of CVD (OR=2.91 for 1st vs. 4th quartile, OR 3.82 for 2nd vs 4th quartile) |



|                                   |                                  |      |  |            |      |  |  |  |
|-----------------------------------|----------------------------------|------|--|------------|------|--|--|--|
| <b>26. (Rosano et al., 2005)</b>  | USA, Cardiovascular health study | 2450 | Longitudinal observational study mean f/u 4years | 74.4 (4.7) | 43   | WMH and ventricular enlargement (graded as minimal, moderate and severe)         | Gait speed over 15ft at usual pace, starting from standing still | Study: Grade of ventricular enlargement was associated with baseline gait speed and mean change in gait speed/year. Gait speed decline was 2.5x that for those with severe VE than minimal VE. ( $p < 0.001$ ). Grade of WMH was associated with baseline gait speed and mean change in gait speed/year ( $p = 0.003$ ). In both analyses adjustment had been made for age, sex, race and education and CV risk factors (BMI, systolic BP, antihypertensive meds, internal carotid wall thickness, and ETOH intake) and prevalent CV disease.  |
| <b>27. (Silbert et al., 2008)</b> | USA, Oregon Brain Aging Study    | 104  | Longitudinal cross-sectional study               | 85.1 (5.6) | 38.5 | PV WMH and s/c WMH, total WMH, brain volume, CSF volume, hippocampal volume, ICV | Gait speed over 9m. Self-selected pace.                          | Study: Adjusted for age and ICV, higher baseline total WMH vol. was associated with increased rate of change in timed walking in seconds ( $r^2 = 0.08$ , $p = 0.0052$ ). This relationship became non-significant after adjustment for multiple comparisons to threshold p value. PVH volume is associated with increased rate of change in timed walk in seconds ( $r^2 = 0.12$ , $p = 0.0039$ ). However, baseline subcortical WMH vol. was not related to change in gait performance over time. Higher rate of PVH accumulation is associated with increased rate of change of time to walk 9m ( $r^2 = 0.15$ , $p = 0.0453$ ). Adjusted for age, ICV and baseline WMH volume:<br><br>Calculated: In an unadjusted GLM, gait speed was predicted by total brain, WMH and hippocampal volume ( $p < 0.001$ ). The relationship remained significant after adjusting for sex, age, ICV and height, for total brain volume ( $t = 3.61$ , $p = 0.004$ , partial eta squared 4.3%) and WMH ( $t = -2.80$ , $p = 0.006$ , partial eta squared 4.4%) but not for hippocampal volume. |
| <b>28. (Marquis et al., 2002)</b> | USA, Oregon Brain Aging          | 108  | Longitudinal study (baseline)                    | 83.2 (7.9) | 37   | Total brain volume, hippocampal volume, ICV                                      | Gait speed over 9m. Self-selected                                | Study: Negative correlation between hippocampal volume and time to walk 30ft ( $r = -.12$ ). No p value given.<br><br>Calculated: See Silbert et al (2008) for Oregon Brain  |

| Study  | data)                            | pace.   | Aging Study data analysis.   |
|--|----------------------------------|---|--|
| <b>Brain structure and gait speed plus grip strength or isometric knee extension strength (IKES)</b> |                                  |   |  |
| <b>29. (Rosano et al., 2010)</b>   | Iceland, AGES-Reykjavik study    | 795   | Longitudinal cohort study<br>(data from wave 2002-3 used in this paper)  |
|  |                                  | M 75.6 (5.4)<br>F 75.6 (5.7)  | 41.1   |
|  |                                  | MTR, ICV, brain parenchyma volume, semiquantitative subcortical WMH and PVH and total WMH volume, brain atrophy index |  |
|  |                                  | Gait speed over 6m usual speed and maximal isometric knee extension strength  | Study: In men: Time to walk 6metres predicted by WMH volume (beta 0.13, p=0.02) but not brain atrophy or peak height MTR (adjusted for age and brain size as includes measure of brain atrophy). In women: Usual walking speed predicted by lower MTR height (ie indicating abnormal brain tissue) (beta -0.14 (p=0.01), increased WMH (beta 0.12, p=0.003) and greater brain atrophy (beta 0.15, p=0.01) (adjusted for age and brain size). Lower muscle strength associated with peak height MTR (p<0.005, beta not given).  |
| <b>30. (Aribisala et al., 2013)</b>  | UK, LBC 1936 study               | 694   | Longitudinal cohort study  |
|  |                                  | 69.5(0.7) wave 1 and 72.5 (0.7) wave 2  | 52.9   |
|  |                                  | TBV, ventricular volume, GM, NAWM and WML at wave 2   |  |
|  |                                  | 6 metre walk (normal walking pace) and grip strength at wave 1 and 2  | Study: Grip strength at wave 1 significantly predicts ventricular volume at wave 2 (standardized beta -0.10), however there was no significant association with other brain volumes. 6metre walk at wave 1 predicted TBV (-0.07), ventricular volume (0.09), NAWM (-0.07) and WML (0.11) all p<0.05. Grip strength at wave 2 was associated with ventricular volume (-0.11) and NAWM (0.08). 6MW at wave 2 was associated with TBV (-0.07), NAWM (-0.09) and WML (0.11) all p<0.05. Change in physical function between wave 1 and 2 (ie decrease in grip strength or increase in 6MW) was not significantly associated with any brain volume measure. GM volume did not significantly associate with any of the physical function variables at wave 1 or 2. All analyses were adjusted for age, ICV, age 11 IQ, years of education, social class, comorbidity and smoking status. Corrected for false discovery rate. |
| <b>31. (Rosano et al., 2011)</b>   | USA, Cardiovascular health study | 643   | Longitudinal observational study   |
|  |                                  | 72.1-72.6 broken down by BP   | 31-42.7 broken down  |
|  |                                  | WMH scale 0-9   |  |
|  |                                  | Gait speed over 15ft, starting from standstill.   | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset.   |

|                                      |                                  |      |   | diagnosis    | by BP diagnosis |   | Grip strength of dominant hand.                            |   |
|--------------------------------------|----------------------------------|------|---|--------------|-----------------|---|--|---|
| <b>32. (Rosano et al., 2008)</b>     | USA, Cardiovascular health study | 3156 | Longitudinal observational study mean f/u 4years                                | 74 (4.6)     | 43.2            | White matter disease score, brain atrophy score (ventricular enlargement) | Gait speed over 15ft and grip strength in dominant hand    | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset.  |
| <b>33. (Longstreth et al., 1996)</b> | USA, Cardiovascular health study | 3658 | Longitudinal observational study<br><br>(data from year 5/6 used in this study) | 70.7 (no sd) | 41.7            | MR WMSA graded 0-9  | Time to walk 15feet, grip strength in dom and non-dom hand | Study: Time to walk 15ft correlated with white matter grade (0-9) ( $r=0.153$ , $p<0.001$ ), with adjustment for age, sex and presence of clinically silent stroke on MRI. Same model showed no significant associated between grip strength in dom hand or non-dom hand and white matter grade ( $p>0.05$ ). |

### 2.3.3 Association of brain function and muscle structure

Fifteen papers were identified which looked at brain function and muscle structure: DEXA was used in eleven of the studies; BIA in two; and CT for thigh muscle CSA and MRI for neck muscle CSA in the final two papers (table 2.4). Measures of brain function were the MMSE, the Community Screening Instrument of Dementia (CSI-D), Trail Making Test (TMT) A and B, digit span and a measure of global cognitive performance (using z scores from multiple cognitive tests). The studies included the Kansas Brain Aging Project (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011) and the MHEM study (Kilgour et al., 2013), both mentioned in the above section, and studies from Canada (Berryman et al., 2013), Chile (Bunout et al., 2005), the Chinese University of Hong Kong (Auyeung et al., 2011, Auyeung et al., 2008), Denmark (Pedersen et al., 2012), Italy (Magri et al., 2006), Lithuania (Lasaite and Krasauskiene, 2009), Taiwan (the I-Lan Longitudinal Aging Study, ILAS) (Liu et al., 2014), and from the USA, the Baltimore Longitudinal Study of Aging (Moore et al., 2014) and the FITKids Study (Kamijo et al., 2014, Kamijo et al., 2012). All the papers included in this section used cross-sectional data except the four papers from the Chinese University of Hong Kong which contain longitudinal data on cognitive test scores and lean mass; the results from these studies have been grouped together at the end of this section.

From the Kansas Brain Aging Project, Burns et al (2010) found a relationship between both MMSE (beta 0.11,  $p=0.009$ ) and global cognitive performance (beta 0.12,  $p=0.007$ ) and TLM, again grouping AD and control subjects together (Burns et al., 2010). They state that in this relationship if the AD subjects are removed from the analysis the results are attenuated, but do not show any results for this. A GLM was performed on the data from the non-demented group supplied to us by the study authors, and I found that neither the global cognitive performance score nor MMSE was predicted by TLM adjusting for age and sex. Adjusting for height and education did not affect this.

The MHEM study used 9 different cognitive tests, which they reduced to two factors using principal components analysis (Kilgour et al., 2013). Total neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor ( $p > 0.05$ ), however, it did predict 10% of the variance in the NART score ( $t = -2.12$ ,  $p < 0.05$ ). The NART score is a measure of childhood intelligence and the authors comment that the finding is the opposite of what they hypothesized, as they found that lower childhood intelligence is associated with larger neck muscle size in old age.

Berryman et al supplied the baseline data from their physical training intervention trial. Subjects had performed a MMSE and modified Stroop test and underwent a DEXA scan at baseline (Berryman et al., 2013). A GLM was performed which showed no association between LBM and MMSE or the Stroop naming, reading or inhibition tasks. However there was an association between the Stroop flexibility task and LBM ( $t$  2.126,  $p=0.039$ , partial eta squared 9.3%), however after adjusting for education and height the effect was attenuated ( $p>0.05$ ). This effect was in the opposite direction than might be expected, ie bigger muscle mass is associated with a worse score (the Stroop test is measured in seconds to perform the task). No details are given in the paper as to how the subjects were recruited, therefore it is unknown if this was a potential source of bias, and there was also quite an extensive exclusion criteria list another source of bias in the results as the subjects are likely to be healthier than the average population in this age group (60-85y).

The two papers from Chile are a study by Bunout et al which includes baseline data for a randomized controlled trial (RCT) investigating an exercise intervention in the elderly (Bunout et al., 2005) and a paper by Bites et al which used baseline trial data from several studies, including the study by Bunout et al, held on their University database (Bites et al., 2013). The paper by Bunout et al was a retrospective analysis of data held from previous studies performed by their University. Recruitment details for the subjects from all the individual studies were lacking and therefore there are several sources of bias attributable to the methodology used in this paper. The authors sent one data sheet for both studies as there is a large amount of overlap between the studies (overlap  $n=203$ ). A GLM was performed which showed total LM ( $t$  2.38,  $p=0.018$ , partial eta squared 1.4%) and leg LM ( $t$  3.53,  $p<0.001$ , partial eta squared 3.1%) were both associated with MMSE score but arm LM is not. After adjusting for height the relationship between TLM and MMSE became non-significant and between leg LM and MMSE is attenuated ( $t$  2.09,  $p=0.038$ , partial eta squared 1.1%). Therefore it seems that leg LM is driving the relationship between total LM and MMSE.

Pedersen et al investigated cognition and physical fitness in normal controls, subjects with impaired glucose tolerance and type 2 diabetes (Pedersen et al., 2012). They supplied the raw data for their control group to be analysed. Subjects underwent DEXA for FFM and six cognitive tests (a cognitive z score was computed as a marker of general cognition). FFM did not predict the cognitive z score with or without adjusting for BMI and childhood intelligence

(Danish Adult Reading Test, DART). The six individual cognitive tests were then analysed. There was no association between FFM and most of the individual cognitive tests. Also, unadjusted there was no significant association between the letter fluency test (using “s”) and FFM ( $P>0.05$ ), however after adjustment for BMI and DART, letter fluency was significantly associated with FFM ( $t\ 2.34$ ,  $p=0.02$ , partial eta squared 7.7%). Letter fluency is a test of executive function and this may indicate that change in this type of cognition with age is associated with FFM in older age. TMT-A test did significantly predict FFM ( $t\ 3.08$ ,  $p=0.003$ , partial eta squared 12.3%), but after adjusting for BMI and DART the relationship became non-significant. The TMT-A test is a measure of processing speed. The subjects were recruited using newspaper adverts, a possible source of bias, and the control group was significantly smaller than the case group. As with all the case-control studies in this review no relevant power calculations were undertaken as the association in the control group was not an outcome measure for this study.

Magri et al (2006) performed a cross-sectional study looking at postmenopausal women and HRT, however their study also contained a control group of young healthy women which were used for our analyses after the study authors kindly supplied their data (Magri et al., 2006). A GLM was performed with MMSE as the outcome/dependent variable and FFM as the predictor/independent variable as measured by BIA. FFM did not significantly predict MMSE after adjustment for age ( $p>0.05$ ) (Magri et al., 2006). Adjustments for BMI and educational level did not further affect these results. There are a couple of methodological issues with this study which could affect the generalisability of these results. Firstly, all the control subjects were recruited from the place of work of the study authors, and secondly the exclusion criteria were extensive including any recent acute or chronic illness, smoking history, no medication and 2 or less caffeinated drinks per day.

Lasaite et al performed an observational case-control study which looked at women with osteoporosis and healthy controls (Lasaite and Krasauskiene, 2009). The study data for the healthy controls was supplied to us on request. The cognitive measures were the TMT-A and B and a digit span test. FFM was measured using BIA. A GLM was performed on the available data. FFM did not predict TMT-A or B adjusting for age +/- height ( $p>0.05$ ). There was a non-significant trend with FFM predicting digit span adjusting for age ( $t\ 1.96$ ,  $p=0.06$ , partial eta squared 13%), however when adjusted for height too, the relationship was attenuated ( $p=0.37$ ). The controls in this study were also all older women, but the way they

were recruited and the exclusion criteria were not documented in the study, therefore represent possible sources of bias.

The I-Lan Longitudinal Aging Study is an ageing cohort study in Taiwan, in the paper identified only baseline data was used (Liu et al., 2014). Within the study they performed a t test comparing mean MMSE in those with a normal relative appendicular skeletal mass ( $RASM=ASM/height^2$ ) with those in the lowest 20% for RASM, and they found a significant difference in both men and women. They also supplied the results of a linear regression on our request for further data, which showed that RASM did not predict MMSE after adjusting for age and sex (beta -0.003,  $p=0.940$ ). This may mean there is a non-linear relationship between cognition and muscle mass. This is a large population based study with random sampling of the population register to recruit subjects and minimal exclusion criteria. It was not clear why they chose to use the lowest 20% of RASM, as guidelines on sarcopenia vary on which cut off to use and, as we now know, when analysed as a continuous variable there was no significant association. It would therefore be interesting to see a scatter plot of this data to look for a non-linear relationship

The Baltimore Longitudinal Aging Study is a large longitudinal cohort study, in which the subjects underwent four cognitive tests and had a mid-femur CT for thigh CSA (Moore et al., 2014). No associations between the cognitive tests and thigh CSA were included in the study, but the authors sent the results of a MLR they had performed on cross-sectional data. They found that none of the cognitive tests predicted thigh CSA, adjusting for age and gender. After adjusting for age, gender and height, the digit-span backward test became significantly negatively associated with thigh CSA (beta -1.55,  $p=0.024$ ), meaning those with bigger thigh muscles perform better on the test (a higher score is better in the digit span tests). As the association between digit-span backward and thigh CSA was only significant after adjusting for height in addition to age and gender, this may represent residual confounding rather than a true association. It doesn't say in this paper how the volunteers in this study were recruited which may indicate a source of bias.

The next study to look at cognition and muscle structure is the FITKids study based in Illinois, USA (Kamijo et al., 2014, Kamijo et al., 2012) which looked at 7-9 year olds. Two papers from this study were identified; however there were no relevant associations in the papers and the study authors kindly provided us with the raw data on which to perform an analysis. As the subjects were all from the same study the authors provided us with one

dataset for the study. I performed a GLM which found that TLM did not predict the Kaufman Brief Intelligence Test, used to assess IQ. The subjects were the control group in a study looking at childhood obesity and as such all had a normal BMI. It doesn't state in the paper how they were recruited which may be a source of bias.

The only longitudinal data identified in this section came from four papers from the Chinese University of Hong Kong which used data from a large prospective longitudinal study looking at bone mineral density in older Chinese adults to assess the relationship between physical and cognitive function (Auyeung et al., 2011, Auyeung et al., 2008, Lee et al., 2011, Auyeung et al., 2013). They used two measures of cognitive function; the MMSE and the cognitive score from the Community Screening Instrument for Dementia (CS-CSID). Only one of the papers included the associations between the cognitive tests and muscle mass (Auyeung et al., 2011). They found that in men, but not in women, lower appendicular skeletal mass (SM) at baseline predicted lower MMSE at follow up (for a 2.54kg increase in appendicular SM, there would a 0.246 change improvement in MMSE,  $p < 0.001$ ). However after adjustment for age, years of education and baseline MMSE, the relationship became non-significant ( $P > 0.05$ ) (Auyeung et al., 2011). The authors from this study kindly supplied further analyses of their data upon our request.

They performed Spearman's partial correlations, adjusting for age and sex. There was no significant relationship between baseline CS-CSID and total LM or appendicular LM at baseline or 4 years. However baseline MMSE predicted both baseline total LM (partial rho 0.058,  $p = 0.002$ ) and appendicular LM (partial rho 0.061,  $p = 0.001$ ) and 4 years follow up total LM (partial rho 0.058,  $p = 0.002$ ) and appendicular LM (partial rho 0.054,  $p = 0.005$ ). However, the effect size is small. They also looked at the whether those with lower MMSE at follow up had lower muscle mass at baseline or follow up but found no significant associations ( $p > 0.05$ ) (Auyeung et al., 2011). Unfortunately the study authors did not supply data for the relationship between change in cognition and change in muscle mass over the four year follow up which would be very interesting in such a large study population. Also, the method of recruitment may have been a source of bias as the subjects were recruited through talks at community centres and housing estates, which may have only accessed a particular sub-population of older people.



**Table 2-4: Studies identified with brain function and muscle structure**

| <b>Author &amp; year</b>          | <b>Country and dataset</b>               | <b>n</b> | <b>Study design</b>  | <b>Mean age (sd)</b>              | <b>Male (%)</b> | <b>Brain Function</b>       | <b>Muscle Structure</b>        | <b>Associations*</b>   |
|-----------------------------------|--|----------|--|-----------------------------------|-----------------|-----------------------------|--------------------------------|--|
| <b>1. (Berryman et al., 2013)</b> | Canada, Training Intervention Study      | 48       | Baseline characteristics from a large physical training intervention study | 70.8 (5.4)                        | 41.67           | MMSE & modified Stroop test | LBM (DEXA)                     | Study: none<br><br>Calculated: A GLM showed no association between LBM and MMSE, Stroop naming, reading or inhibition tasks, adjusted for sex and age. However there was an association between the Stroop flexibility task and LBM (t 2.126, p=0.039, partial eta squared 9.3%), however after adjusting for education and height the effect was attenuated (p>0.05).   |
| <b>2. (Bites et al., 2013)</b>    | Chile                                    | 306      | Retrospective study  | M 74.9 (61-91),<br>F 75.5 (69-90) | 24.5            | MMSE                        | TLM, Arm LM and Legs LM (DEXA) | Study: none<br><br>Calculated: Authors sent one data sheet for this study and Bunout et al, as there is a large amount of overlap between the studies. N=401, mean age 75.3 (sd 4.8), males 28.7%. GLM performed adjusting for sex and gender. Total LM (t 2.38, p=0.018, partial eta squared 1.4%) and Leg LM (t 3.53, p<0.001, partial eta squared 3.1%) were both associated with MMSE score but Arm LM is not. After adjusting for height the relationship between total LM and MMSE is non-significant and between leg LM and MMSE is attenuated (t 2.09, p=0.038, partial eta squared 1.1%). |
| <b>3. (Bunout et al., 2005)</b>   | Chile                                    | 298      | RCT  | M 75.4 (4.8)<br>F 75.8 (4.7)      | 29.2            | MMSE                        | TLM, Arm LM and Legs LM (DEXA) | Study: none<br><br>Calculated: See Bites et al 2013 for analysis using this dataset  |
| <b>4. (Auyeung et al., 2013)</b>  | Chinese University of Hong Kong - 4y f/u | 3153     | Prospective observational study  | M 71.76 (4.67)<br>F 72.03 (5.07)  | 49.7            | CSI-D and MMSE              | ASM, LLMM, FFM (DEXA)          | Study: none<br><br>Given: CS-CSID did not predict TLM or ASM at baseline or at 4 years (all p>0.05). However baseline MMSE was associated with baseline  |

| Author & year                     | Country and dataset                        | n           | Study design                    | Mean age (sd)                         | Male (%) | Brain Function  | Muscle Structure      | Associations*  |
|-----------------------------------|--|-------------|---------------------------------|---------------------------------------|----------|---|-----------------------|--|
|                                   |  |             |                                 |                                       |          |   |                       | TLM (rho=0.058, p=0.002) and ASM (rho=0.061, p=0.001) and at follow-up (TLM rho=0.058, p=0.002, ASM rho=0.054, p=0.005). MMSE at follow up was not associated with TLM or ASM at baseline or follow-up (p>0.05).   |
| <b>5. (Auyeung et al., 2011)</b>  | Chinese University of Hong Kong - 4y f/u   | 2737        | Prospective observational study | M 71.6 (4.58)<br>F 71.5 (4.85)        | 55.3     | CSI-D and MMSE  | ASM (DEXA)            | Study: In men, low baseline ASM predicted lower MMSE score after 4 years (B = 0.246, p<0.01) however after adjustment for age, years of education and baseline MMSE it no longer did (p>0.05). In women, ASM did not significantly predict MMSE after 4 years, either before adjustment or after (p>0.05).<br><br>Given: see Auyeung et al (2013) for analysis using this dataset  |
| <b>6. (Lee et al., 2011)</b>      | Chinese University of Hong Kong            | 4000        | Prospective observational study | M 72.3 (5.0)<br>F 72.5 (5.3)          | 50       | CSI-D and MMSE  | ASM, LLMM, FFM (DEXA) | Study: none<br><br>Given: see Auyeung et al (2013) for analysis using this dataset   |
| <b>7. (Auyeung et al., 2008)</b>  | Chinese University of Hong Kong - baseline | 4000        | Prospective observational study | M 72.3 (5.0)<br>F 72.5 (5.3)          | 50       | CSI-D and MMSE  | ASM (DEXA)            | Study: none<br><br>Given: see Auyeung et al (2013) for analysis using this dataset   |
| <b>8. (Pedersen et al., 2012)</b> | Denmark                                    | 72 controls | Cross-sectional study           | Median 53 (48-60 interquartile range) | 46       | DART, WAIS-III information subtest, TMT-A&B, Rey Auditory Verbal Learning Test (RAVLT), | FFM (DEXA)            | Study: None<br><br>Calculated: FFM did not predict the cognitive z score with or without adjusting for BMI and childhood intelligence (Danish Adult Reading Test, DART). The six individual cognitive tests were then analysed: FFM did not predict RAVLT, SDMT, category fluency (using animals) or TMT-b test, with or without adjusting for BMI and childhood intelligence (DART). Unadjusted, there was no significant |

| Author & year                               | Country and dataset                    | n                   | Study design                                | Mean age (sd)        | Male (%) | Brain Function   | Muscle Structure  | Associations*  |
|---|--|---------------------|---|----------------------|----------|--|---|--|
|   |  |                     |   |                      |          | Symbol Digit Modalities Test (SDMT), and fluency tests |   | association between the letter fluency test (using “s”) and FFM (P>0.05), however after adjustment for BMI and DART, letter fluency was significantly associated with FFM (t 2.34, p=0.02, partial eta squared 7.7%). TMT-a test did significantly predict FFM (t 3.08, p=0.003, partial eta squared 12.3%). After adjusting for BMI and DART the relationship became non-significant. |
| <b>9. (Magri et al., 2006)</b>              | Italy                                  | 27 controls         | Cross-sectional case-control study          | Controls 33.3 (7.15) | 0        | MMSE   | FFM (BIA)   | Study: none<br>Calculated: FFM did not significantly predict MMSE (p>0.05), adjusting for age. Adjustments for BMI and educational level did not significantly affect the results.   |
| <b>10. (Lasaite and Krasauskiene, 2009)</b> | Lithuania                              | 29 healthy controls | Observational case-control study            | 66.2(6.3)            | 0        | TMT-A and B and digit span                             | FFM (BIA)   | Study: none<br>Calculated: FFM does not significantly predict TMT-A or B adjusting for age +/- height (p>0.05). Trend with FFM predicting digit span (t 1.96, p=0.06, partial eta squared 13%) but attenuated when adjusted for height in addition to age (p=0.37).  |
| <b>11. (Liu et al., 2014)</b>               | Taiwan, I-Lan Longitudinal Aging Study | 983                 | Population based ageing cohort study        | 65.2 (9.3)           | 50.6     | MMSE   | LBM and Relative ASM (=ASM/height <sup>2</sup> ) (DEXA) | Study: A t test comparing mean MMSE in those with normal RASM and those within the lowest 20% of RASM found a significant difference in men and women of all ages (p<0.05).<br>Given: In a MLR, RASM did not predict MMSE after adjusting for age and sex (beta - 0.003, p=0.940). Adjusting for education in addition did not affect the results.                                     |
| <b>12. (Moore et al., 2014)</b>             | USA, Baltimore Longitudinal            | 786                 | Longitudinal cohort study – cross-sectional | 66.3 (range 26-96)   | 51.9     | California Verbal Learning                             | Mid-femur thigh CSA                                     | Study: none<br>Given: In a linear regression, none of the  |

| Author & year                    | Country and dataset | n                   | Study design  | Mean age (sd) | Male (%) | Brain Function                          | Muscle Structure | Associations*  |
|----------------------------------|---------------------|---------------------|---|---------------|----------|---|------------------|--|
|                                  | Study of Aging      |                     | analysis  |               |          | Test (CVLT), digit-span test, TMT A & B | (CT)             | cognitive tests predicted thigh CSA, adjusting for age and gender. After adjusting for age, gender and height, the digit-span backward test became significantly associated with thigh CSA (beta -1.55, p=0.024).  |
| <b>13. (Kamijo et al., 2014)</b> | USA, FITKids Study  | 37 (healthy weight) | Cross-sectional study (case-control substudy comparing obese and healthy weight children) | 8.8 (0.6)     | 46       | Kaufman Brief Intelligence Test (K-BIT) | TLM (DEXA)       | Study: none<br>Calculated: Authors sent one data sheet for the FITKids study as there is considerable overlap in subjects between the two Kamijo et al papers (Kamijo et al., 2014, Kamijo et al., 2012), (n= 139, mean age 8.8 (sd 0.6), male 51.1%). A GLM found that TLM did not predict K-BIT after adjustment for age and gender (p>0.05). Adjusting for BMI in addition did not alter the results. |
| <b>14. (Kamijo et al., 2012)</b> | USA, FITKids Study  | 126                 | Cross-sectional study   | 8.9 (0.5)     | 50       | Kaufman Brief Intelligence Test (K-BIT) | TLM (DEXA)       | Study: none<br>Calculated: as per Kamijo et al (Kamijo et al., 2014)   |

*Table 2-5: Studies identified with measures of brain structure or function and muscle structure or function but no associations given in paper or on request*

| <b>Author &amp; year</b>  | <b>Country and dataset</b>   | <b>n</b>             | <b>Study design</b>                                       | <b>Mean age (sd)</b> | <b>Male (%)</b> | <b>Brain structure or function</b>   | <b>Muscle structure or function</b>          |
|---|--|----------------------|---|----------------------|-----------------|--|--|
| <b><i>Studies with brain structure and muscle structure (re: table 2.2)</i></b> |  |                      |   |                      |                 |  |  |
| <b>1. (Chowdhury et al., 1994)</b>  | Sweden   | 8                    | Methodology paper   | 35 (8)               | 100             | Brain volume (CT)  | Calculated skeletal muscle volume (CT)       |
| <b><i>Studies with brain structure and muscle function (re: table 2.3)</i></b>  |  |                      |   |                      |                 |  |  |
| <b>2. (Liu-Ambrose et al., 2010)</b>  | Canada, Exercise RCT in Vancouver                                  | 155                  | RCT, prospective over 52 weeks                            | 69.6 (2.9)           | 0               | Whole brain volume (MRI)   | Gait speed, quads strength and muscle power  |
| <b>3. (Nadkarni et al., 2012)</b>   | Canada, Sunnybrook Dementia Study                                  | 20 controls          | Cross-sectional substudy of longitudinal study            | 75 (9)               | 40              | Score on Age-Related White Matter Change Scale (MRI)   | Self-selected speed on a treadmill           |
| <b>4. (Sullivan et al., 2005)</b>   | USA, California, Stanford  | 51                   | Case-control study  | 45.2 (13.9)          | 100             | Caudate, putamen, nucleus accumbens and medial septal / diagonal band volumes and ICV (MRI)                      | Bilateral grip strength                      |
| <b><i>Studies with brain function and muscle structure (re: table 2.4)</i></b>  |  |                      |   |                      |                 |  |  |
| <b>5. (Guthrie et al., 2004)</b>  | Australia, The Melbourne Women's Midlife Health Project            | 1897                 | 9 year prospective, observational population based sample | Median 50            | 0               | Episodic verbal memory using a 10 word recall task (CERAD)   | Body composition (DEXA)                      |
| <b>6. (Ellis et al., 2009)</b>  | Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging | 768 healthy controls | Longitudinal case control study (AD vs MCI vs normal)     | 70.0 (7.0)           | 43              | CVLT-II, Logical memory, RCFT, digit span, digit symbol coding, D-KEFS verbal fluency, BNT, clock, WTAR, Stroop. | Body composition (DEXA) in subgroup in Perth |
| <b>7. (Dao et al., 2013)</b>  | Canada, Exercise RCT in Vancouver                                  | 114                  | Secondary analysis of RCT data                            | 69.4 (2.9)           | 0               | Stroop test, MMSE  | Sub-total lean mass (DEXA)                   |

| Author & year                      | Country and dataset          | n                                   | Study design  | Mean age (sd)                                  | Male (%)               | Brain structure or function                     | Muscle structure or function                          |
|------------------------------------|------------------------------|-------------------------------------|---|--|------------------------|---|---|
| 8. (Schwartz et al., 2013)         | Canada, Saguenay Youth Study | 983                                 | Longitudinal cohort study                                   | M 14.9 (1.8),<br>F 15.1 (1.9)                  | 48.8                   | Executive function and Memory                   | FFM (BIA)   |
| 9. (Bagger et al., 2004)           | Denmark, PERF study          | 5607                                | Prospective, observational cohort study                     | 71.1 (6.6)                                     | 0                      | Short Blessed Test                              | TLM (DEXA)  |
| 10. (Abellan van Kan et al., 2013) | France, EPIDOS study         | 3025                                | Prospective multi-centre cohort study                       | 80.51(3.9)                                     | 0                      | SPMSQ   | Lean mass and ALM (DEXA)                              |
| 11. (Nourhashemi et al., 2002)     | France, EPIDOS study         | 7105                                | Cross-sectional study                                       | 80.3 (3.65) (SPMSQ $\geq$ 8)                   | 0                      | SPSMQ for orientation, concentration and memory | FFM (DEXA)  |
| 12. (Nourhashemi et al., 2001)     | France, EPIDOS study         | 7364                                | Prospective multicentre study                               | Broken down by ADLs; means 79.9-82.7 years     | 0                      | Pfeiffer's test (aka SPMSQ)                     | Body composition (DEXA)                               |
| 13. (Paolisso et al., 1997)        | Italy, Naples                | 30 (>50y),<br>30 (75-99y) 19 (>99y) | Observational study   | 44.5(1.8),<br>78(0.7),<br>102(0.8)             | 46.8                   | MMSE  | FFM (BIA)   |
| 14. (Malaguarnera et al., 2007)    | Italy, Sicily                | 66                                  | Placebo controlled, randomized, double-blind, 2-phase study | 101(1.3) treatment,<br>101(1.4) placebo        | 31.8                   | MMSE  | Total muscle mass (BIA)                               |
| 15. (Jacobsen et al., 2012)        | Netherlands                  | 318                                 | RCT   | Mean for each arm given range 73.4-74.0        | 0                      | 15 words test and Trails B test                 | BIA and DEXA  |
| 16. (Genton et al., 2011)          | Switzerland                  | 213 in 1999 and 112 in 2008         | Cross-sectional study with 9 year f/u visit                 | 1999 M 71.7(5.2)<br>2008 M 80.3(5.2)<br>1999 F | 1999 49.3<br>2008 49.1 | MMSE  | FFM (BIA), ASMM (DEXA) and BCM (total body potassium) |

| Author & year                | Country and dataset                            | n             | Study design  | Mean age (sd)  | Male (%)                           | Brain structure or function                       | Muscle structure or function  |
|------------------------------|--|---------------|---|--|------------------------------------|---|---|
|                              |  |               |   | 73.2(5.5)<br>2008 F<br>82.2(5.6)                               |                                    |   |   |
| 17. (Donaldson et al., 1996) | USA, Baltimore                                 | 73            | Cross-sectional study   | 68.8 (7.2)   | 31.5                               | MMSE  | FFM (DEXA)  |
| 18. (Bove et al., 2013)      | USA, Boston, Harvard                           | 12            | Cross-sectional study   | 31.6 (6.4)   | 0                                  | Multiple tests broken down to 5 cognitive domains | Cross sectional area of mid-thigh (CT)  |
| 19. (Papadakis et al., 1995) | USA, California, San Francisco                 | 104           | Cross-sectional study   | 75.5(4.9)  | 100                                | MMSE, Trails B and DSST                           | Lean tissue mass (DEXA)   |
| 20. (Janssen, 2006)          | USA, Cardiovascular health study               | Baseline 5036 | Longitudinal observational study (over 8 years)               | 65-70 42.7%,<br>71-76 32.7%,<br>83-89 18.2%,<br>>=90 6.4%      | 43.6                               | MMSE  | Whole body muscle mass (BIA) and normalized for height to the skeletal muscle index (SMI, kg/m <sup>2</sup> ) |
| 21. (Masley et al., 2008)    | USA, Florida                                   | 56            | RCT   | Controls 43.5 (11.2),<br>Intervention 47.1 (9.4)               | Control 39.3,<br>Intervention 53.6 | CNS vital signs battery                           | FFM (BIA)   |
| 22. (Houston et al., 2012)   | USA, Health, Aging, and Body Composition study | 2641          | Longitudinal cohort study                                     | 74.7 (2.9)   | 48.9                               | MMSE  | Lean mass (DEXA)  |
| 23. (Middleton et al., 2011) | USA, Health, Aging, and Body Composition study | 197           | Cross-sectional study from a 9 year longitudinal cohort study | Separated into tertile of activity, means range from 73.9-75.8 | Not given                          | 3MS   | FFM (DEXA)  |
| 24. (Koster et al., 2010)    | USA, Health, Aging, and Body Composition study | 2949          | Cross-sectional study from a 9 year longitudinal cohort study | Age 70-79 at baseline  | 48.5                               | 3MS   | Total bone-free lean mass, trunk lean mass, appendicular lean mass (DEXA)                                     |

| <b>Author &amp; year</b>                 | <b>Country and dataset</b>                                     | <b>n</b>   | <b>Study design</b>   | <b>Mean age (sd)</b>                                    | <b>Male (%)</b>                | <b>Brain structure or function</b>   | <b>Muscle structure or function</b>                                  |
|--|--|--|---|---|--------------------------------|--|--|
| <b>25. (de Rekeneire et al., 2003a)</b>  | USA, Health, Aging, and Body Composition study                 | 2926   | Baseline data from a 9 year longitudinal cohort study               | Diabetes mellitus (DM) 73.6 (2.9) and non-DM 73.6 (2.9) | DM 55.9, Non-DM 46.9           | MMSE and DSST  | Lean mass and lean soft tissue mass (ie lean mass minus bone) (DEXA) |
| <b>26. (de Rekeneire et al., 2003b)</b>  | USA, Health, Aging, and Body Composition study                 | Fallers 652, non-fallers 2398                        | Baseline data from a 9 year longitudinal cohort study               | Range 70-79   | Fallers 41.7, non-fallers 50.3 | Teng Mini-mental State Examination and DSST  | Total muscle mass and skeletal muscle mass in the legs (DEXA)        |
| <b>27. (Watts et al., 2013)</b>          | USA, Kansas, Brain Aging Project                               | 74 healthy controls                                  | Longitudinal case-control study (Alzheimer's dementia vs. controls) | 74.0 (7.2)  | 43                             | MMSE   | Lean mass (DEXA)   |
| <b>28. (Canon and Crimmins, 2011)</b>    | USA, National Health and Nutrition Examination Survey (NHANES) | 867  | Cross-sectional longitudinal study                                  | Range 60-85   | 44.8                           | Digit-symbol coding test   | Lean tissue mass (DEXA)  |
| <b>29. (Garry et al., 2007)</b>          | USA, New Mexico Aging Process Study                            | 809 rolling participants (average 302 seen per year) | Longitudinal Aging study (1979-2003)                                | 60+ Varied between years                                | 40                             | 3MS (annual), WAIS R digit span, Fuld object memory evaluation, Color Trails 1 and 2, clock drawing (all less than annual) | Annual skeletal tissue mass (DEXA)                                   |
| <b>30. (Haren et al., 2008)</b>          | USA, St Louis, African-American Health Study                   | 124  | Population based longitudinal study                                 | 56.1(4.4)   | 100                            | MMSE, TMT A&B  | TLM and ASM (DEXA)   |
| <b>31. (Dvorak &amp; Poehlman, 1998)</b> | USA, Vermont   | 30   | Case-control study  | 73(7)   | 43.3                           | MMSE   | ASM and FFM (DEXA)   |



## 2.4 Discussion

This systematic review looked at the evidence for whether: a) brain structure is related to muscle structure, b) brain structure is related to muscle function and c) brain function is related to muscle structure in healthy humans over the life course.

### 2.4.1 Brain volumes and muscle mass

The relationship between brain structure and muscle structure was first reviewed (summary in table 2.6). Three studies tested for an association between whole brain volume and muscle mass; the three papers from the Kansas Brain Aging Project are treated as one study (Heymsfield et al., 2012, Kilgour et al., 2013, Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011). Two studies found a positive association between WBV and muscle mass (Heymsfield et al., 2012, Kilgour et al., 2013) and one study found no significant association (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011). However, this study found a significant positive association between WM volume and FFM but no association between GM volume and FFM (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011). A different study looked at regional GM volume and found four areas negatively associated with FFM but found most areas to have no association with FFM (Weise et al., 2013). Two studies found no association between hippocampal volume and muscle mass (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011, Kilgour et al., 2013). One study looked at ventricular volume and cerebellar volume and muscle size and found no association either (Kilgour et al., 2013). Four of the studies were of older adults and two were of younger adults, and it may be that the relationship between brain and muscle structure varies over the life course. Furthermore if there is a relationship between whole brain volume and muscle size it looks like it may be regional brain volume that drives this relationship rather than total volume. The studies are all cross-sectional and a large longitudinal study is needed to explore these relationships further.

**Table 2-6: Number of studies<sup>#</sup> of brain structure and muscle structure, direction of effect and number of subjects**

|  | Negative Association<br>( <i>n</i> ) | No Association<br>( <i>n</i> ) | Positive Association<br>( <i>n</i> ) |   |
|--|--------------------------------------|--------------------------------|--------------------------------------|---|
| <b>Whole brain size and muscle size</b>    | -                                    | 1 (70)                         | 2 (311)                              | (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011, Heymsfield et al., 2012, Kilgour et al., 2013) |
| <b>White matter volume and muscle size</b> | -                                    | -                              | 1 (70)                               | (Burns et al., 2010, Weise et al., 2013, Honea et al., 2009, Wetmore et al., 2011)                            |
| <b>Grey matter volume and muscle size</b>  | 1 (76)*                              | 2 (146)*                       | -                                    | (Burns et al., 2010, Honea et al., 2009, Wetmore et al., 2011, Weise et al., 2013)                            |
| <b>Hippocampal volume and muscle size</b>  | -                                    | 2 (121)                        | -                                    | (Burns et al., 2010, Kilgour et al., 2013, Honea et al., 2009, Wetmore et al., 2011)                          |
| <b>Cerebellar volume and muscle size</b>   | -                                    | 1 (51)                         | -                                    | (Kilgour et al., 2013)  |
| <b>Ventricular volume and muscle size</b>  | -                                    | 1 (51)                         | -                                    | (Kilgour et al., 2013)  |

\*this study found negative associations between some areas of grey matter volume (the right temporal pole and bilateral vmPFC) and muscle size but found the majority of GM areas had no association

<sup>#</sup>Number of studies reflects the result found for each study not each paper (ie for whole brain size and muscle size all the papers from the Kansas Brain Aging Project (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011) have been grouped as one study as they all use the same data). This was to prevent the repetition of results from a single study that may occur if several papers from the same study repeated analyses.

## 2.4.2 Brain structure and muscle function

Next evidence for an association between muscle function and brain structure was reviewed (summary in table 2.7 & 2.8). Muscle function was either grip strength (5 studies) or gait speed (13 studies) apart from in one paper where isometric knee extensor strength (IKES) was used (Rosano et al., 2010).

### 2.4.2.1 Brain structure and grip strength

Only one study looked at the relationship between whole brain, GM or WM volume and grip strength (Aribisala et al., 2013). There were no significant associations except for a positive relationship between WM volume and grip strength at wave 2 (age 73) (Aribisala et al., 2013). This could mean that the relationship between WM volume and grip strength only becomes important with age, once a volumetric threshold is passed. Another study found no association between caudate volume and grip strength (Hardan et al., 2003). However the basal ganglia may be expected to play less of a role in grip strength than in gait speed. Two studies found a negative association with markers of brain atrophy and grip strength (Doi et al., 2012, Aribisala et al., 2013), however one of these studies also looked at change in grip

strength over 3 years and found no association with ventricular volume (a marker of brain atrophy) (Aribisala et al., 2013). This means that whilst cerebral atrophy and grip strength appear to be associated, decline in grip strength does not predict cerebral atrophy. A longitudinal study including both measures would help explain this relationship further.

One study found an association between WMH and grip strength (Sachdev et al., 2005). They found that location of the WMH is important, with some brain areas correlating with grip strength and others not (Sachdev et al., 2005). On looking at the data separated by sex, this relationship persisted in men, but not women, but the study authors think this is due to a sex difference present in their study population, with the men having higher volumes of WMH (Sachdev et al., 2009). Two larger studies found no association between WMH and grip strength, however one of these studies used a visual rating scale from 0-9 to measure WMH, which may lead to differing results than using WMH volumes (Aribisala et al., 2013, Longstreth et al., 1996). The other study also looked at change in grip strength over 3 years and WMH volume at follow up and found no association (Aribisala et al., 2013). WMH are known to predict dementia and cerebrovascular disease but their relationship to physical function is less well understood (Debette and Markus, 2010).

*Table 2-7: Number of studies of brain structure and grip strength, direction of effect and number of subjects*

|  | <b>Negative Association<br/>(n)</b> | <b>No Association<br/>(n)</b> | <b>Positive Association<br/>(n)</b> |   |
|--|-------------------------------------|-------------------------------|-------------------------------------|---|
| <b>Grip strength and whole brain volume</b>                    | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Grip strength and WM volume</b>                             | -                                   | 1 (694) <sup>a</sup>          | 1 (694) <sup>a</sup>                | (Aribisala et al., 2013)  |
| <b>Grip strength and GM volume</b>                             | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Grip strength and caudate volume</b>                        | -                                   | 1 (41)                        | -                                   | (Hardan et al., 2003)   |
| <b>Grip strength and ventricular volume/brain atrophy</b>      | 2 (804)                             | -                             | -                                   | (Doi et al., 2012, Aribisala et al., 2013)                              |
| <b>Grip strength and WMH</b>                                   | 1 (478)                             | 2 (4352)                      | -                                   | (Sachdev et al., 2005, Aribisala et al., 2013, Longstreth et al., 1996) |
| <b>Change in grip strength over f/u and whole brain volume</b> | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Change in grip strength over f/u and WM volume</b>          | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Change in grip strength over f/u and GM volume</b>          | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Change in grip strength over f/u and ventricular volume</b> | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |
| <b>Change in grip strength over f/u and WMH volume</b>         | -                                   | 1 (694)                       | -                                   | (Aribisala et al., 2013)  |

a. at wave 1 in this study there was no association and at wave 2 there was a positive association (Aribisala et al., 2013)

#### **2.4.2.2 Brain structure and gait speed**

Two studies found a positive association between WBV and gait speed (Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013), whereas studies investigating the relationship between WM and GM volume and gait speed found less unanimous results. Three studies found a positive association between WM volume and gait speed (Hajjar et al., 2010, Novak et al., 2009, Aribisala et al., 2013) and two studies found no association (Elbaz et al., 2013, Moscufo et al., 2012). Four studies found no association between GM volume and gait speed (Dumurgier et al., 2012, Manor et al., 2012, Hajjar et al., 2010, Aribisala et al., 2013) but three studies found a positive relationship (Novak et al., 2009, Moscufo et al., 2012, Rosano et al., 2012). There was no evidence that hippocampal volume or cerebellar volume were associated with gait speed (Silbert et al., 2008, Marquis et al., 2002, Piguet et al., 2006). It may be that specific sub-regions of the white and grey matter are associated with gait speed, for example one paper found an association between basal ganglia volume and gait speed but no association with total GM and gait speed. Further studies looking at regional brain areas will help to clarify these relationships. Five studies looked at markers of brain atrophy and gait speed; two found a negative association (ie more atrophy associated with a slower gait speed) (Moscufo et al., 2012, Rosano et al., 2006, Rosano et al., 2005) and one found no association (Rosano et al., 2010), with one finding an association at wave 1 but not at wave 2 (Aribisala et al., 2013).

No association was found between change in gait speed over follow up and whole brain, WM and GM volume (mean length of follow up in each study, 3 and 7 years) (Elbaz et al., 2013, Aribisala et al., 2013). However one large study did find an association between ventricular volume and change in gait speed over follow up (mean 4 years) (Rosano et al., 2005) but another study found no association (mean follow up 3 years) (Aribisala et al., 2013). Only one study looked at the relationship between change in gait speed and change in brain structure over time (mean follow up 30.6 months) (Callisaya et al., 2013). They found a positive association between change in gait speed and WM and hippocampal atrophy but no association with GM atrophy. The well-established relationship between cognitive decline and gait speed and cognitive decline and brain atrophy could underpin the possible relationship between brain atrophy and gait speed (Abellan van Kan et al., 2009, Atkinson et al., 2007, Scherder et al., 2007, Drago et al., 2011, Moran et al., 2013). It is interesting that the only study to look at both variables in a longitudinal study found significant associations between brain structure and gait speed and further studies like this are needed.

Eleven studies were found which looked at gait speed and WMH, making it the most studied relationship in our review. Seven of these studies found that greater levels of WMH were associate with slower gait speed (Soumare et al., 2009, Moscufo et al., 2012, Wolfson et al., 2005, Rosano et al., 2006, Rosano et al., 2005, Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013, Rosano et al., 2010, Longstreth et al., 1996), but four other smaller studies found no association (Starr et al., 2003, Novak et al., 2009, Moscufo et al., 2012, Guttmann et al., 2000). Two of these studies found that this is primarily due to the volume of PVH and not subcortical WMH lesions (Soumare et al., 2009, Silbert et al., 2008) and two papers found that volume of brainstem WMH was associated with gait speed (Starr et al., 2003, Rosano et al., 2006). One small study (n=14) found no association between gait speed and WMH progression over follow up (19-22 months) (Wolfson et al., 2005). However, change in gait speed was found to be associated with WMH volume in two large studies (Soumare et al., 2009, Rosano et al., 2005) with another study showing no association (Aribisala et al., 2013). Two studies looked at change in both variables; one found that greater decline in gait speed was associated with greater WMH progression (Callisaya et al., 2013), whereas the other found no association (Moscufo et al., 2012). Further studies looking not just at total WMH volume but their rate of accumulation and location within the brain, and their association with gait speed should help clarify this area.

*Table 2-8: Number of studies of brain structure and gait speed, direction of effect and number of subjects*

|  | Negative Association<br>(n) | No Association<br>(n)  | Positive Association<br>(n) |  |
|--|-----------------------------|------------------------|-----------------------------|--|
| <b>Gait speed and whole brain volume</b> | -                           | -                      | 2 (885)                     | (Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013)   |
| <b>Gait speed and WM volume</b>          | -                           | 2 (1587)               | 3 (813) <sup>b</sup>        | (Elbaz et al., 2013, Hajjar et al., 2010, Novak et al., 2009, Moscufo et al., 2012, Aribisala et al., 2013)  |
| <b>Gait speed and GM volume</b>          | -                           | 4 (2449) <sup>c</sup>  | 3 (367) <sup>b,d</sup>      | (Dumurgier et al., 2012, Manor et al., 2012, Hajjar et al., 2010, Novak et al., 2009, Moscufo et al., 2012, Rosano et al., 2012, Aribisala et al., 2013) |
| <b>Gait speed and hippocampal volume</b> | -                           | 1 (191)                | -                           | (Silbert et al., 2008, Marquis et al., 2002)   |
| <b>Gait speed and cerebellar volume</b>  | -                           | 1 (111)                | -                           | (Piguet et al., 2006)  |
| <b>Gait speed and WMH volume</b>         | 7 (7145) <sup>e</sup>       | 4 (278) <sup>e,f</sup> | -                           | (Soumare et al., 2009, Starr et al., 2003, Novak et al., 2009, Moscufo et al., 2012,   |

|   |                       |                       |          |   |
|---|-----------------------|-----------------------|----------|---|
|   |                       |                       |          | Wolfson et al., 2005, Guttman et al., 2000, Rosano et al., 2006, Rosano et al., 2005, Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013, Rosano et al., 2010, Longstreth et al., 1996) |
| <b>Gait speed and CSF volume/ventricular volume/brain atrophy</b> | 3 (3221) <sup>g</sup> | 2 (1489) <sup>g</sup> | -        | (Moscufo et al., 2012, Rosano et al., 2006, Rosano et al., 2005, Aribisala et al., 2013, Rosano et al., 2010)   |
| <b>Gait speed and WMH progression over f/u</b>                    | -                     | 1 (14)                | -        | (Wolfson et al., 2005)  |
| <b>Change in gait speed over f/u and whole brain volume</b>       | -                     | 1 (694)               | -        | (Aribisala et al., 2013)  |
| <b>Change in gait speed over f/u and WM volume</b>                | -                     | 2 (1622)              | -        | (Elbaz et al., 2013, Aribisala et al., 2013)  |
| <b>Change in gait speed over f/u and GM volume</b>                | -                     | 1 (694)               | -        | (Aribisala et al., 2013)  |
| <b>Change in gait speed over f/u and CSF/ventricular volume</b>   | -                     | 1 (694)               | 1 (2450) | (Rosano et al., 2005, Aribisala et al., 2013)   |
| <b>Change in gait speed over f/u and WMH volume</b>               | -                     | 1 (694)               | 2 (4152) | (Soumare et al., 2009, Rosano et al., 2005, Aribisala et al., 2013)   |
| <b>Change in gait speed over f/u and WM atrophy</b>               | -                     | -                     | 1 (225)  | (Callisaya et al., 2013)  |
| <b>Change in gait speed over f/u and GM atrophy</b>               | -                     | 1 (225)               | -        | (Callisaya et al., 2013)  |
| <b>Change in gait speed over f/u and hippocampal atrophy</b>      | -                     | -                     | 1 (225)  | (Callisaya et al., 2013)  |
| <b>Change in gait speed over f/u and WMH progression</b>          | -                     | 1 (77)                | 1 (225)  | (Callisaya et al., 2013, Moscufo et al., 2012)  |

b. one study only looked at frontal WM/GM volume not total WM volume (Novak et al., 2009)

c. basal ganglia volume was positively associated with gait speed in this study (Dumurgier et al., 2012)

d. one study only looked at prefrontal area volume within GM (Rosano et al., 2012)

e. this study found usual walking speed was negatively associated with WMH but that maximum walking speed was not (Moscufo et al., 2012)

f. except brainstem WMH which were negatively associated with gait speed in this study (Starr et al., 2003)

g. at wave 1 there was a negative association and at wave 2 there was no association in this study (Aribisala et al., 2013)

### 2.4.3 Cognitive function and muscle mass

Nine studies were found which looked at cognitive function and muscle structure (table 2.9). Three studies looked at a measure of global cognitive performance (a composite score of several tests used in their study) and muscle size and all 3 found no association (Kilgour et al., 2013, Wetmore et al., 2011, Pedersen et al., 2012). The Kaufman Brief Intelligence Test can also be used as a marker of general cognition and it too found no association with muscle size (Kamijo et al., 2014, Kamijo et al., 2012). Seven studies looked at muscle size and MMSE score, which is a useful screening tool for dementia but is not a robust test of cognitive function. Four of the studies found no association between MMSE and muscle mass (Wetmore et al., 2011, Berryman et al., 2013, Magri et al., 2006, Liu et al., 2014) and one found an association but with a very small effect size (Auyeung et al., 2013). However in one study which showed no association between MMSE and FFM, when comparing subjects with normal RASM and those within the lowest 20% of RASM this study found a significant difference in mean MMSE (Liu et al., 2014). Several of the included studies did not include those with cognitive impairment and it may be that an association does exist between MMSE and muscle size but in a non-linear relation, affecting the frailer older adult more, but that it was not picked up in these studies due to the method of analysis in a linear regression. Overall though in healthy individuals it seems that no such association exists. The final study found an association between leg LM and MMSE but not between total or arm LM (Bites et al., 2013). Sarcopenia is known to affect leg and arm muscles differently which perhaps explains this effect (Janssen et al., 2000). Another screening tool for dementia, the cogscore part of the CSI-D, also found no association with muscle mass (Auyeung et al., 2013). It is well established that gait speed and cognition are associated in older age and these results appear to show that muscle size is not a driving force behind this relationship (Abellan van Kan et al., 2009, Atkinson et al., 2007, Scherder et al., 2007).

With regard to the individual cognitive tests (which measure processing speed and executive function), there were no significant associations (Lasaite and Krasauskiene, 2009, Moore et al., 2014, Berryman et al., 2013, Pedersen et al., 2012), except for the NART (a measure of childhood IQ, which showed a negative association with neck muscle CSA (Kilgour et al., 2013). The authors comment that perhaps subjects with higher cognition are more likely to have sedentary jobs and therefore more likely to lose their muscle mass over time. None of the studies looking at cognition and muscle size contained longitudinal associations therefore whilst these results appear to support no association between muscle mass and cognition; it

may be that longitudinal data would show an association, whereby those that lose more muscle with age have a sharper slope of decline in their cognition also. Longitudinal studies will help to elucidate these complex relationships further.

*Table 2-9: Number of studies of cognition and muscle size, direction of effect and number of subjects*

|  | <b>Negative Association<br/>(n)</b> | <b>No Association<br/>(n)</b> | <b>Positive Association<br/>(n)</b> |   |
|--|-------------------------------------|-------------------------------|-------------------------------------|---|
| <b>Muscle size and global cognitive score</b>          | -                                   | 3 (193)                       | -                                   | (Kilgour et al., 2013, Wetmore et al., 2011, Pedersen et al., 2012)   |
| <b>Muscle size and MMSE</b>                            | -                                   | 5 (1434) <sup>h,i</sup>       | 2 (3459) <sup>h</sup>               | (Wetmore et al., 2011, Berryman et al., 2013, Bites et al., 2013, Auyeung et al., 2013, Magri et al., 2006, Liu et al., 2014) |
| <b>Muscle size and California Verbal Learning Test</b> | -                                   | 1 (786)                       | -                                   | (Moore et al., 2014)  |
| <b>Muscle size and CSI-D</b>                           | -                                   | 1 (3153)                      | -                                   | (Auyeung et al., 2013)  |
| <b>Muscle size and digit span</b>                      | -                                   | 2 (815) <sup>j</sup>          | -                                   | (Lasaite and Krasauskiene, 2009, Moore et al., 2014)  |
| <b>Muscle size and Kaufman Brief Intelligence Test</b> | -                                   | 1 (139)                       | -                                   | (Kamijo et al., 2014, Kamijo et al., 2012)  |
| <b>Muscle size and modified Stroop test</b>            | -                                   | 1 (48)                        | -                                   | (Berryman et al., 2013)   |
| <b>Muscle size and NART</b>                            | 1 (51)                              | -                             | -                                   | (Kilgour et al., 2013)  |
| <b>Muscle size and TMT-a</b>                           | -                                   | 3 (887)                       | -                                   | (Pedersen et al., 2012, Lasaite and Krasauskiene, 2009, Moore et al., 2014)   |
| <b>Muscle size and TMT-b</b>                           | -                                   | 3 (887)                       | -                                   | (Pedersen et al., 2012, Lasaite and Krasauskiene, 2009, Moore et al., 2014)   |

- h. Leg LM was associated with muscle size but not total LM or arm LM in this study (Bites et al., 2013)
- i. In an MLR there was no association between MMSE and FFM but when comparing subjects with normal RASM and those within the lowest 20% of RASM this study found a significant difference (Liu et al., 2014)
- j. Digit span forwards unadjusted and adjusted was not associated with thigh muscle CSA, but adjusted backwards digit span was negatively associated with thigh muscle CSA in this study (Moore et al., 2014)

## 2.4.4 Limitations

In the review protocol the decision was made to write to study authors for relevant associations or data that were not given in the study but could be calculated using the recorded variables. This expanded the number of articles included in the review and the scope that they covered, however this may have led to some bias in which articles responded to the request and therefore what was reported, as study authors who found an association may have been more likely to reply. Of the 79 articles written to 59 replied therefore 25% did not respond. However the studies that did respond included both those which showed a



significant association and those that did not. The associations which were sent to us and the associations performed by us have not undergone peer review (eg for variable selection when adjusting the models), however I have included this information in our review and the statistical technique used to remain as transparent as possible.

The studies included in the review used a wide variety of techniques to record the variables of interest which means it is difficult to compare them (eg in a meta-analysis). For example, gait speed was recorded over multiple lengths, using automated and manual techniques and different levels of speed (ie maximum or usual pace). The large differences in how gait speed was measured combined with the fact that over longer distances it can become a test of cardiovascular fitness etc more than a test of muscle function, makes it difficult to compare the results of these studies directly. Another example would be the relationship between brain size and muscle size where we found three studies investigating this relationship. One study used brain mass (MRI) and lean mass (DEXA), one used brain volume (MRI) and neck muscle CSA (MRI) and one used brain volume (MRI) and lean mass (DEXA). Therefore none of the studies were directly comparable and hence meta-analysis could not be performed. These issues of variation in measurement technique persisted across the other relationships also; therefore meta-analysis was not possible. Hopefully more standardized testing will come about in the wake of resources like the NIH toolbox which may allow meta-analysis of studies in this area in the future (NIH, 2014).

When looking at the relationship between brain size and muscle size or function it is important to make sure that the size of the individual being studied is not acting as a confounding factor (ie that large people have large brains and large muscles). This meant that it was important to ensure that some measure of body size had been adjusted for in each association (eg ICV, height, BMI). In most of the studies I looked at this occurred but in some it did not and this may lead to a false relationship being reported.

A relatively wide range of ethnicities are represented in the study, however Caucasian subjects were by far the most commonly studied and there were no studies including those of Arabic or Indian ethnicity. Also most of the studies used subjects in their sixties, seventies or eighties, meaning the validity of our findings for other age groups, particularly children and adolescents is limited.

Finally, while a few of the studies included longitudinal data, it would be very useful to have more studies looking at the relationships over time as these may be able to highlight potential modifiable factors.

## 2.5 Conclusions

An increasing body of research has now linked brain function (cognition) and muscle function (eg gait speed) (Abellan van Kan et al., 2009, Atkinson et al., 2007, Scherder et al., 2007, Drago et al., 2011, Moran et al., 2013), however less well studied is the role of muscle and brain structure in this relationship. This systematic review looks at the evidence for whether: brain structure is related to muscle structure; brain structure is related to muscle function; and brain function is related to muscle structure in healthy humans across the lifecourse.

The review found evidence of a positive association between whole brain volume and total white matter volume with muscle size, and evidence that some areas of regional grey matter volume (right temporal pole and bilateral vmPFC) are negatively associated with muscle size (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011, Heymsfield et al., 2012, Kilgour et al., 2013, Weise et al., 2013).

The review found no evidence of a relationship between grip strength and whole brain volume, however there was some evidence of a positive association between grip strength and WM volume. Markers of brain ageing, that is brain atrophy and greater WMH accumulation, were associated with grip strength (Doi et al., 2012, Aribisala et al., 2013, Sachdev et al., 2005). Unlike grip strength, there is evidence that gait speed is positively associated with whole brain volume; this relationship may be driven by total WM volume or regional GM volumes, specifically the hippocampus (Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013). Like grip strength, gait speed is also associated with markers of brain ageing; WMH accumulation, brain atrophy and WM atrophy all show evidence of either a temporal association with gait speed or change in gait speed with time, with PVH and brainstem WMHs playing a particularly important role, but not subcortical WMH (Rosano et al., 2005, Soumare et al., 2009).

The evidence overwhelmingly points to no association between cognition and muscle size, except in the case of MMSE where it is mixed, but MMSE is more a screening tool for dementia than a true marker of cognitive function (Kilgour et al., 2013, Wetmore et al., 2011, Pedersen et al., 2012, Berryman et al., 2013, Bites et al., 2013, Auyeung et al., 2013, Magri et al., 2006, Liu et al., 2014).

I have identified some evidence which supports the common cause hypothesis, in so far as associations were identified between brain structure and muscle size, and physical function and some measures of brain structure. However most of the data is from cross-sectional studies therefore there may be contributing factors other than ageing *per se* which have caused these associations. Also, some relationships showed no evidence of a convincing association (eg cognition and muscle size) implying that ageing processes are affecting these tissues differently and the common cause hypothesis may not play a large role. This may be because structure and function do not decline in parallel and differing extrinsic and intrinsic factors may be more important for structure or function leading to these discordant results. Interestingly whilst there was no evidence of an association between grip strength and whole brain volume there was an association between grip strength and markers of brain ageing (eg brain atrophy). For any measure such as grip strength or whole brain volume, there are two factors which will affect their magnitude in older adults: their peak in young adulthood and their trajectory of decline with ageing. Therefore as most of the studies that were identified in the review were cross-sectional both of these factors will affect the relationships we looked at, whereas longitudinal studies will allow correction for baseline measures and a better assessment of whether the trajectories are in parallel or not. Large longitudinal studies should ultimately be able to prove or disprove the common cause hypothesis and these studies are therefore needed to explore these relationships over time, which will allow a better understanding of the potential causal relationships.

# Chapter 3 Methods for the Lothian Birth Cohort 1936 study

## 3.1 Introduction

The Lothian Birth Cohort 1936 study is an example of a longitudinal ageing cohort study. It has a particular focus on cognitive ageing but also includes data on a large number of potential covariates. By developing the technique described in chapter 4 to measure neck muscle cross-sectional area on the brain scans performed as part of LBC 1936, I was able to use data already collected as part of the study and investigate its relationship with this measure of muscle size. The LBC 1936 study was therefore an extremely important resource for my thesis. In this chapter the premise of the LBC 1936 study and its structure are described, along with the methods used to measure the main brain and muscle variables and important covariates used in this thesis.

## 3.2 Background and sample

On 4<sup>th</sup> June 1947 nearly all children attending school in Scotland sat a general mental ability test, called the Moray House Test number 12 (MHT), this was called the Scottish Mental Survey 1947 (Education, 1949). It was intended to repeat, and for its results to be compared with, the Scottish Mental Survey 1932, which again was a population wide measure of 11 year olds in Scotland using the same test but in 1932. There was concern that the mean intelligence level of the population might be decreasing, however they found the opposite, with children in the 1947 study scoring slightly better than children in the 1932 study. There were 70,805 children tested out of a possible 75,211 born in 1936 in the total population. This information is highly valuable as Scotland is the only country to have ever performed a population wide intelligence test.

In 2004 a cohort study was set up to follow up around 1000 of these participants who were currently resident in the Lothian area of Scotland (Deary et al., 2007b). The primary aim of the study was to test for genetic, medical, physiological, demographic and other determinants of individual differences in non-pathological cognitive ageing over the lifecourse, from age 11 to age 70. Potential participants were contacted and after consenting to take part underwent a series of cognitive, physical and biochemical tests, at the Wellcome Trust Clinical Research Facility (WTCRF) at the Western General Hospital, Edinburgh (Wave 1 2004-2007, n=1091), and returned for further testing at age 73 (Wave 2 2007-2010, n=866).

All participants were Caucasian, all born in 1936 and almost all lived independently in the Lothian region (Edinburgh city and surrounding area) of Scotland. The participants have recently completed wave 3 of testing (2011-2013, n=697) and wave 4 of testing is due to commence at the end of 2014.

The following list represents a summary of the variables recorded within waves 1 and 2 of the LBC 1936 study:

- Social and demographic data
- Physical and medical measures
- Blood and urine analysis
- Inflammatory and immunological markers
- Cognitive test scores
- Volumetric MR brain imaging, quantitative and qualitative measures of white matter hyperintensities and diffusion-tensor-MR derived variables of long-range tracts (eg fractional anisotropy)
- Doppler ultrasound of the carotid arteries
- Retinal and facial photography
- Personality tests
- Genetic analysis (including GWAS, telomere length and methylation analysis)
- Other (including questionnaire on social environment in childhood and adulthood, smoking and alcohol history, food frequency questionnaire)

### **3.3 Ethics**

Ethics permission for the LBC 1936 study protocol was obtained from the Multi-Centre Research Ethics Committee for Scotland (MREC/01/0/56) and from the Lothian Research Ethics Committee (LREC/2003/2/29) and covered the sub-studies in this thesis because the ethics approval included the use of the data for future research purposes. The research was carried out in compliance with the Helsinki Declaration. All participants gave written, informed consent.

## **3.4 Brain variables**

### **3.4.1 Cognitive testing**

#### **3.4.1.1 Moray House Test No. 12**

This was taken at about age 11 on June 4<sup>th</sup> 1947 and was re-administered when participants were seen again at about age 70 (Education, 1949). It contained the following items: following directions (14 items), same-opposites (11), word classification (10), analogies (8), practical items (6), reasoning (5), proverbs (4), arithmetic (4), spatial items (4), mixed sentences (3), cypher decoding (2), and other items (4). The maximum score was 76 and scores were used to construct age 11 and age 70 IQ.

#### **3.4.1.2 Mini-Mental State Examination**

The MMSE was originally designed as a bedside test to screen for cognitive impairment, but is now widely used as a screening tool for dementia (Folstein et al., 1975). It comprises items on attention and orientation (10 points), verbal registration (3 points), working memory (5 points), short-term verbal memory (3 points), naming (2 points), verbal repetition (1 point), verbal comprehension (3 points), writing (1 point), reading a sentence (1 point) and constructional praxis (1 point). A score of less than 24 out of 30 is used to indicate possible dementia, however it should be noted that greater age, pre-morbid IQ and number of years of formal education are all known to be associated with lower MMSE scores and therefore a score of 24 may well be considered normal for a 90 year old with little formal education, but abnormal for a 65 year old architect.

#### **3.4.1.3 National Adult Reading (NART) and the Wechsler Test of Adult Reading (WTAR)**

These tests are used to estimate prior cognitive ability. The participant pronounces 50 irregular words from a sheet; these words use the discordance between graphemes (the smallest semantically distinguishable unit in a written language) and phonemes (significant sounds) which occur within the English, and other, languages (eg the b in subtle is not pronounced) (Holdnack, 2001, Nelson and Willison, 1991). The NART test has been validated against true pre-morbid IQ using data from the 1932 Scottish Mental Survey, giving it a retrospective validity covering a 66 year interval (Crawford et al., 2001).

#### **3.4.1.4 Logical Memory I and II**

These two tests from the Wechsler Memory Scale-IIIUK (WMS-IIIUK) are designed to test immediate and delayed verbal declarative memory (Wechsler, 1998b). Participants are read

two short stories each containing 25 elements, and are asked to recall information about the stories immediately after they have listened to them and again later. These tests are two of 5 subtests used from the Wechsler Memory Scale within the LBC 1936 study. The Wechsler Memory Scale was first designed in 1945 and was revised in 1987 and 1997; the UK version was used for the LBC 1936 study. The Wechsler Memory Scale was designed to test five types of memory function: auditory memory, visual memory, visual working memory, immediate memory, and delayed memory, when all seven subtests are administered.

#### **3.4.1.5 Backward digit span**

The backward digit span tests working memory and is also from the WMS-IIIUK (Wechsler, 1998b). The participant needs to repeat backwards increasingly long strings of numerical digits.

#### **3.4.1.6 Verbal paired associates**

These are used to test verbal learning and memory and are also from the WMS-IIIUK (Wechsler, 1998b). The participant has to remember a list of pairs which include words having no obvious connection.

#### **3.4.1.7 Spatial span**

This is used to test non-verbal, spatial learning & memory and are also from the WMS-IIIUK (Wechsler, 1998b). The participant has to repeat a sequence of touching the top of a number of blocks in a pattern, first in the same order and then in reverse order.

#### **3.4.1.8 Digit symbol coding**

This is used to test speed of information processing and is from the Wechsler Adult Intelligence Scale-IIIUK (WAIS-IIIUK) (Wechsler, 1998a). The participant must fill in as many the associated symbols as possible in two minutes. The Wechsler Adult Intelligence Scale was originally published in 1955 and third revision was published in 1997. It uses 10 subtests to create index scores representing four major components of intelligence: verbal comprehension, perceptual reasoning, working memory and processing speed. The UK version was used in LBC 1936.

#### **3.4.1.9 Block design**

The block design tests constructional ability and is from the WAIS-IIIUK (Wechsler, 1998a). The participants are given two minutes to use blocks to copy specific designs.



#### **3.4.1.10 Symbol search**

This tests speed of information processing and is also from the WAIS-IIIUK (Wechsler, 1998a). The participant checks a row of symbols to see if it contains one of a pair of target symbols, completing as many as possible within an allocated time.

#### **3.4.1.11 Letter-number sequencing**

This tests working memory and is also from the WAIS-IIIUK (Wechsler, 1998a). The participant listens to increasingly long, strings of numbers and letters. They then repeat them, with the numbers first, in numerical order, and then the letters in alphabetical order.

#### **3.4.1.12 Matrix reasoning**

This tests non-verbal reasoning and is also from the WAIS-IIIUK (Wechsler, 1998a). The participant has to complete a pattern displayed as a matrix with one piece missing.

#### **3.4.1.13 Verbal fluency**

Verbal fluency is used to assess executive function (Lezak, 2004). The participant is asked to name as many words as possible in one minute beginning with a given letter (C, F and L were used in LBC 1936).

#### **3.4.1.14 Simple and four-choice reaction time**

These tasks were used to assess speed and variability of simple information processing (Deary et al., 2001). The tasks were administered using a box with an LCD screen and five response keys (1, 2, 0, 3, 4). In the simple task only the 0 key is used and the other four keys are used in the four-choice task.

#### **3.4.1.15 Inspection time**

This was used to test speed of elementary visual processing. The participant indicated, with no pressure on response time, which of two parallel, vertical lines of markedly different lengths was longer after increasingly short lengths of exposure (Deary et al., 2004a).

### **3.4.2 G cognition, G processing speed and G memory**

For a previous study three composite cognitive scores were derived using principal-components analyses (PCA) to represent three distinct cognitive domains (Corley et al., 2011). Regression scores were calculated for the first unrotated principal component of the tests in each domain. In each case, the scree slopes and eigenvalues suggested that a single component could be extracted.

### **3.4.2.1 G Cognition**

A cognition factor score, representing general cognitive ability, was derived from a PCA of scores on six tests; Letter–Number Sequencing, Matrix Reasoning, Block Design, Digit Symbol, Digit Span Backwards, and Symbol Search. The first unrotated principal component explained 53% of the variance, and all subtests had high loadings.

### **3.4.2.2 G Processing speed factor**

A processing speed factor was derived from a PCA of scores on a set of speed of processing measures, namely, Symbol Search, Digit Symbol, inspection time and simple and choice reaction time. The first unrotated principal component for the speed factor explained 51% of the variance, and all tests had high loadings.

### **3.4.2.3 Memory factor**

A memory factor was derived from a PCA of scores on a set of memory measures; Logical Memory I and II, Spatial Span, and Verbal Paired Associates I and II. The first unrotated principal component explained 43% of the variance, and all tests had high loadings.

## **3.4.3 Imaging Protocol**

The volumetric MR brain scans from LBC 1936 used in this thesis had already been performed, as a primary outcome for that study was brain volume measurement (Wardlaw et al., 2011). The MR imaging was performed with participants in the supine position on a 1.5 tesla MR imaging unit (Signa HDxt, GE Healthcare, Milwaukee, USA) at the Brain Research Imaging Centre ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)). A phased array eight channel head coil was used and inversion recovery prepared volumetric T1 weighted images were acquired on a coronal plane for each patient. For this set of images, the alignment was perpendicular to the long axis of the hippocampus determined from a preliminary T2 weighted sagittal sequence. The flip angle was 8°, bandwidth 15.63 KHz, echo time (TE) 4ms minimum to 13ms maximum, repetition time (TR) 9.6ms and inversion or preparation time (TI) 500ms. The field of view (FOV), fixed superiorly at the cranial vertex, was 25.6cm x 25.6 cm, slice thickness 1.3mm with no slice gap leading to 160 slices, displayed on a 192 x 192 matrix. These images took 8.13 minutes to acquire per patient.

## **3.4.4 Brain volume and WMHs**

The intracranial volume (ICV) was chosen to include the contents within the inner skull table. The inferior limit was the axial slice just superior to the tip of the odontoid peg at the foramen

magnum and superior to the inferior limits of the cerebellar tonsils. It was obtained semi-automatically using the T2\*W sequence. All brain volume images were corrected for ICV in final models. This is to ensure that the associations I found between brain volumes and muscle size and function were not just due to body size (eg people with bigger skulls might be expected to have larger neck muscle CSA).

The CSF, white matter lesions (WML) and normal-appearing white matter (NAWM) volumes were calculated from binary masks generated by an in-house developed software tool written in MATLAB that applies a technique named MCMxxxVI, which stands for Multispectral Colouring Modulation and Variance Identification and also represents the number “1936”, reflecting the Lothian Birth Cohort 1936 for which it was developed (Hernandez et al., 2010). The brain tissue volume was estimated by subtracting the volume of the ‘CSF+veins+meninges’ from the ICV.

Ventricular volume was obtained by thresholding the T1W images. The optimum threshold to delineate the boundaries of the ventricles was calculated as the mean intensity value from four sample regions: two inside the lateral ventricles and two in the superior temporal cortex.

In those subjects with a stroke lesion the total hyperintense WML volume was manually edited to exclude stroke by thresholding on the FLAIR volumes. I used only the “True WML” variable in our analyses which did not include stroke lesion volumes.

WMLs were also scored on the FLAIR and T2W images using the Fazekas scale, which codes separately for deep and periventricular lesions (Fazekas et al., 1987). The left and right hemispheres were scored separately and combined to give an overall value indicating the highest WML score.

## **3.5 Muscle variables**

### **3.5.1 Neck muscle CSA**

See chapter 4 for the development of the tool for measuring neck muscle CSA on volumetric MR brain scans.

### **3.5.2 Physical function measures**

#### **3.5.2.1 Grip strength**

Grip strength is a commonly used measure of physical function in large cohort studies. This is because: the equipment used to test it is easily transportable and inexpensive; it correlates well with more other more complicated to measure measures of muscle strength (eg lower extremity muscle power and isometric knee extension strength) (Lauretani et al., 2003); and it has been found to correlate with disability associated with activities of daily living (ADLs) (Al Snih et al., 2004, Rantanen et al., 1999).

Grip strength was measured using a North Coast Hydraulic Hand Dynamometer (JAMAR). Each measurement was taken three times and the highest reading was recorded for both hands.

#### **3.5.2.2 6 metre walk test (6MWT)**

Gait speed is another commonly recorded variable of muscle function within large cohort studies. Again this is because it is inexpensive and easy to record and is associated with a range of adverse health outcomes, including: severe mobility limitation, hospitalisation events and mortality (Cesari et al., 2009).

The 6MWT was measured by hand using a stopwatch. Subjects were asked to start from standing still and walk 6 metres down a corridor as quickly as possible. Subjects were allowed to use any usual walking aid if they wished to.

## **3.6 Covariates from LBC 1936 used in the thesis**

### **3.6.1 Age and sex**

Sex at birth was recorded at wave 1 of the study as Male =1 and Female =2. Age used for all analyses in the thesis pertaining to LBC 1936 was age in days at time of MR brain scan at wave 2.

### **3.6.2 Height and weight**

Height (in centimetres) was measured with a SECA stadiometer, after shoes had been removed, and weight (in kilograms) was measured using electronic SECA scales with a digital readout, without outer clothing or shoes.

### **3.6.3 Social factors**

Participants gave details of their social history at the initial interview at wave 1 of LBC 1936. A trained psychologist collected details of: number of years of full-time formal education completed; occupational history or for women their husband's occupational class if it was higher than their own (allowing categorisation within HMSO social class, categorised from I, professional, to V, unskilled); details of overcrowding at age 11 (ie the number of rooms in the participant's house at this time and the number of people living there, an index was created by dividing the number of people in the house by the number of rooms); and lastly they were asked if they had indoor or outdoor toilet facilities at age 11. Social deprivation is well established to be associated with negative health outcomes, and therefore considering the role it has to play in muscle and brain ageing is crucial (Smith et al., 1998, Pickett and Pearl, 2001).

### **3.6.4 Physical measures**

Sitting and standing systolic and diastolic blood pressure was recorded using an Omron 705IT monitor, three times each. The average of the sitting systolic blood pressure was used for the analyses in this thesis.

Brachial systolic pressure was measured in the right arm after 5 min of rest using a Doppler ultrasound and a random zero sphygmomanometer (to reduce the risk of digit preference) placed just above the elbow. Ankle systolic pressure was measured in the posterior tibial artery of the right leg using a Doppler ultrasound and a random zero sphygmomanometer with the cuff position just above the malleolus. The ABPI was calculated by dividing the systolic blood pressure in the ankle by that in the arm.

### **3.6.5 Comorbidity**

Disease history was recorded at wave 1 and 2 of the study and I used the data from wave 2 of the study for the analyses in this thesis. Participants were asked to answer yes or no to a series of questions, including: have you ever been treated for high blood pressure; have you ever been diagnosed as having diabetes; have you ever been told that you have high cholesterol in your blood; have you ever had a heart attack, angina, heart valve problem, abnormal heart rhythm or any other heart problem; and have you ever had a stroke or mini stroke? These answers were then coded as no=1 and yes=2 for history of hypertension, diabetes mellitus, hypercholesterolaemia, cardiovascular disease and stroke.

### **3.6.6 Blood markers**

Blood samples were obtained on the same day as the psychological and physical testing.

#### **3.6.6.1 Total cholesterol and total cholesterol:high density lipoprotein ratio**

Non-fasting blood was analyzed with an enzymatic Quinoneimine dye method using the Abbott Architect c16000 General Chemistry analyser (Abbott Laboratories, USA) at the Western General Hospital, Edinburgh. An increased ratio of total cholesterol to HDL cholesterol (chol:HDL) provides a better prediction of coronary heart disease risk than the individual measures, because the components are related to risk in opposite directions (Castelli et al., 1983, Castelli, 1984).

#### **3.6.6.2 Glycated haemoglobin (HbA1c)**

Glycated haemoglobin can be used both to diagnose diabetes and to monitor glucose control in diabetic patients (Farmer, 2012). They act as a marker of blood glucose over the previous 2-3months and are associated with both macro and microvascular disease in diabetes. HbA1c levels were measured using the Menarini HA8160 analyser, and results were reported in DCCT (Diabetes Control and Complications Trial) units which are expressed as a percentage with <6.5% considered normal.

#### **3.6.6.3 C reactive protein (CRP)**

CRP is an acute phase response protein synthesised in the liver, which has been found to be predictive of serious health outcomes, including cardiovascular disease and death (Ridker et al., 2003, Sigdel et al., 2014). CRP was measured using a dry slide immuno-rate method on the OrthoFusion 5.1 F.S. Analyser (Vitros Chemistry Products CRP slides, Ortho Clinical Diagnostics, Buckinghamshire, UK). As the CRP assay is designed for detecting raised levels

of CRP it cannot distinguish values less than 3 mg/L and all readings less than 3 mg/L (n = 467) were assigned a value of 1.5 mg/L.

#### **3.6.6.4 Estimated glomerular filtration rate (eGFR)**

eGFR was calculated using the subject's Creatinine, age and sex. Creatinine was measured using the Abbott Architect c16000 General Chemistry analyser (Abbott Laboratories, USA) at the Western General Hospital, Edinburgh. If the eGFR was greater than 60ml/min it was coded as 65.0. eGFR is known to be associated with cardiovascular and all-cause mortality (Matsushita et al., 2010).

#### **3.6.6.5 Cortisol**

Salivary cortisol was measured in a subset of the male participants (n=89). Saliva was collected using Salivette devices (Sarstedt, Numbrecht, Germany) at home on waking and at 10 PM (ie evening) to characterize the diurnal cortisol profile. Diurnal slope was calculated by subtracting the waking measure from the evening measure. Thus, a negative value denotes a decreasing slope. The enzyme-linked immunosorbent assays (ELISAs) were carried out by Dresden LabService GmbH (Dresden, Germany) in accordance with a Material Transfer Agreement.

#### **3.6.6.6 S100 beta**

The calcium-binding protein S100 beta is predominantly expressed by astrocytes in the human brain and circulating levels are used as a marker of blood brain barrier permeability and central nervous system injury or disease (Sen and Belli, 2007). A two-site chemiluminescence immunoassay was used to quantify S100 beta in human serum on a Liaison chemiluminescence analyser (Diasorin, Berkshire, UK). The normal range for S100B protein is 0.02 - 0.2 µg/L

#### **3.6.6.7 Interleukin-6 (IL-6)**

IL-6 levels were analysed at the University of Glasgow using high sensitivity ELISA from R&D Systems. The minimum detectable dose ranged from 0.016-0.110 pg/mL (mean=0.039 pg/mL). The intra-assay CV ranged from 6.9 to 7.8%, while the inter-assay coefficient of variance ranged from 6.6 to 9.6%.

#### **3.6.6.8 CMV serostatus and titres**

CMV was measured in plasma samples collected at age 70, using a CMV ELISA assay. Mock and viral-infected lysate was coated onto ELISA plates and incubated overnight.

Standards (a mixture of three CMV positive plasma samples) and plasma samples were added to the plates and incubated for one hour before washing. An anti-IgG horseradish peroxidase conjugated secondary antibody was then added to the plate to incubate for one hour. After washing, TMB substrate was added and the reaction stopped by addition of 1M HCL. The sample was assessed using an ELISA reader at 450nm. To determine CMV titres, mock values were first subtracted from lysate values. The data were then analysed in PRISM, and CMV titres were calculated with reference to the standard curve. Values above 10 were considered to be seropositive. To ensure accuracy, all samples were tested in duplicate.

### **3.6.6.9 Telomere length**

Telomere shortening is associated with age-related diseases and mortality (Blasco, 2005). Telomere length was measured as abundance of telomeric template versus a single gene (glyceraldehyde 3-phosphate dehydrogenase) by quantitative real-time polymerase chain reaction (PCR). Four internal control DNA samples were run within each plate to correct for plate to plate variation. These internal controls are cell lines of known absolute telomere length whose relative ratio values (telomere starting quantity/glyceraldehyde 3-phosphate dehydrogenase starting quantity) were used to generate a regression line by which values of relative telomere length for the actual samples were converted into absolute telomere lengths. Measurements were performed in quadruplicate. All PCRs were carried out on an Applied Biosystems (Pleasanton, CA, USA) 7900HT Fast Real Time PCR machine with 384-well plate capacity.

### **3.6.6.10 Apolipoprotein E4 genotype**

APOE4 carrier status is a well-established risk factor for Alzheimer's dementia, vascular dementia and mild cognitive impairment (Chuang et al., 2010, Kim et al., 2009). It has also been associated with telomere shortening (Starr et al., 2008). DNA was isolated from whole blood, and the APOE SNPs rs7412 and rs429358 were genotyped with TaqMan technology. These two SNPs form the APOE E2/E3/E4 haplotype. Participants were then classified as either no E4 allele=0 or E4 allele present=1.

### **3.6.7 Lifestyle factors**

As part of the initial interview at wave 2 alcohol and smoking history were recorded by the interviewer using a structured questionnaire. In the analyses I used current smoking status (never=0, ex=1, current=2) but decided not to use number of cigarettes smoked per day. This is because the percentage of participants who were current smokers in wave 2 was small



(8.4%) and it was felt this would lead to skewed results. For alcohol history I used three variables; whether the participant drank alcohol at all (no=0, yes=1); the number of days alcohol was consumed on per week and the number of units consumed per week.

Participants were asked to fill in questionnaires after their clinic visit and return these to the study team using a stamped addressed envelope provided. Participants were sent a reminder if the questionnaires had not been received within 6 weeks of their clinic visit. In one of the questionnaires, participants were asked to indicate how many days in an average month they took part (for more than 20 minutes at a time) in any vigorous sport or physical exercise, which was recorded as “number of days exercise per month”.

# **Chapter 4 Design and Validation of a Novel Method to Measure Cross-Sectional Area of Neck Muscles Included During Routine MR Brain Volume Imaging**

## **4.1 Introduction**

Low muscle mass secondary to disease and ageing is an important cause of excess mortality and morbidity (Roubenoff et al., 2003, Anker et al., 1997, Tisdale, 2002, Janssen et al., 2002). Studies investigating correlates of muscle loss or potential interventions to slow or reverse muscle loss require accurate measurements of muscle size. Current imaging techniques used to measure muscle size include whole body or regional DEXA scans and volumetric or cross-sectional area measurements on MR or CT scans of the arm or leg (Cruz-Jentoft et al., 2010). Arm and thigh cross-sectional area (CSA) have been used in previous studies as they are large and are viewed to be used in everyday tasks. However thigh muscle CSA has been shown to correlate well with total muscle mass and it may be that other muscle groups around the body are equally useful as a guide of general muscle bulk (Ohkawa et al., 2000, Lerner et al., 1986). Whilst the above techniques remain the current gold standard, they are not commonly employed in clinical practice or in studies out with those directly investigating muscle mass (eg studies of sarcopenia or cachexia). Volumetric MR brain scans are commonly used in both research and clinical practice. These scans often include much of the posterior neck muscles. A technique to measure posterior neck muscle CSA on volumetric MR brain scans would therefore enhance the value of volumetric MR brain scans: both brain and muscle size could be measured without additional scanning.

Recent studies have shown a correlation between grip strength and cognition, which has implications for studying rates of ageing (Boyle et al., 2009, Deary et al., 2011). However, few studies have investigated the relationship between muscle size and brain size. This is likely due in part to the fact that two different scans would be required in each subject to obtain these data. Both brain and muscle size are known to decrease with age, therefore studying the pattern of their inter-relationship would allow investigation of their shared risk factors which, in turn, may suggest underlying mechanisms. Many longitudinal ageing studies include a volumetric MR brain scan (Blumenthal et al., 1985, Deary et al., 2007b, Ellis et al., 2009). If it were possible to measure muscle CSA reliably from volumetric MR

brain scans and this measure was representative of general body muscle bulk, the relationship between muscle and brain size could be investigated using a single scan.

MR measurement of neck muscle cross sectional area (CSA) has been shown to be feasible in young healthy adults using scans dedicated to this purpose (ie MR imaging of the neck), but older adults have not been studied (Elliott et al., 2007, Ulbrich et al., 2011, Cagnie et al., 2010). These studies have demonstrated good inter-rater reliability (Ulbrich et al., 2011, Cagnie et al., 2010). Moreover, I found no previous studies documenting a technique to measure neck muscle size on MR brain scans. The limited data that are available suggest that neck muscle CSA and strength are correlated, indicating that neck muscle CSA has good construct validity (Tsuyama et al., 2001). I aimed to establish a novel method for measuring neck muscle CSAs from routine MR brain volume acquisitions. This chapter details the technique I developed and further studies to test its reliability, validity and repeatability.

## **4.2 Methods**

### **4.2.1 Study 1: Feasibility study**

#### **4.2.1.1 Goal**

To investigate whether it is feasible to measure neck muscle CSA on MRI volumetric brain scans.

#### **4.2.1.2 Ethics & Sample**

The volumetric MR brain scans used in this study had already been performed as part of the Lothian Birth Cohort 1936 (LBC) study (see chapter 3: Methods for cohort studies), as a primary outcome for that study was brain volume measurement. Twenty consecutive scans from a final total of 735 were selected between 02/02/09 and 30/03/09. Ethics gained for the LBC 1936 study covers this sub-study because the ethics approval included the use of the data for future research purposes.

#### **4.2.1.3 Imaging Protocol**

See chapter 3: Methods for the Lothian Birth Cohort 1936 study

#### **4.2.1.4 Development of neck muscle cross sectional area measurement technique**

The image data were transferred to a Kodak Carestream picture archiving and communication systems (PACS) workstation where 3-D multiplanar reconstructions were performed.

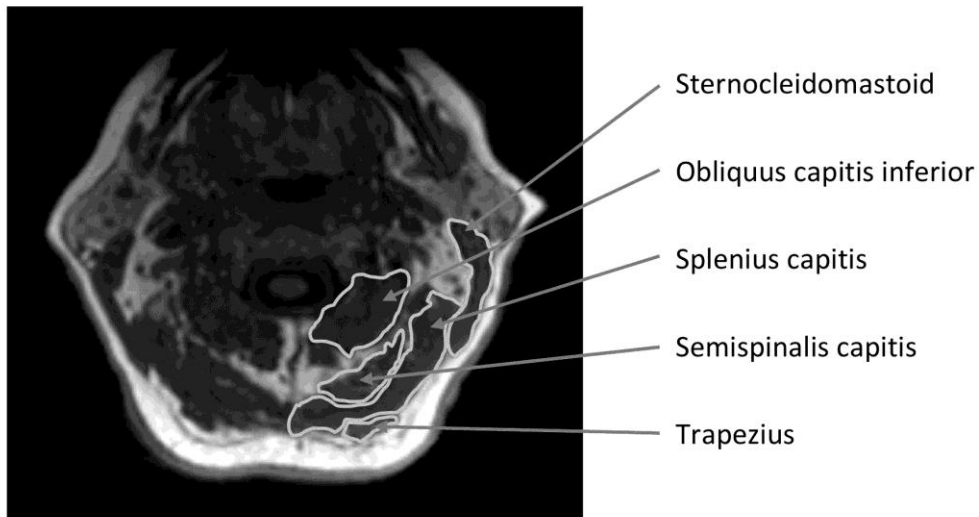
Freehand cursor was used to draw a region of interest (ROI) around the neck muscle of interest in the axial plane to obtain the cross sectional area on each side separately.

Two raters tested feasibility in ten of the participants. I sought to develop a technique that ensured raters found the same level from which to make their CSA measurements. Our first attempt involved finding the MR slice in which the CSA of the obliquus capitis inferior was at its maximum in the axial plane. We chose the obliquus capitis inferior because it is a short muscle and its width varies more along its length than the other neck muscles in the scan. We then measured the CSAs for the largest muscles in that slice of the scan; sternocleidomastoid (SCM), obliquus capitis inferior, semispinalis capitis, splenius capitis and trapezius for both right and left sides (Figure 4.1). Although it was possible to measure the CSA of neck muscles using this technique, there were occasional large discrepancies between raters indicating that this method lacked reliability, particularly with regard to finding the level to take the measurements.

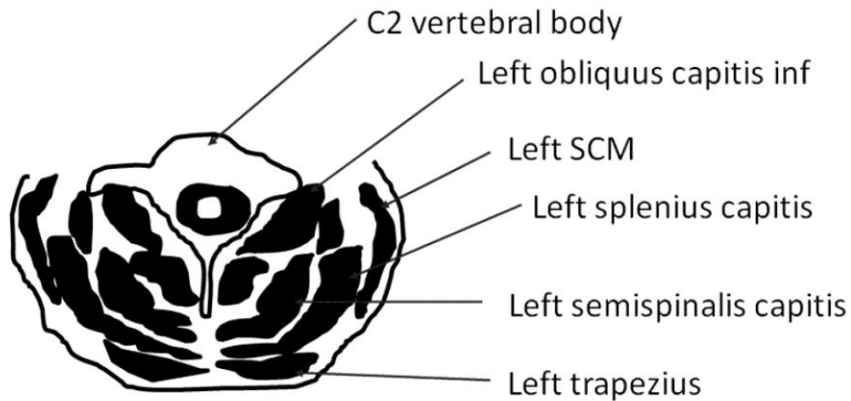
**Figure 4-1: Figure of the posterior neck muscles and diagram demonstrating how the measurement plane was selected.**

- A.** Non-contrast T1-weighted MR of transverse plane of the neck at mid-infero-superior-C2 level.
- B.** Outline diagram showing the neck muscles whose cross-sectional areas were measured.
- C.** Outline diagram demonstrating how measurement plane is selected with an example C2 height of 42mm.

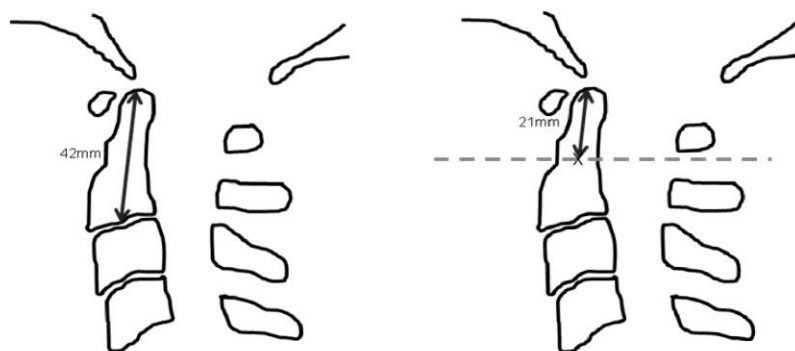
**A**



**B**



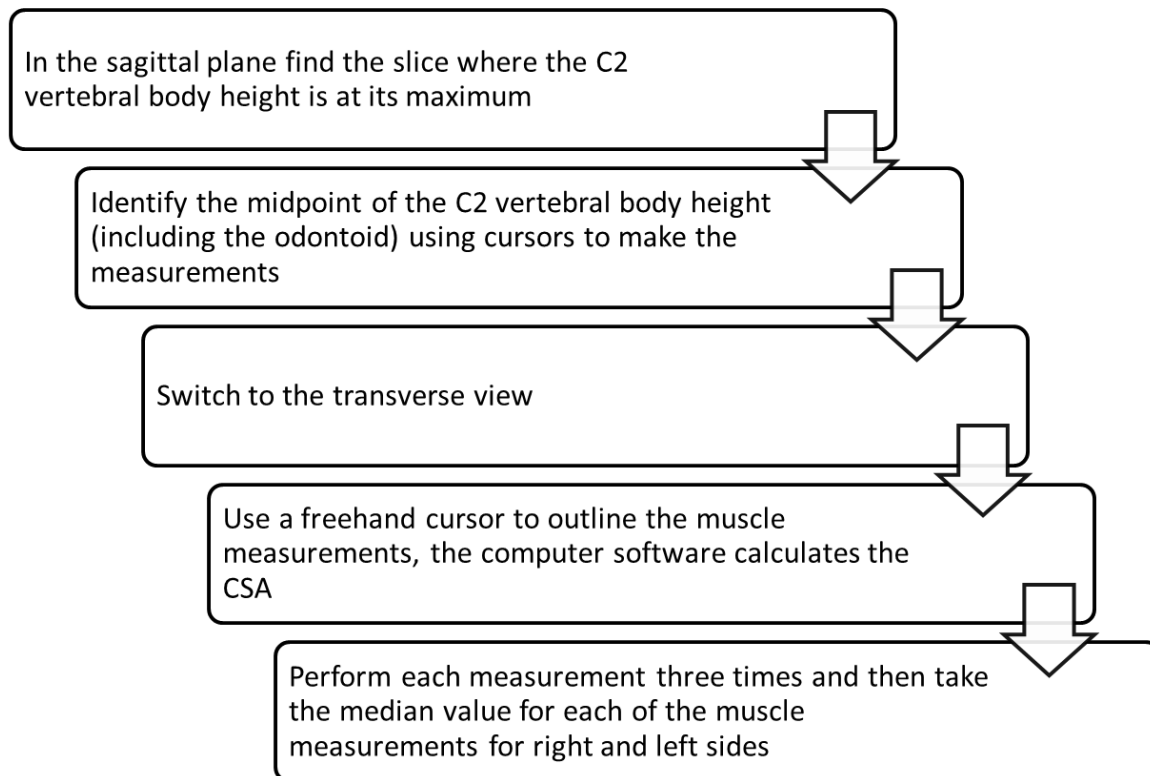
**C**



Therefore, in the second attempt, we measured the neck muscles' CSAs of a further ten participants, but this time we started with the images in the sagittal view with the volume images loaded into the multiplanar reformat view. We chose the slice where the C2 vertebral body height was at its maximum. We then identified the midpoint of the C2 vertebral body height including the odontoid by measuring along its vertical length from the odontoid tip to lower end plate using the cursor and then marked the midpoint. We then switched to the axial view of the multiplanar reformat at the vertical midpoint of C2 and measured the CSAs of the neck muscles in that axial image.

We initially attempted to standardise the plane of the axial image on a line parallel to C2 end plates, but the variability of tilt in endplates meant that occasionally that line could go as high as suboccipital level posteriorly and thereby miss the muscles of interest. Setting the axial slice perpendicular to the vertical line of measurement through C2 did not work either as it proved difficult to manipulate the axial slice by small angle changes precisely enough. Therefore, we used the midpoint of C2 in the sagittal plane while viewing the images in the multiplanar reformat and then clicked on the corresponding axial image. This resulted in the axial slice being parallel to the lower border of the volume scan, but not related to any particular line in the participant. This time we measured the three posterior neck muscles (trapezius, splenius capitis and semispinalis capitis) individually and in combination. See Figure 4.2 for the chosen method.

*Figure 4-2: Flowchart summarizing method to measure craniad muscles cross-sectional areas.*



## **4.2.2 Study 2: Study to measure inter-rater reliability**

### **4.2.3 Goal**

To investigate and quantify whether two raters using this technique would produce the same measurements.

#### **4.2.3.1 Sample**

A further 20 scans from the LBC 1936 study were studied in addition to the 20 from the feasibility study, to give us a total of 40 scans to measure with the newly-developed technique.

#### **4.2.3.2 Neck muscle cross sectional area measurements**

We performed the measurements with the chosen technique, as described in the feasibility study, on all 40 MRI scans (Figure 4.2). Each scan was measured three times by both raters and the median value for C2 body height and each of the three muscle measurements (SCM, obliquus capitis inferior and combined trapezius, splenius capitis and semispinalis capitis) were recorded separately for right and left sides for analysis. We noted that, unlike thigh muscles, there was only minimal intramuscular fat in the images of these neck muscles, so we

did not seek to adjust for this or the small area of interfascial fat between trapezius, splenius capitis and semispinalis capitis in the combined group measure.

#### **4.2.3.3 Analysis**

To compare inter-rater reliability, I calculated the percentage difference in CSA as measured by the two raters and the two-way random effects absolute agreement intraclass correlation coefficients. Multiple linear regression analysis was used to estimate effects of sex and body mass index (BMI) on neck muscle CSA. Principal components analysis was used to extract a general trait for neck muscle CSA from the three individual muscle CSA measures: I accepted components with eigenvalues greater than unity and inspected the scree plot to identify the number of components. All analyses were performed using the SPSS 16.0 statistical package.

### **4.2.4 Study 3: Study to measure repeatability of technique**

#### **4.2.4.1 Goal**

To assess whether the technique would provide the same results on scans measured:

- on the same subject and the same MRI scanner on different days
- on the same subject and different MRI scanners on different days

#### **4.2.4.2 Ethics**

All subjects provided written consent and ethics approval was gained by the local ethics research committee for the original CaliBrain study (REC 05/S0801/105) (Gountouna et al., 2010, Moorhead et al., 2009). This included further use of the data for research purposes and therefore further ethics permission for this study was not required.

#### **4.2.4.3 Sample**

The CaliBrain study investigated the reliability of repeat volumetric brain MR measures with the same scanner and between different scanners (Gountouna et al., 2010). We therefore used these data to test the reliability of repeat neck muscle CSA measurement from volumetric brain MR scans. The participants of the CaliBrain study were 14 normal volunteers from the three participating centres aged between 25 and 51 years, see below for details of the centres. As the data had been collected as part of the CaliBrain study no power calculations were carried out and we analysed all the available data. Exclusion criteria for the CaliBrain study were: previous history of a diagnosed neurological disorder or a major psychiatric disorder, treatment with psychotropic medication, including treatment for substance misuse and not meeting the MR safety criteria.



#### **4.2.4.4 Imaging protocol**

Each of the fourteen participants twice underwent a structural and functional MR brain scan at three imaging research centres around Scotland; The Department of Radiology, University of Aberdeen; The Brain Research Imaging Centre, Western General Hospital, University of Edinburgh; and The Neuroradiology Department, Southern General Hospital, NHS Greater Glasgow South University Hospitals Division. Therefore each participant underwent 6 separate scans. Each scan took place on a separate day and there were nominally 2 weeks between the scans at the same site. We only used the structural data for our study. The three scanners used were all manufactured by General Electric (GE Healthcare, Milwaukee, Wisconsin) and had primary field strengths of 1.5T however the machines had differing software and hardware. Images were taken in the coronal plane at a slice thickness of 1.7mm with no slice gap. 3D reconstruction was used to make the measurements with our technique. Further details of the imaging protocol can be found in the paper by Moorhead et al (Moorhead et al., 2009).

#### **4.2.4.5 Cross-sectional area measurement technique**

The measurements were performed using Analyze, the biomedical image analysis software (Mayo Foundation, Rochester, Minnesota, USA).

The CaliBrain images were aligned with the ACPC line, an anatomical line which runs between the superior surface of the anterior commissure and the centre of the posterior commissure. The feasibility and reliability studies had used images which were perpendicular to the MR table. Therefore the images underwent pre-processing prior to the measurements being made. This involved the images being tilted 15 degrees forward on the axial axis. This was actually preferable to the original study where the angle of the brain as viewed on PACS was not standardised, but just depended on how the patient placed their head in the scanner, as all the images in the CaliBrain study were standardised to an anatomical landmark, the ACPC line. Neck muscle CSA was measured as described in the feasibility study.

#### **4.2.4.6 Analysis**

ICCs were calculated for comparing within scanner and between scanner variations. When calculating the ICC for within scanner variation the measurements taken from the first and second scans were compared for each of the three individual measurements (ie combined group, SCM and obliquus) and the total neck muscle CSA (ie all three measurements for right and left sides added together). The ICC for between scanner variation were calculated using

the mean total muscle CSA for each measurement on that site (eg (Total neck muscle CSA Edinburgh scan 1 + Total neck muscle CSA Edinburgh scan 2)/2).

All data were analysed using the SPSS 17.0 statistics package. Three nonsynchronous sets of measurements were taken for each scan and the median values were used for the analysis.

## **4.2.5 Study 4: External validity study**

### **4.2.5.1 Goal**

To assess and quantify whether neck CSA is related to mid-thigh CSA, which has been previously shown to be related to general muscle mass (Dai et al., 2001, Ohkawa et al., 2000).

### **4.2.5.2 Ethics**

Ethics permission for the MR brain scans undertaken as part of the Lothian Birth Cohort (LBC) 1936 project had already been obtained as per study 1. A substantial amendment was submitted to allow us to recruit 25 subjects from the LBC 1936 pool and perform a MR scan of their mid-thigh. This was approved in April 2010.

### **4.2.5.3 Sample**

735 LBC 1936 participants had brain MR data available. Power calculations indicated that for a minimum Pearson correlation coefficient of 0.6 at  $\alpha=0.05$ ,  $n=20$  provided 80% power and  $n=26$  provided 90% power. I therefore chose to scan 25 subjects. I contacted the subjects who had most recently had their MR brain scan, within a few weeks, to ensure that the effect of any variable on the thigh muscle bulk in the time lapsing between the MR brain and thigh scans was as small as possible. Participants were excluded if they had severe osteoarthritis affecting the knee or hip or a previous stroke, previous total hip replacement, or a history of any degenerative neurological disorder

### **4.2.5.4 Imaging protocol**

The MR brain scans had been collected as part of the LBC 1936 study. See study 1 (above) for details of the protocol.

I used anatomical landmarks to identify the midpoint of the femur. I palpated for the protuberance of the greater trochanter and the upper border of the patella and then measured down the lateral aspect of the thigh using these landmarks and marked the midpoint. A cod

liver oil capsule was then taped there to allow us to identify the corresponding MR slice. This was performed separately for each leg.

The scan was performed using a 3.0 tesla Siemens Verio research MR scanner (Siemens Medical, Germany) at the Clinical Research Imaging Centre within the Queen's Medical Research Institute. Images were acquired using a combination of body and spine matrix coil elements. The subjects lay supine for the scan. A coronal scouting scan was performed and then 5-10 axial images were taken with the cod liver oil capsules in the middle slices. Slice thickness was 3mm with no slice gap.

#### **4.2.5.5 Cross-sectional Area Measurements**

Measurements for sternocleidomastoid, obliquus and the combined group (trapezius, splenius and semispinalis) were made using the above described technique on a PACS workstation.

The thigh muscle CSA measurements were also performed on a PACS workstation using the slice on each side where the cod liver oil capsule was at its widest which should indicate the anatomically chosen midpoint. Three measurements were taken on each leg: the anterior group, the medial group and the posterior group. The anterior group consisted of the quadriceps (vastus lateralis, intermedius and medialis and rectus femoris) and sartorius, the medial group of gracilis and the adductors (longus, brevis and magnus) and the posterior group of the hamstrings (biceps femoris, semitendinosus and semimembranosus).

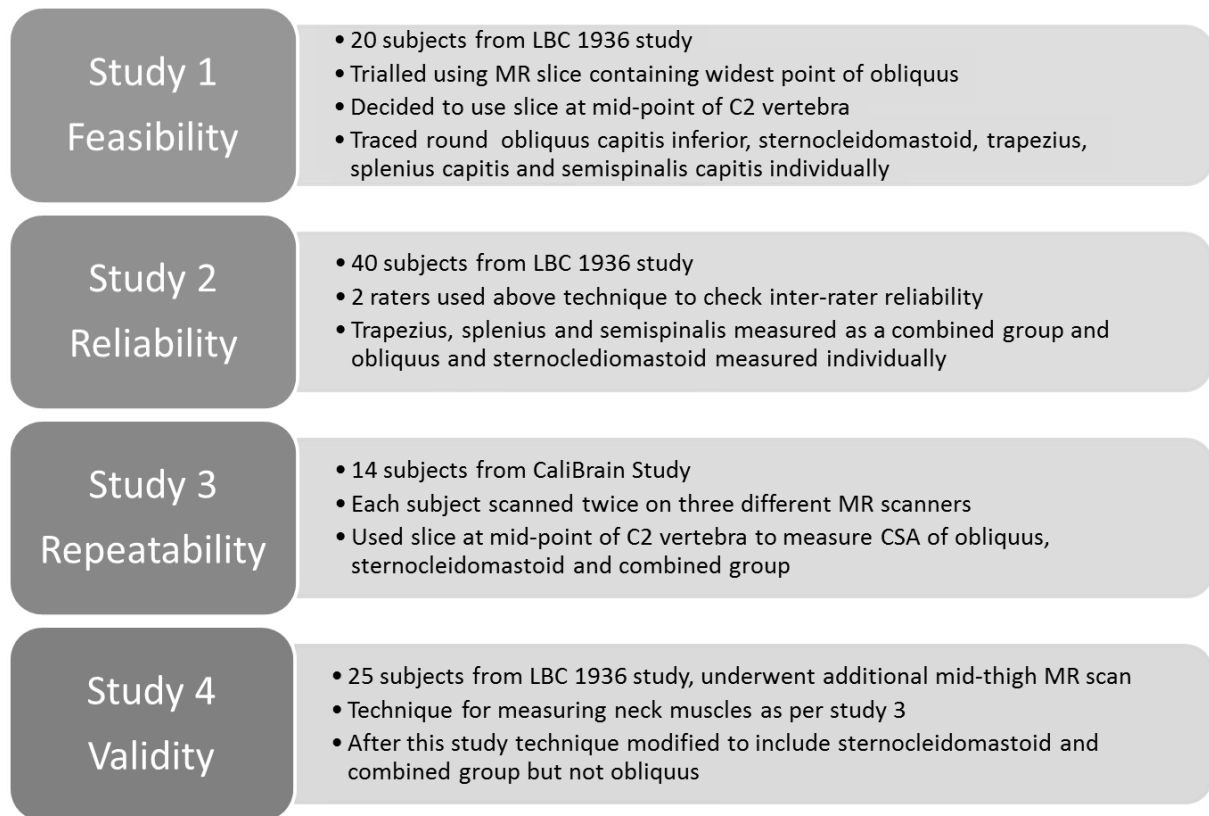
Both the thigh and neck measurements were repeated 3 times for the left and right sides.

#### **4.2.5.6 Analysis**

All data were analysed using the SPSS 17.0 statistics package. Three nonsynchronous sets of measurements were taken for each subject and the median values were used for the analysis.

Please see figure 4.3 for a summary of the methods for the above four studies.

*Figure 4-3: Diagram summarizing the methods for the four studies.*



## 4.3 Results

### 4.3.1 Study 1: Feasibility study

The measurements made with the chosen technique were used to calculate intra-class correlation coefficients (ICC) to compare the median value of 3 measurements made by rater A against rater B. In this study we measured each of the three posterior muscles (trapezius, splenius and semispinalis) separately and together in a single measurement as a combined group. The boundaries between these three muscles are not clear and we thought that the differences in CSA measurements were a reflection of where the boundary was taken to be rather than true measurement differences in the size of the muscles themselves. The ICC and associated 95% confidence intervals support this view, as the values for the ICC for the three respective individual muscles were 0.78 (CI 0.16-0.94), 0.86 (CI 0.48-0.97) and 0.90 (CI 0.60-0.97) and the combined group ICC was much stronger at 0.99 (CI 0.95-1.00). Therefore we decided to use the combined measurement from thereon with individual measurements of the stand alone muscle, obliquus and sternocleidomastoid.

*Table 4-1: Intra-class correlation coefficients for the second technique trialled in the feasibility study*

| Measurement                                     | ICC   | 95% CI      |
|---|-------|-------------|
| Trapezius                                       | 0.778 | 0.160-0.944 |
| Splenius  | 0.861 | 0.475-0.965 |
| Semispinalis                                    | 0.895 | 0.603-0.974 |
| Summation of trapezius, splenius & semispinalis | 0.978 | 0.916-0.994 |
| Single measurement of combined group            | 0.986 | 0.946-0.996 |
| Obliquus  | 0.900 | 0.623-0.975 |
| SCM   | 0.894 | 0.598-0.973 |

### 4.3.2 Study 2: Study to measure inter-rater reliability

Of the 40 scans from the LBC 1936 cohort, one proved to be a duplicate and was excluded. One rater considered one scan to be unmeasurable whilst the other considered two scans unmeasurable. Scans were thus measured for 37 (18 male, 19 female) participants of mean age 72.0 (standard deviation 0.38) years when weighed and mean age 72.2 (sd 0.25) years when scanned. Men had a mean height of 1.73m (sd 0.07), mean weight of 85.0kg (sd 11.2)

and mean BMI of 28.2 kg/m<sup>2</sup> (sd 3.2). Women had a mean height of 1.59m (sd 0.04), mean weight of 71.0kg (sd 14.0) and mean BMI of 27.9 kg/m<sup>2</sup> (sd 5.2).

Rater A measured mean C2 height as 3.7cm (sd 0.5) in men and 3.6cm (sd 0.2) in women. Rater B measured mean C2 height as 4.0cm (sd 0.4) in men and 3.7cm (sd 0.2) in women. These differences had no effect on slice chosen as midpoint of C2 for muscle CSA measurement (Figure 4.4).

*Figure 4-4: Plot of MR slice chosen as representing the mid-point of C2 for both raters*

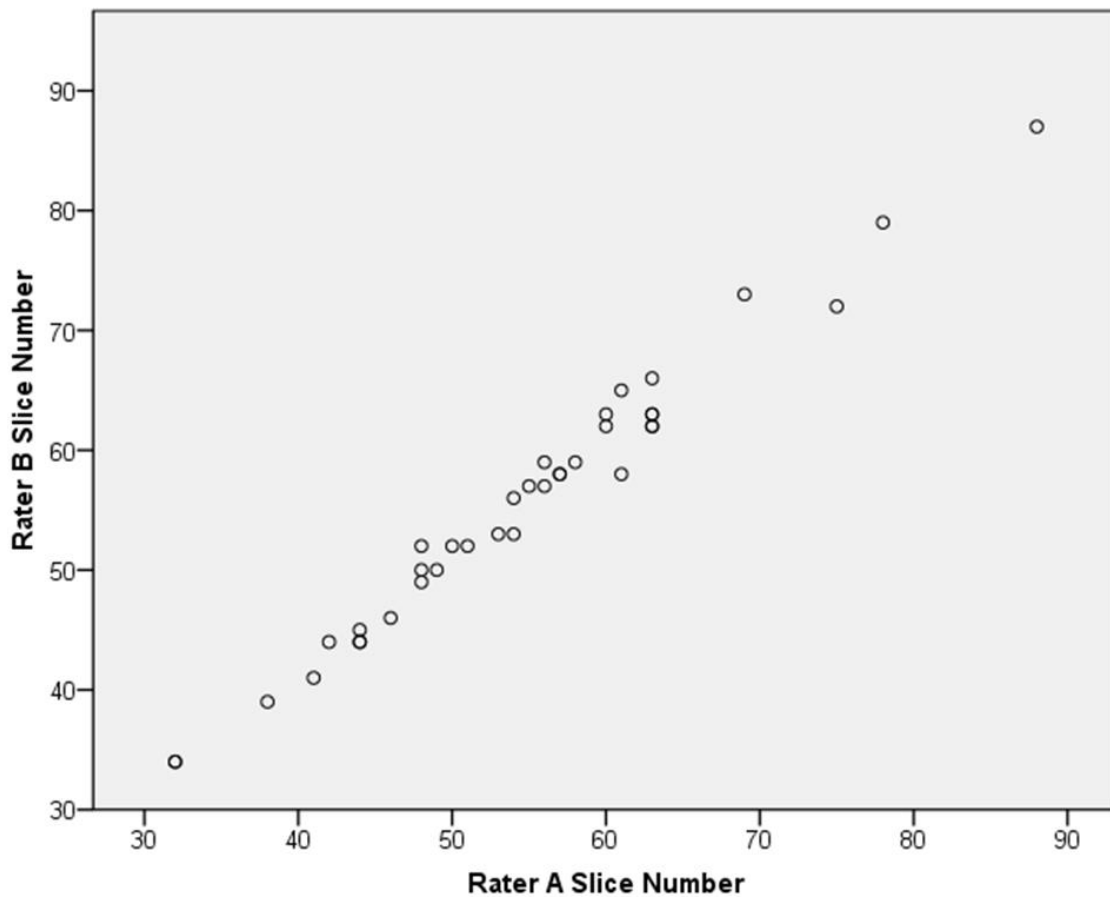


Table 4.2 shows the mean CSAs per rater, absolute mean difference and mean difference as percentage of CSA.

*Table 4-2: Mean cross-sectional areas (CSAs) as measured by each rater summed for left and right, together with absolute mean difference and mean difference as percentage of CSA between raters*

|  | <b>Combined Group</b> | <b>Obliquus Capitis Inferior</b> | <b>Sternocleidomastoid</b> |
|--|-----------------------|----------------------------------|----------------------------|
| <b>Mean CSA rater A (mm<sup>2</sup>)</b>                     | 1850                  | 773                              | 422                        |
| <b>Mean CSA rater B (mm<sup>2</sup>)</b>                     | 1847                  | 753                              | 376                        |
| <b>Mean inter-rater difference (95% CI) (mm<sup>2</sup>)</b> | 3 (-30, 36)           | 20 (-33, 73)                     | 46 (-29, 63)               |
| <b>Mean difference as percentage of mean CSA (95% CI)</b>    | 0.3 (-1.5, 2.0)       | 4.1 (-6.3, 14.4)                 | 11.3 (7.1, 15.5)           |

Intraclass correlation coefficients between raters were 0.99 (95% CI 0.98-1.00) for the combined group CSA, 0.92 (95% CI 0.85-0.96) for obliquus CSA and 0.92 (95% CI 0.85-0.96) for sternocleidomastoid CSA (Table 4.3). Obliquus CSA was predicted by sex (beta=-0.54 for women, p<.001) and BMI (beta=0.36, p=.01) adjusted R<sup>2</sup> for model =0.40, sternocleidomastoid CSA by sex (beta=-0.60 for women, p<.001) and BMI (beta=0.41, p=.001) adjusted r<sup>2</sup> for model =0.52, and combined CSA by sex (beta=-0.74 for women, p<.001 only) adjusted r<sup>2</sup> for model =0.55.

*Table 4-3: Intra-class correlation coefficients with 95% confidence intervals for the reliability study*

| <b>Muscle Measurement</b>        | <b>ICC</b> | <b>95% Confidence Intervals</b> |
|----------------------------------|------------|---------------------------------|
| <b>Combined group</b>            | 0.99       | 0.98-0.995                      |
| <b>Obliquus capitis inferior</b> | 0.92       | 0.85-0.96                       |
| <b>SCM</b>                       | 0.92       | 0.85-0.96                       |

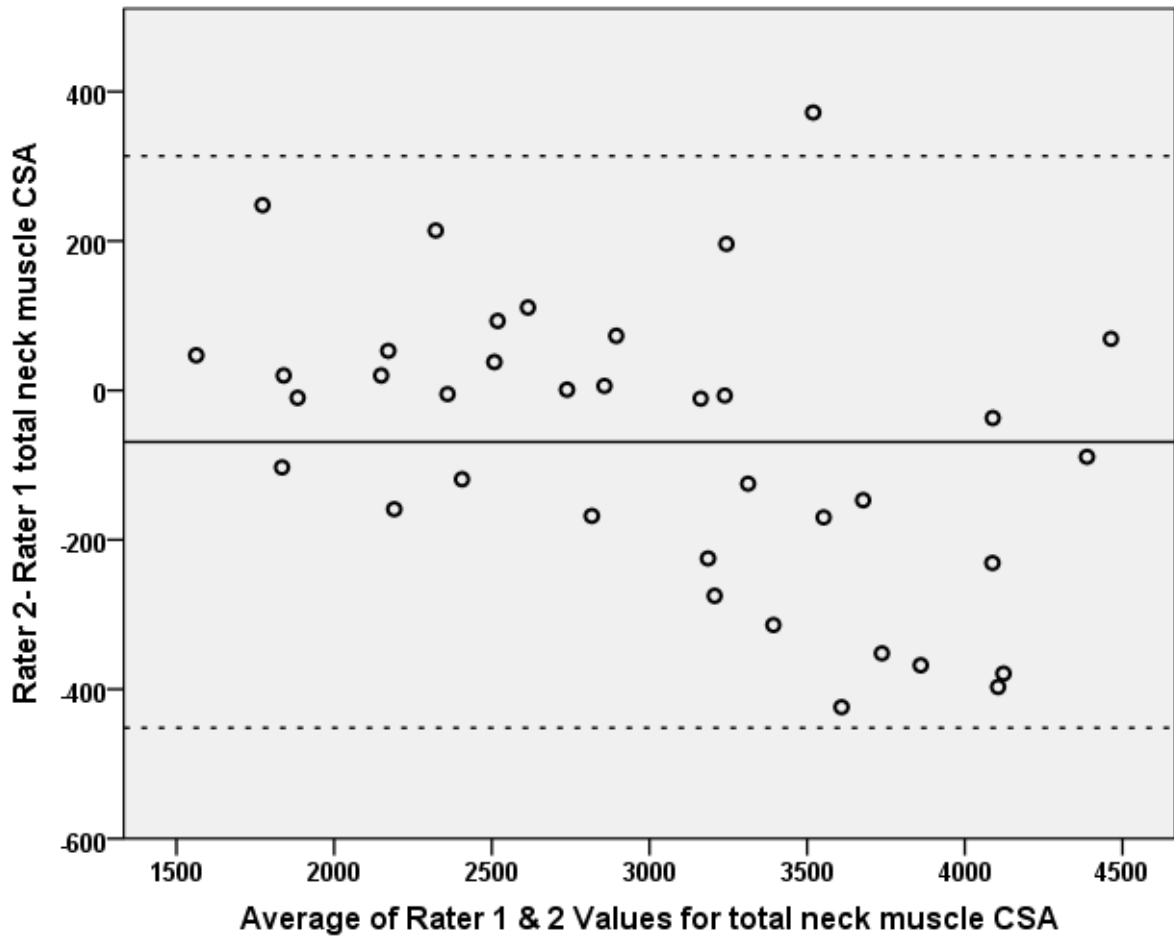
There were no significant associations between inter-rater CSA difference and mean CSA for the combined group (r=0.08, p=.66), but larger inter-rater differences were significantly associated with smaller CSAs for both obliquus (r=-0.61, p<.001) and sternocleidomastoid (r=-0.39, p=.018). CSAs all correlated highly significantly with each other (p<.001): combined-obliquus (r=0.59), combined-sternocleidomastoid (r=0.66), obliquus-sternocleidomastoid (r=0.50).

A Bland-Altman plot for total neck muscle CSA demonstrates a degree of linear bias with Rater 2 reporting bigger measurements for the small neck muscle CSAs and smaller measurements for the bigger neck muscle CSAs (Figure 4.5A). If obliquus is removed,

leaving the combined group plus SCM, this linear bias appears to resolve (Figure 4.5B). However the bias of measurement is small for both graphs.

*Figure 4-5: Bland-Altman plots for total neck muscle CSA and SCM + combined CSA measured by 2 raters. Bias of measurement between 2 different raters (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively*

A. *Bland-Altman plot for measurements of total neck muscle CSA by 2 different raters*





**B. Bland-Altman plot for measurements of SCM + combined CSA by 2 different raters**

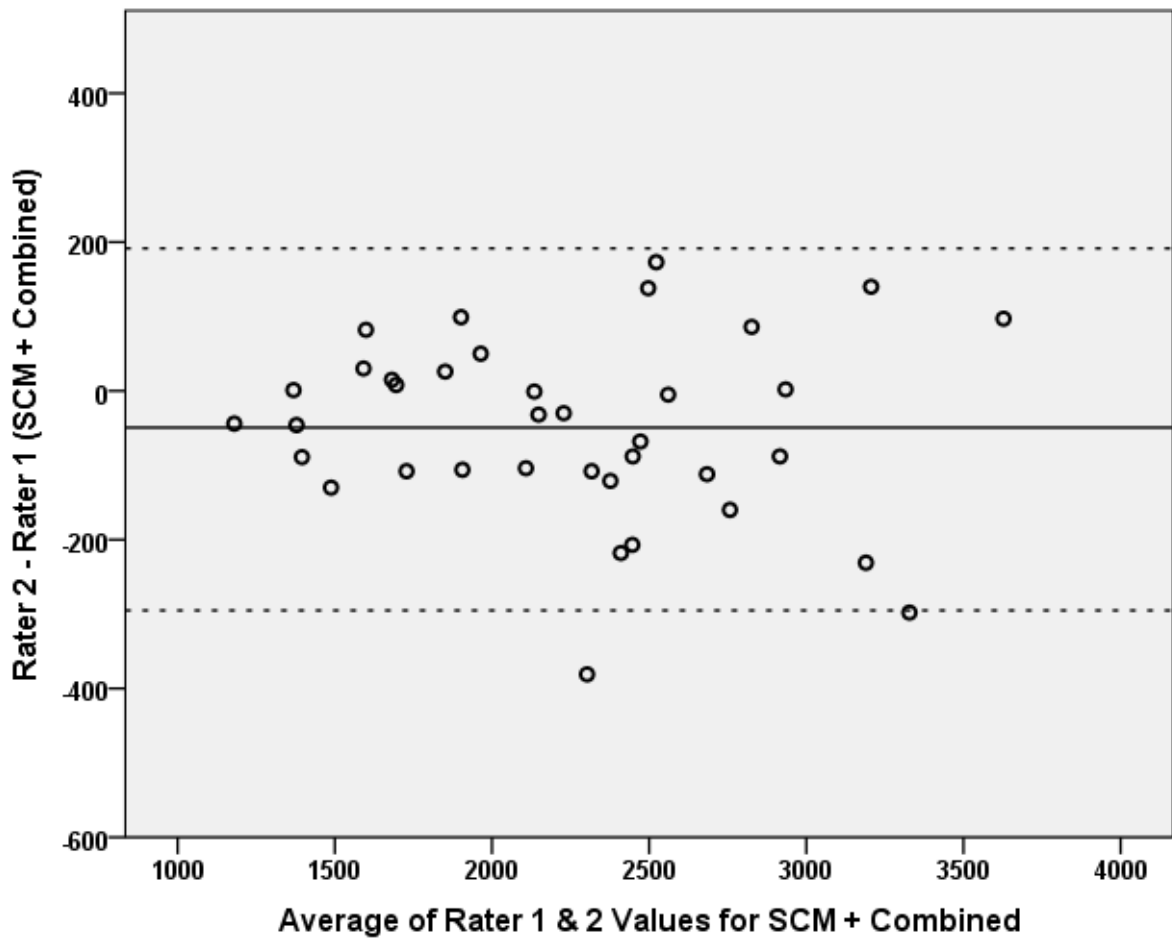


Table 4.4 shows coefficients of variation (CV) for both raters and a Levene's test for homogeneity which found no significant difference between the CV for the two raters for any of the muscle measurements.

*Table 4-4: Coefficients of variation (%) with 95% CI and Levene's significance test between the two raters*

| Measurement                  | Rater | Coefficients of Variation (CV) (%) | 95% CI for CV | Levene's test (Significance) |
|------------------------------|-------|------------------------------------|---------------|------------------------------|
| <b>Combined group</b>        | 1     | 28.1                               | 22.9-36.5     | 0.96                         |
|                              | 2     | 28.5                               | 23.2-37.0     |                              |
| <b>Obliquus</b>              | 1     | 43.8                               | 35.6-56.9     | 0.10                         |
|                              | 2     | 32.3                               | 26.3-42.0     |                              |
| <b>SCM</b>                   | 1     | 31.0                               | 25.2-40.3     | 0.82                         |
|                              | 2     | 29.8                               | 24.2-38.7     |                              |
| <b>Total neck muscle CSA</b> | 1     | 28.2                               | 22.9-36.6     | 0.47                         |
|                              | 2     | 25.9                               | 21.1-33.6     |                              |

The first unrotated principal component explained 72.2% of total CSA variance for the three muscles (loadings were 0.89 for the combined group, 0.81 for obliquus and 0.85 for sternocleidomastoid) and correlated positively with grip strength of both right ( $r=0.52$ ,  $p=.001$ ) and left ( $r=0.50$ ,  $p=.002$ ) hands. The second principal component had an eigenvalue of 0.51.

### **4.3.3 Study 3: Study to measure repeatability of technique**

Data were analysed for all 14 participants. Thirteen of the participants had undergone all 6 scans and one had undergone 5 of the scans having not completed their second scan in one location. There were 10 men and 4 women. The mean age was 36.3 years (range 25-51).

Mean values (sd) for the measurements across all six scanners for right and left sides added together were: SCM  $4.97\text{cm}^2$  (1.11), combined group  $20.12\text{cm}^2$  (5.74), obliquus  $9.88\text{cm}^2$  (3.23) and total neck muscle CSA  $34.97\text{cm}^2$  (8.67). ICCs were calculated for within scanner and between scanner variability (Tables 4.5 & 4.6). Within scanner ICCs for the Edinburgh scanner used for studies 1, 2 and 4 ranged from 0.83 for SCM to 0.96 for the combined group.

*Table 4-5: Between scanner intra-class correlation coefficients for the repeatability study*

| <b>Groups</b>                   | <b>ICC</b> | <b>95% Confidence Intervals</b> | <b>Sig</b> |
|---------------------------------|------------|---------------------------------|------------|
| <b>E, A &amp; G Total means</b> | 0.94       | 0.86-0.98                       | p<0.001    |
| <b>E, A &amp; G SCM means</b>   | 0.76       | 0.53-0.90                       | p<0.001    |
| <b>E, A &amp; G Comb means</b>  | 0.95       | 0.89-0.98                       | p<0.001    |
| <b>E, A &amp; G Obliq means</b> | 0.78       | 0.56-0.92                       | p<0.001    |

*Table 4-6: Within scanner intra-class correlation coefficients for the repeatability study*

| <b>Groups</b>            | <b>ICC</b> | <b>95% Confidence Intervals</b> | <b>Sig</b> |
|--------------------------|------------|---------------------------------|------------|
| <b>E1 &amp; E2 Total</b> | 0.95       | 0.86-0.98                       | p<0.001    |
| <b>A1 &amp; A2 Total</b> | 0.97       | 0.92-0.99                       | p<0.001    |
| <b>G1 &amp; G2 Total</b> | 0.96       | 0.86-0.99                       | p<0.001    |
| <b>E1 &amp; E2 SCM</b>   | 0.83       | 0.55-0.94                       | p<0.001    |
| <b>A1 &amp; A2 SCM</b>   | 0.80       | 0.48-0.93                       | p<0.001    |
| <b>G1 &amp; G2 SCM</b>   | 0.90       | 0.70-0.97                       | p<0.001    |
| <b>E1 &amp; E2 Comb</b>  | 0.96       | 0.88-0.99                       | p<0.001    |
| <b>A1 &amp; A2 Comb</b>  | 0.97       | 0.92-0.99                       | p<0.001    |
| <b>G1 &amp; G2 Comb</b>  | 0.96       | 0.88-0.99                       | p<0.001    |
| <b>E1 &amp; E2 Obliq</b> | 0.93       | 0.79-0.98                       | p<0.001    |
| <b>A1 &amp; A2 Obliq</b> | 0.83       | 0.56-0.94                       | p<0.001    |
| <b>G1 &amp; G2 Obliq</b> | 0.83       | 0.53-0.95                       | p<0.001    |

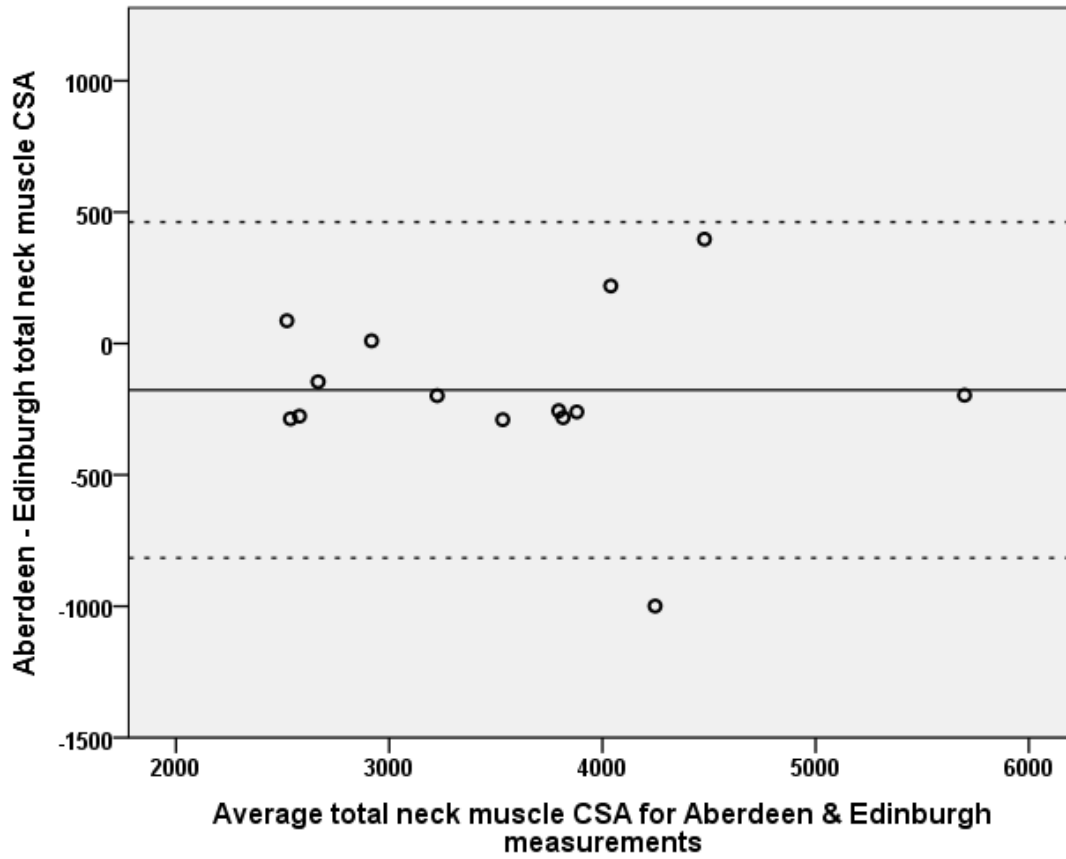
Key for Table 4.5 & 4.6:

- E = scan performed in Edinburgh
- A = Scan performed in Aberdeen
- G = Scan performed in Glasgow
- 1 & 2 = 1<sup>st</sup> and 2<sup>nd</sup> scan on that site
- SCM = Sternocleidomastoid
- Comb = Combined group (Trapezius, Splenius capitis, Semispinalis capitis)
- Obliq = Obliquus Capitis Inferior
- Total = Total neck muscle CSA

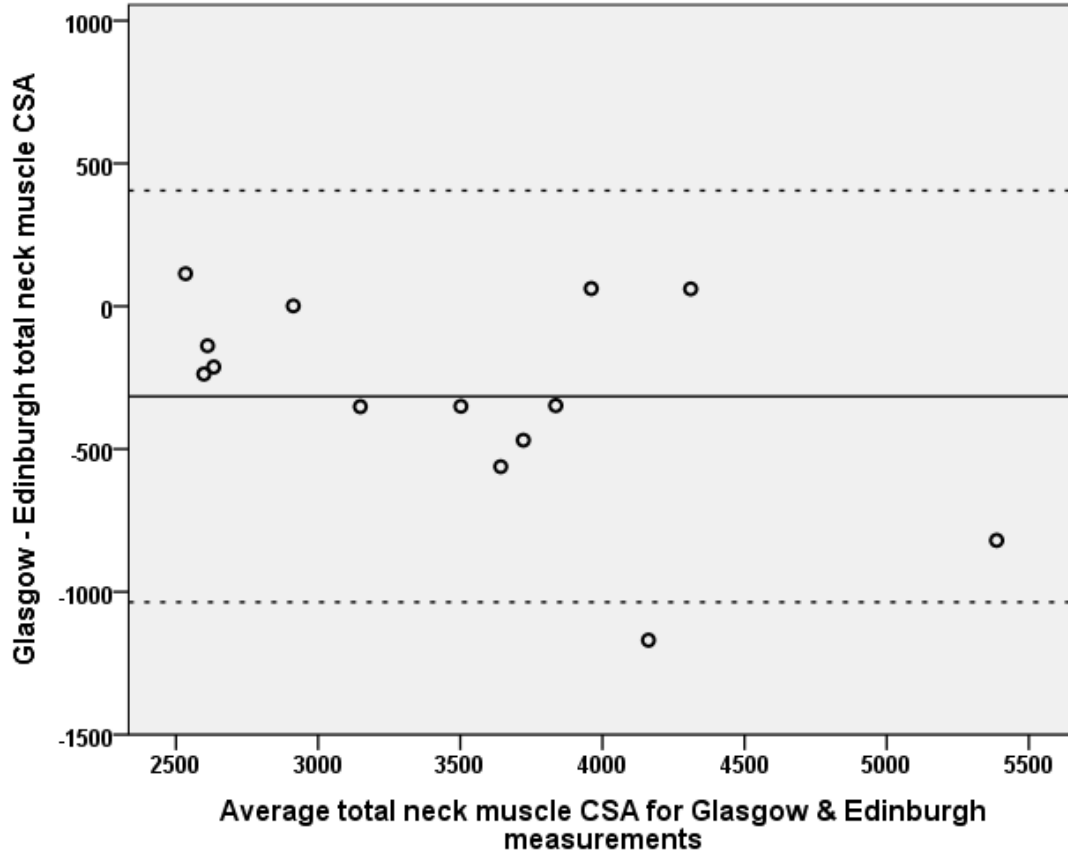
Bland-Altman plots show no definite linear bias between the Edinburgh and Glasgow scanners and the Aberdeen and Edinburgh scanners (Figures 4.6A & B). The Aberdeen-Glasgow plot indicates that the Aberdeen scanner may overestimate larger neck muscle CSA and underestimate smaller neck muscle CSA (Figure 4.6C). However the numbers involved in this study were small (n=14).

Figure 4-6: Bland-Altman plots for total neck muscle CSA measured on 3 different MRI scanners. Bias of measurement between different MRI scanners (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively.

A. Bland-Altman plot for total neck muscle CSA measured on the Aberdeen and Edinburgh MR images



B. *Bland-Altman plot for total neck muscle CSA measured on the Edinburgh and Glasgow MR images*



C. *Bland-Altman plot for total neck muscle CSA measured on the Glasgow and Aberdeen MR images*

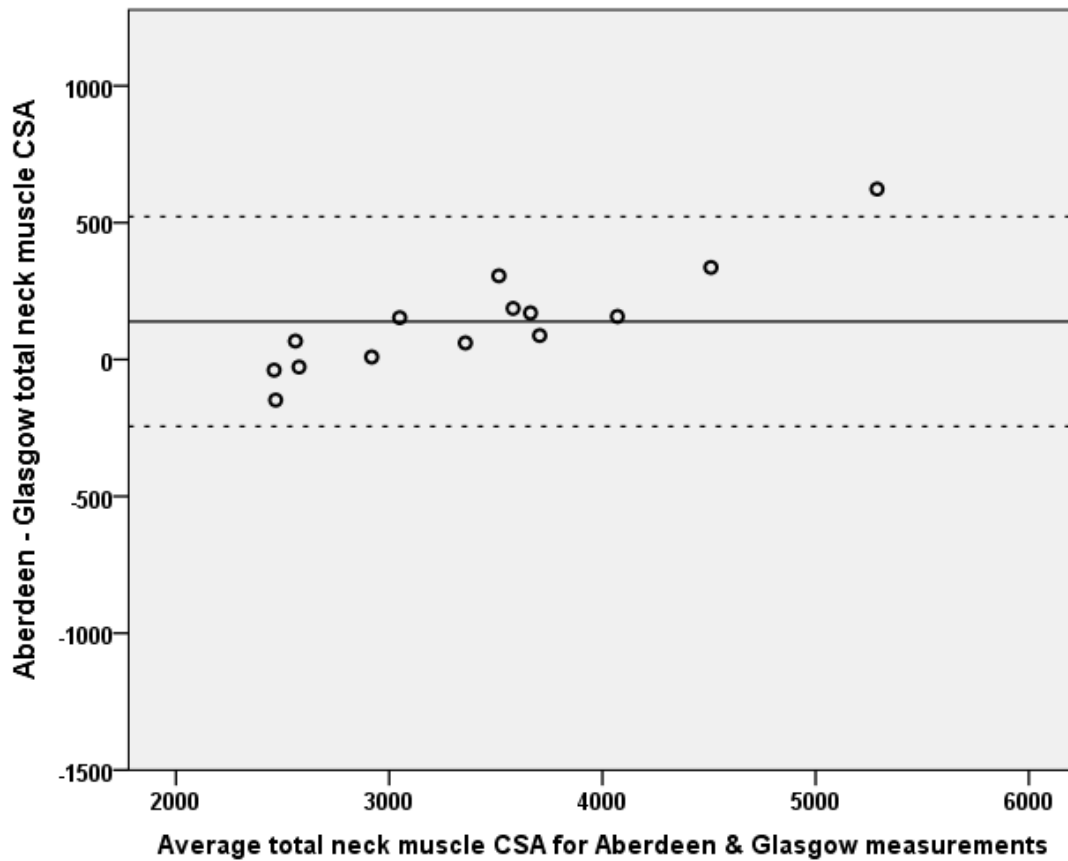


Table 4.7 shows the coefficients of variance (CV) for the mean values for each of the three scanners and a Levene's test of homogeneity which found no significant difference in CV for any of the three scanners, for any of the muscle measurements.

*Table 4-7: Coefficients of variation (%) with 95% CI and Levene's significance test between the three MRI scanners*

| Measurement                  | Scanner   | Coefficients of Variation (CV) (%) | 95%CI for CV | Levene's test (Significance) |
|------------------------------|-----------|------------------------------------|--------------|------------------------------|
| <b>Combined</b>              | Aberdeen  | 32.2                               | 23.3-51.9    | 0.89                         |
|                              | Edinburgh | 27.1                               | 19.7-43.7    |                              |
|                              | Glasgow   | 26.0                               | 18.9-41.9    |                              |
| <b>Obliquus</b>              | Aberdeen  | 30.6                               | 22.2-49.3    | 0.53                         |
|                              | Edinburgh | 36.7                               | 26.6-59.1    |                              |
|                              | Glasgow   | 28.1                               | 20.4-45.3    |                              |
| <b>SCM</b>                   | Aberdeen  | 21.7                               | 15.7-35.0    | 0.95                         |
|                              | Edinburgh | 20.6                               | 14.9-33.2    |                              |
|                              | Glasgow   | 22.1                               | 16.0-35.6    |                              |
| <b>Total neck muscle CSA</b> | Aberdeen  | 26.4                               | 19.1-42.5    | 0.84                         |
|                              | Edinburgh | 25.5                               | 18.5-41.1    |                              |
|                              | Glasgow   | 22.3                               | 16.2-35.9    |                              |

#### 4.3.4 Study 4: External validity study

25 subjects underwent the additional thigh scan; however, only 24 could be used in the analyses as one patient had not tolerated the full MR brain scan so we were unable to make the neck muscle CSA measurements. There was no overlap between subjects in study 2 and study 4. Of these 24 subjects, 11 were female and 13 male. Mean age (sd) was 73.8 years (0.27). Mean weight (sd) for the women was 63.2kg (15.4) and for the men was 85.6kg (10.9).

Mean total neck muscle CSA (sd) was 22.5cm<sup>2</sup> (3.7) for the female subgroup and 38.1cm<sup>2</sup> (6.3) for the male subgroup. Mean total thigh muscle CSA was 184.3cm<sup>2</sup> (36.5) for the female subgroup and 277.0cm<sup>2</sup> (31.3) for the male subgroup. An independent t test showed that both total neck muscle CSA (p<0.0005) and total thigh muscle CSA (p<0.0005) were significantly different between the female and male subgroups. The correlation coefficient for

all subjects for total thigh CSA and total neck CSA was 0.88 indicating that each explained at least 77.4% of the variance of the other.

## 4.4 Discussion

### 4.4.1 Summary of findings

This study sought to develop a technique to measure neck muscle cross-sectional area on volumetric MR brain scans. An initial feasibility study led to the formation of the technique (Figure 4.2). The reliability study then demonstrated that the technique had high inter-rater reliability for measurement of the CSA of the combined trapezius, splenius and semispinalis group in older adults. Obliquus capitis inferior and sternocleidomastoid CSAs are smaller muscles and measurements were less reliable between raters, though intraclass correlation coefficients remained high.

Study 3 demonstrated that the technique has good within scanner and between scanner repeatability. The confidence intervals for the measurements of the combined group and the total neck muscle area are quite narrow however the confidence intervals for the SCM and obliquus capitis inferior measurements are wider. This is because the cross-sectional areas of the SCM and obliquus muscles are smaller than either the combined or the total measurements. This means that any measurement errors will account for a greater proportion of the CSA than for muscles with a large area.

The obliquus is a short muscle whose cross-sectional area varies greatly over its length, unlike the other four muscles, as it has a wide belly and comparatively narrow tails. Our technique meant that in some subjects we were measuring across the belly of the obliquus and in some just the tail end. It also runs at an oblique angle and we identified that this angle varies between subjects. This meant that the area of obliquus we were measuring was an inexact proxy. For these reasons I conclude that the obliquus measurement should not be included in our technique. The ICCs are not as strong for the SCM as the combined group in the repeatability and reliability studies, which is likely a reflection of its small size. However as there are not the same intrinsic anatomical problems in measuring this muscle as there are with the obliquus and as total neck muscle CSA including the SCM appears to have stronger ICCs than the combined group alone, it is probably beneficial to include the SCM in addition to the combined group to provide a better estimate of general muscle bulk.

The final study was designed to measure the external validity of the technique. It shows that there is a strong correlation between neck muscle cross sectional area and thigh muscle cross sectional area, which is often used as a proxy for general muscle mass (Kongsgaard et al., 2004, Godard et al., 2002, McIntyre et al., 2006). The percentage variance (ie r-squared) is



76.7% and the 1<sup>st</sup> unrotated principal component of neck muscle CSA was found in the reliability study to explain 72.2% of variance. Extracting principal components is useful to reduce random measurement errors that might be associated with individuals' neck positions for example. The principal component correlated positively with grip strength, providing further support for neck muscle CSAs' validity as an index of sarcopenia. This means that posterior neck muscles can be used equally as well as thigh muscles as an index of general muscle bulk (Ohkawa et al., 2000, Lerner et al., 1986).

#### **4.4.2 Previous research on quantifying muscle mass**

Previous studies quantifying neck muscle CSA have only focused on young subjects and have used scans performed specifically for that purpose. They have however shown good reliability in the techniques used (Cagnie et al., 2010, Ulbrich et al., 2011). I found no previous studies which measured neck muscle CSA on MRI scans on elderly subjects and none which used MRI brain scans for this purpose.

When considering the validity of using a cross-sectional measurement of muscle size to infer general muscle bulk I referred to previous studies on body composition. Studies investigating how differing muscle groups relate to each other have tended to compare upper and lower limb muscle mass alone (Janssen et al., 2000, Gallagher et al., 1997, Gallagher and Heymsfield, 1998). I found no studies which compared muscle CSA, mass or volume between any other two or more areas of the body, including the neck. Three large studies on body composition suggest that the distribution of muscle between the upper and lower limbs varies with gender, age, height, weight and ethnicity (Janssen et al., 2000, Gallagher et al., 1997, Gallagher and Heymsfield, 1998).

#### **4.4.3 Strengths and limitations of the studies**

Although there is no reason to suppose that this methodology is not applicable in younger adults and older adults (ie 75y+), three of the studies were restricted to a narrow age cohort around 72 years old and the study of younger subjects only had a n=14. The study participants were all community-resident volunteers and thus relatively healthy and were not diverse in terms of geography or ethnicity. The narrow geographical location of the subjects is important as it has been shown that anthropometric measurements vary across the UK. Bannerman et al collected data from residents of Edinburgh and compared their results with anthropometric reference data from South Wales and Nottingham. They found significant

differences between the three groups confirming their hypothesis that anthropometric measurements vary across geographical area (Bannerman et al., 1997).

Skeletal muscle can be split into two groups; postural and phasic. Postural muscles have a larger percentage of type 1 fibres and show less fatigability. Phasic muscles are primarily involved in movement and have a higher proportion of type 2 muscle fibres. A feature of ageing muscle is that type 2 fibre width decreases more than type 1 fibre width, therefore the relation between neck CSA (mainly postural, ie more type 1 fibres) and thigh CSA (a mixture of postural and phasic) will change with age (Doherty, 2003, Larsson, 1978, Andersen, 2003, Lexell et al., 1988). Also, the neck muscles which we use in our final model may have differing percentages of type 1 and 2 fibres as a reflection of their differing roles: the sternocleidomastoid muscle is primarily used for rotation and flexion of the neck, whereas the three more posterior neck muscles (trapezius, splenius capitis and semispinalis capitis) are all primarily involved in neck extension. However, at present the fibre type composition of the neck muscles has not been studied and it seems likely that they all represent a mainly postural function with a lesser degree of phasic function. This could mean that by only studying neck muscles, changes which affect phasic muscles more with age may not be identified (ie affecting type 2 fibres more than type 1 fibres).

Despite not standardising the angle of the axial measurement slice relative to the patient, we still achieved very high inter-rater reliability. However, it is possible that the measurement variability would be larger in a longitudinal study if the patients were in different positions in the scanner on each occasion. Such differences are usually only slight because head, neck and back are passively supported during scanning leaving the neck muscles in a relaxed state. Lateral changes are unlikely to have a major effect because muscle CSAs are summed for both left and right so that reductions on one side could be compensated by the accompanying increase on the other. Such compensation does not apply to antero-posterior positioning; however, small differences in this plane are also unlikely to be important. A difference in angle of C2 between two repeated scans in the sagittal plane of 5° would result in a CSA difference of 0.4%, 10° increases CSA by 1.5% and 15° by 3.5% all within the limits for inter-rater SCM CSA difference; even a 20° angle increases CSA by only 6.4%, probably at the limit of utility of the technique to detect medium effect sizes. If reliability in longitudinal studies was not acceptable (i.e. mean differences in plane angles measured at C2 >20°),

increased positional standardization would be necessary which might include using more than one anatomical marker from a T1-weighted volume scan for standardization.

Most of these limitations could be addressed by a larger study which included a wider spread of age, geographical area, ethnicity, health status and an equal gender balance. It would be interesting to look at muscles from elsewhere in the body also. For example including a measure of upper arm CSA and calf muscle CSA and to investigate how the comparative size of these muscles varies with age.

#### **4.4.4 Implications for future research**

This new technique is particularly interesting because several of the longitudinal studies investigating ageing involve an MR brain scan, therefore the method could be used to measure changes in muscle size and consequently estimate sarcopenia in these studies without any further imaging. This will allow the wealth of variables already collected as part of these studies to be researched as possible correlates of sarcopenia. Longitudinal studies are important sources of information for researchers interested in age associated disease to allow identification of key risk factors. These in turn allow hypotheses to be generated which can lead to both an understanding of the mechanisms underlying these diseases, which may lead on to development of treatments, and the possibility of generating advice to prevent or slow down some of the disease processes. Although we developed the technique on MR volume brain images, it is now common to acquire volume data when performing a CT brain scan and, as there is often good differentiation between muscle and fat in the neck, the same approach could possibly work on CT scans as well. Further testing is required.

I then used this technique on two longitudinal studies to investigate correlates of sarcopenia and identify possible causative factors from lifestyle and biomedical data which have been collected concurrently with the MRI scans.

### **4.5 Conclusion**

We have developed a feasible, valid and repeatable method for measuring neck muscle cross-sectional area on MR brain scans which has good inter-rater reliability. This technique can be used to measure neck muscle CSA which can serve as a proxy measure of muscle bulk as shown by the above factor analysis and shared variance measures. We have demonstrated that neck muscle CSA correlates strongly with grip strength, a commonly used functional measure. The development of a reliable method to measure neck muscle CSA from

volumetric MR brain scans potentially opens up a new field of radiological ageing research. This in turn will allow sarcopenia to be investigated in studies which include a MR brain scan but no measure of muscle bulk without involving any additional scanning. This will be of particular use in studies wishing to investigate both brain and muscle ageing within subjects, and will allow synchronous measurements of brain and muscle structure using the same scan. I now plan to use this measurement technique on longitudinal ageing cohorts which have already included a volumetric MR brain scan to investigate the relationship between muscle and brain structure in these datasets. Longitudinal data will also allow further exploration of the common cause hypothesis by looking at the trajectories of change within brain and muscle structure over time.

# **Chapter 5 Neck muscle cross-sectional area, brain volume and cognition in healthy older men; a cohort study**

## **5.1 Background**

Chapter 2 identified only three studies investigating the relationship between brain and muscle structure and it was postulated that this was because the imaging required to investigate both measures does not overlap and only very few studies undertook imaging of both brain and muscle. In chapter 4 I described the development of a technique to measure neck muscle CSA, as a measure of muscle bulk, on volumetric MR brain scans, allowing quantification of brain and muscle size on one scan. Here I used this novel technique to investigate the relationship between muscle structure, brain structure and cognition in healthy older men.

Until recently, muscle and brain ageing had rarely been studied in tandem; however, studies demonstrating improved cognition in later life with increased physical activity have highlighted this important area of research. For example a Cochrane review of randomised controlled trials found evidence that aerobic physical activities improved cognitive function in healthy older adults, with effects observed for motor function, cognitive speed, and auditory and visual attention (Angevaren et al., 2008). Observational studies have also found positive relationships between physical activity and cognitive function (Coley et al., 2008, Weuve et al., 2004). There is some evidence that both grey and white matter brain volume can be significantly improved by aerobic exercise in older adults (Colcombe et al., 2006). It was hypothesized that these relationships were due solely to cardiovascular fitness, and although this may play a role, animal studies have found other underlying mechanisms including: increased levels of brain-derived neurotrophic factor which may contribute to neurogenesis, effects on neurotransmitter systems and increased insulin-like growth factor 1 (Kramer et al., 2006).

It is known that muscle size and function (ie strength or power) do not age in parallel (Young et al., 1985, Skelton et al., 1994), therefore the association between muscle size and either brain structure (eg whole brain volume) or cognitive function requires independent study. We found only one study which investigated the relationship between muscle bulk and brain structure in older adults; however, this study included subjects with Alzheimer's disease

along with normal controls (Burns et al., 2010). The studies investigating muscle size and cognition have largely relied on simple cognitive screening tools (eg Mini Mental State Examination (MMSE)) and do not usually contain a measure or estimate of prior cognitive ability (Auyeung et al., 2011, Wirth et al., 2011, Nourhashemi et al., 2002).

Here we studied community-dwelling healthy older men, measuring: neck muscle cross-sectional area (CSA), multiple cognitive domains including estimated prior cognitive ability and neuroimaging volumes. We hypothesised that lower muscle bulk is associated with structural markers of brain ageing, and poorer cognitive ability in healthy older people.

## **5.2 Methods**

### **5.2.1 Participants**

Participants were 51 community-dwelling men involved in a longitudinal ageing study investigating healthy ageing, glucocorticoid status and brain structure (MacLulich et al., 2012, MacLulich et al., 2005). The study was approved by the Lothian Health Ethics Committee. All subjects gave written informed consent and the research was carried out in compliance with the Helsinki Declaration. Data from the second wave of the study were used because the smaller MR head coil used in the first wave excluded the neck muscles. Exclusion criteria were previously provided (MacLulich et al., 2005, MacLulich et al., 2012). Participants were healthy, lacking of significant illness, including dementia, stroke, ischaemic heart disease, depressive illness, excessive alcohol intake, and cancer. No participants were taking psychotropic medication.

### **5.2.2 MR brain imaging**

The full MR brain imaging protocol has been previously published (MacLulich et al., 2012). In summary, imaging was performed on a GE Signa LX 1.5 T (General Electric) MR scanner. Participants received a sagittal T<sub>1</sub>-weighted spin echo sequence covering the whole head (TR 450msec, TE 9msec, FOV 24 cm, matrix 256 × 224, slice thickness 5 mm (no gap)) and a volume scan consisting of a T<sub>1</sub>-weighted 3D inversion recovery prepared sequence (3D IR\_PREP) acquired in the coronal plane with slices perpendicular to the long axis of the hippocampus and covering the whole head (TI 600msec with TE set to minimum, FOV 22 cm, matrix 256 × 192, slice thickness 1.7 mm (no gap)).

### **5.2.3 Brain structure measurements**

Image analysis was performed using Analyze v7.0 for Windows (Mayo Clinic, Rochester, MA). Whole brain, hippocampal and ventricular volumes (MacLulich et al., 2012), and intracranial area (a validated estimate of intracranial volume (Ferguson et al., 2005)) were obtained by an experienced rater.

### **5.2.4 Neck muscle cross-sectional area**

We used neck muscle cross-sectional area (CSA) as a measure of muscle size. We have previously shown in a study of 24 subjects that neck muscle CSA is strongly correlated with thigh muscle CSA ( $R^2$  0.77), which is often used as a proxy for general muscle bulk (Kilgour et al., 2012). Neck muscle CSA is generally available in brain MRI studies, whereas thigh

muscle CSA is not usually measured within longitudinal cognitive ageing studies. Neck muscle CSA was measured using a validated technique (Kilgour et al., 2012). Full details can be obtained in chapter 4; however, in summary, the mid-point of the C2-vertebra was located in the sagittal slice of a 3D reconstructed image. The image was then converted to a transverse view and the posterior neck muscles were outlined using a cursor. The software then calculated the contained area. The muscles measured were the semispinalis capitis, splenius capitis and trapezius (measured as a combined group), and the sternocleidomastoid.

### **5.2.5 Tests of cognitive function**

The following tests of cognitive function were performed as part of the original study, as previously described (MacLulich et al., 2012): the Mini-Mental State Examination (MMSE, a screening test for cognitive impairment); the Controlled Word Association Test (CWAT, tests verbal fluency, which is an aspect of executive function); the Digit-Symbol Substitution Test (DSST, tests attention and processing speed) from the Wechsler Adult Intelligence Scale; Raven's Standard Progressive Matrices (RSPM, tests non-verbal reasoning, an important aspect of fluid intelligence); Logical Memory (tests immediate and delayed verbal declarative memory); Visual Reproduction (tests immediate and delayed visual memory); Rey's Auditory-verbal Learning Test (tests verbal memory and learning); Benton's Visual Retention Test (tests visual memory); and the National Adult Reading Test (NART, provides an estimate of prior general cognitive ability). Due to the strong correlation between pre-morbid cognitive ability (of which NART provides an estimate) and educational achievement (Deary et al., 2007a), it was decided to include only NART and not educational achievement as a predictor variable. One participant had a MMSE below 24 and was excluded from the analysis as this score may be reflective of an incipient diagnosis of dementia.

### **5.2.6 Statistical analysis**

Descriptive statistics, exploratory analyses, general linear modelling (Analysis of Covariance; ANCOVA) and principal components analysis were performed on SPSS version 18.0 for Windows. Missing values were excluded listwise. For the ANCOVA we constructed baseline models with the measures of brain structure and cognitive ability as dependent (i.e. outcome) variables and neck muscle CSA as an independent variable, adjusting for intracranial area (ICA) and age. ICA is thought not to change after the onset of age-related neuronal loss, particularly in men, therefore can be used as a marker of peak brain size, thus allowing the outcome variable to be more reflective of brain atrophy (Mori et al., 1997). Also, without



adjusting for ICA it could be argued that those with bigger skulls require larger neck muscles for support or that they are just larger in proportion; therefore, this adjustment also controls for this. We also adjusted for NART since this was found to correlate significantly with neck muscle CSA, brain volumes and current cognition. Furthermore, in the models with current cognitive ability as an outcome variable, adjusting for the NART score allows the model to reflect the degree of cognitive ageing that has taken place.

## 5.3 Results

All participants (n=51) were male with a mean age of 73.8 years (sd 1.5). Descriptive statistics for participant neuroimaging and neck muscle cross-sectional area data are in Table 5.1. Of the 51 MR brain scans reviewed, we were unable to measure neck muscle CSA on one scan as the scan did not extend far enough inferiorly to include the neck muscles at the required level for measurement (the mid-point of the C2-vertebra). In a further two cases, the hippocampal volume could not be measured.

*Table 5-1: Neuroimaging and muscle cross-sectional area data*

|  | N  | Mean   | Std. Deviation |
|--|----|--------|----------------|
| Whole brain volume (cm <sup>3</sup> )        | 50 | 1149.8 | 90.4           |
| Intracranial area (cm <sup>2</sup> )         | 50 | 157.9  | 9.6            |
| Total Ventricular volume (cm <sup>3</sup> )* | 50 | 31.2   | 24.0           |
| Total Hippocampal volume (cm <sup>3</sup> )  | 48 | 7.0    | 0.8            |
| Cerebellar volume (cm <sup>3</sup> )         | 50 | 135.4  | 14.3           |
| Total SCM muscle area (cm <sup>2</sup> )     | 49 | 5.1    | 1.1            |
| Total Comb muscle area (cm <sup>2</sup> )    | 49 | 22.8   | 3.1            |
| Total muscle area (cm <sup>2</sup> )         | 49 | 28.0   | 3.4            |
| Valid N (listwise)                           | 47 |        |                |

\*Non-parametric data, median and inter-quartile range presented

Table 5.2 contains the descriptive statistics for the cognitive test data. To reduce the risk of type 1 statistical error by testing multiple associations, we performed principal components analysis. The Kaiser-Meyer-Olkin measure verified sampling adequacy. Two principal components were extracted employing varimax rotation; the first had an eigenvalue of 2.58, explaining 36.9% of the variance and the second had an eigenvalue of 2.4, explaining 34.3% of the variance. For comprehensibility, we refer to these components as ‘factors’ as is general usage. Together, the factors explain 71.2% of the variance in the cognitive test scores. The cognitive tests which focused on memory (ie Logical Memory, Visual Reproduction, AVLT and BVRT) had high factor loadings for Factor 1; therefore, we hereafter call this factor the Memory Factor. Factor 2 had high factor loadings for the three other tests: CWAT, DSST and RSPM and hereafter is referred to as the Cognitive Processing Factor.

*Table 5-2: Prior and current cognition data*

|                                       | <b>N</b> | <b>Mean</b> | <b>Std. Deviation</b> |
|---------------------------------------|----------|-------------|-----------------------|
| National Adult Reading Test *         | 47       | 42.0        | 13.0                  |
| Mini Mental State Examination *       | 50       | 28.0        | 4.0                   |
| Controlled Word Association Test      | 47       | 40.4        | 10.9                  |
| Digit Symbol Substitution test        | 46       | 43.3        | 10.7                  |
| Raven's standard progressive matrices | 46       | 40.0        | 9.1                   |
| Logical Memory                        | 47       | 51.6        | 15.0                  |
| Visual Reproduction                   | 47       | 46.4        | 14.8                  |
| Auditory-Verbal Learning Test         | 47       | 49.9        | 9.0                   |
| Benton Visual Retention Test *        | 47       | 17.0        | 4.0                   |
| Factor 1 (Memory)                     | 45       | 0.0         | 1.0                   |
| Factor 2 (Cognitive processing)       | 45       | 0.0         | 1.0                   |
| Valid N (listwise)                    | 45       |             |                       |

\*Non-parametric data, median and inter-quartile range presented

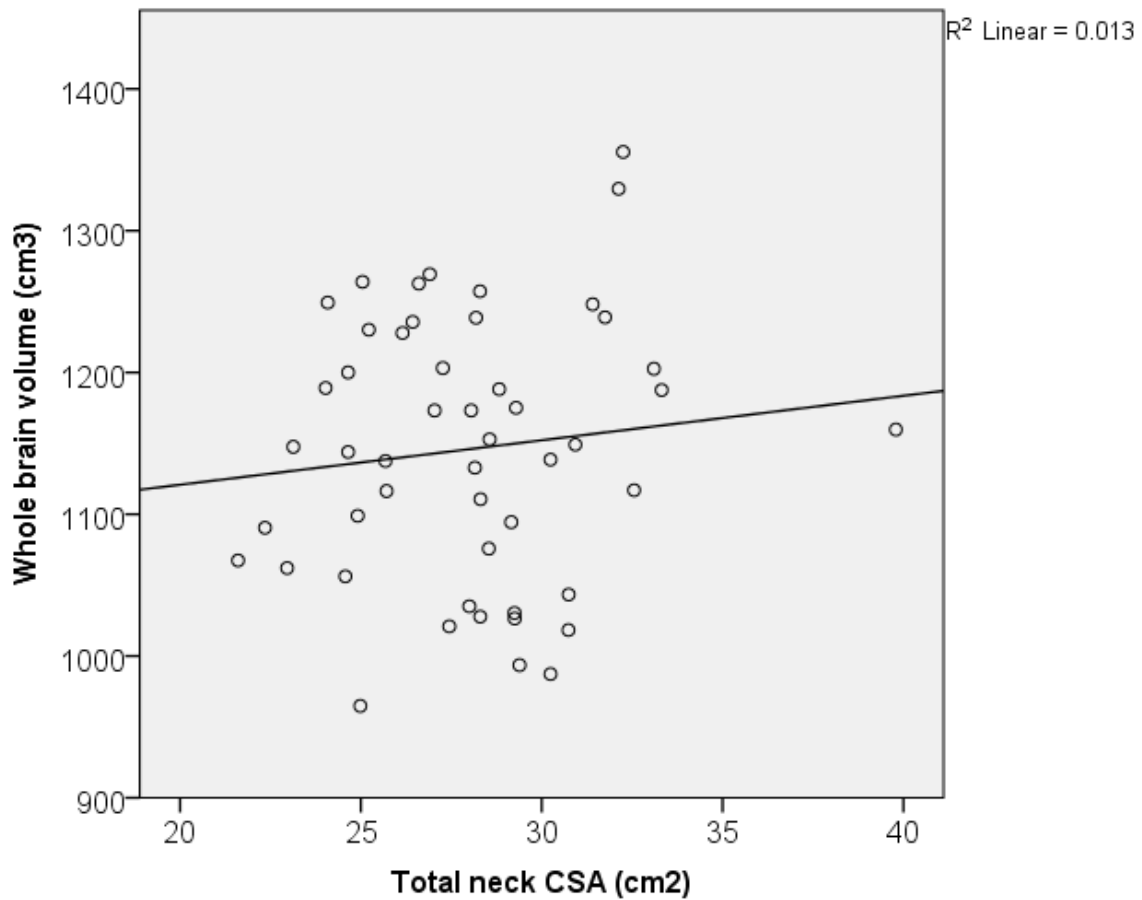
Initially bivariate statistics were performed (Spearman's rho) (Table 5.3). The only variable to significantly correlate with total neck muscle CSA was NART (rho  $-0.36$ ,  $p = .01$ ). NART was also found to correlate strongly with the following variables: intracranial area; unadjusted whole brain, hippocampal, and cerebellar volumes; and the cognitive processing factor.

*Table 5-3: Spearman's rho correlations between neuroimaging, muscle and cognitive variables (p values)*

|                             | <b>Total neck<br/>muscle CSA</b> | <b>NART</b>     |
|-----------------------------|----------------------------------|-----------------|
| Age                         | .21<br>(.15)                     | -.03<br>(.82)   |
| Whole brain volume          | .07<br>(.62)                     | .49<br>(<0.001) |
| Intracranial area           | -.11<br>(.44)                    | .56<br>(<0.001) |
| Total ventricular volume    | .13<br>(.10)                     | .10<br>(.50)    |
| Hippocampal volume          | -.07<br>(.67)                    | .48<br>(<0.001) |
| Cerebellar volume           | .03<br>(.83)                     | .46<br>(<0.001) |
| Memory factor               | -.28<br>(.07)                    | .13<br>(.40)    |
| Cognitive processing factor | -.03<br>(.83)                    | .52<br>(<0.001) |
| NART                        | -.36<br>(.01)                    | 1.00<br>-       |

A scatter plot confirmed no significant relationship between whole brain volume and total neck muscle CSA (figure 5-1).

Figure 5-1: Plot of whole brain volume against total neck muscle CSA



ANCOVA was performed to check for shared variance among neck muscle CSA and the neuroimaging measures, the cognitive factors, and NART. Models were corrected for age, intracranial area (ICA, to correct for head size) and NART, except in the model for NART where only age and ICA were adjusted for. Total neck muscle CSA was found to predict 17% of the variance in whole brain volume ( $t=2.86$ ,  $p=0.01$ ) (Table 5.4). However, total neck muscle CSA did not significantly predict the variance in ventricular, hippocampal or cerebellar volumes ( $p>0.05$ ).

*Table 5-4: ANCOVA for whole brain volume with total neck muscle area, intracranial area, age and prior cognition as predictor variables*

| Source  | Degrees of Freedom | F     | Unstandardised B* | t    | Sig. | Partial Eta Squared |
|---|--------------------|-------|-------------------|------|------|---------------------|
| <b>Corrected Model</b>                        | 4                  | 12.85 |                   |      | <.01 | .56                 |
| <b>Intercept</b>                              | 1                  | 1.06  | 491.40            | 1.03 | .31  | .03                 |
| <b>Age (years)</b>                            | 1                  | 0.79  | -5.64             | -.89 | .38  | .02                 |
| <b>Intracranial area (cm<sup>2</sup>)</b>     | 1                  | 13.13 | 0.04              | 3.62 | <.01 | .24                 |
| <b>NART (Score out of 50)</b>                 | 1                  | 8.43  | 3.85              | 2.90 | .01  | .17                 |
| <b>Total neck muscle CSA (cm<sup>2</sup>)</b> | 1                  | 8.16  | 0.09              | 2.86 | .01  | .17                 |
| <b>Corrected total</b>                        | 45                 |       |                   |      |      |                     |

\*Unstandardised B coefficients reflect change in whole brain volume in cm<sup>3</sup>

Neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor ( $p > 0.05$ ) (Tables 5.5 & 5.6). Using the NART score as an outcome variable, we found that total neck muscle CSA predicts 10% of the variance in the NART score ( $t = -2.12$ ,  $p < 0.05$ ) after adjusting for ICA and age.

*Table 5-5: ANCOVA for Memory Factor with total neck muscle area, intracranial area, age and prior cognition as predictor variables*

| Source  | Degrees of Freedom | F    | Unstandardised B* | t     | Sig. | Partial Eta Squared |
|---|--------------------|------|-------------------|-------|------|---------------------|
| <b>Corrected Model</b>                        | 4                  | 1.23 |                   |       | .31  | .11                 |
| <b>Intercept</b>                              | 1                  | 1.39 | 8.91              | 1.18  | .25  | .03                 |
| <b>Age (years)</b>                            | 1                  | 1.30 | -.12              | -1.14 | .26  | .03                 |
| <b>Intracranial area (cm<sup>2</sup>)</b>     | 1                  | 0.02 | <-.01             | -.15  | .88  | .00                 |
| <b>NART (Score out of 50)</b>                 | 1                  | 1.56 | .03               | 1.25  | .22  | .04                 |
| <b>Total neck muscle CSA (cm<sup>2</sup>)</b> | 1                  | 0.28 | <.01              | -.53  | .60  | .01                 |
| <b>Corrected total</b>                        | 43                 |      |                   |       |      |                     |

\*Unstandardised B coefficients reflect change in factor score for the Memory Factor

*Table 5-6: ANCOVA for General Processing Factor with total neck muscle area, intracranial area, age and prior cognition as predictor variables*

| <b>Source</b>                                 | <b>Degrees of Freedom</b> | <b>F</b> | <b>Unstandardised B*</b> | <b>t</b> | <b>Sig.</b> | <b>Partial Eta Squared</b> |
|---|---------------------------|----------|--------------------------|----------|-------------|----------------------------|
| <b>Corrected Model</b>                        | 4                         | 5.19     |                          |          | <.01        | .35                        |
| <b>Intercept</b>                              | 1                         | 0.23     | 3.06                     | .48      | .63         | .01                        |
| <b>Age (years)</b>                            | 1                         | 1.60     | -.11                     | -1.26    | .21         | .04                        |
| <b>Intracranial area (cm<sup>2</sup>)</b>     | 1                         | 0.15     | <.01                     | .39      | .70         | .00                        |
| <b>NART (Score out of 50)</b>                 | 1                         | 11.86    | .06                      | 3.44     | <.01        | .23                        |
| <b>Total neck muscle CSA (cm<sup>2</sup>)</b> | 1                         | 2.86     | <.01                     | 1.69     | .10         | .07                        |
| <b>Corrected total</b>                        | 43                        |          |                          |          |             |                            |

\*Unstandardised B coefficients reflect change in factor score for the General Processing Factor

## 5.4 Discussion

We found that in healthy elderly men, preservation of whole brain volume was associated with larger total neck muscle cross-sectional area. Therefore in an elderly cohort, those that have a smaller muscle bulk have undergone more brain atrophy. This finding supports the common cause hypothesis, by demonstrating that the rate of sarcopenia and ARCD may occur in parallel within individuals, driven by core underlying biological processes. However, we found no significant association between total neck muscle CSA and ventricular volume (a different measure of brain atrophy), or hippocampal or cerebellar volumes. Also, whilst the relationship between whole brain volume and total neck muscle CSA was significant when studied within a general linear model, the unadjusted Spearman's correlation found no significant association for the same relationship; therefore it is possible that the relationship we found was a result of residual confounding rather than representing a true association. It will now be important to repeat these analyses using data from a different study to see if the results are repeatable to help investigate this complex relationship further.

We unexpectedly found that total neck muscle CSA was negatively associated with estimated prior cognitive ability (NART) after adjustment for ICA and age, but we found no significant association between total neck muscle CSA and current cognitive abilities. This suggests that those with lower prior cognitive ability may have larger muscles in old age. Muscle mass in old age is determined by 2 factors. Firstly, peak muscle bulk obtained in young adulthood, and secondly, rate of muscle atrophy with ageing. Therefore we hypothesise that those with lower cognitive abilities may have undertaken more manual work (Neisser et al., 1996, Mackintosh, 1998) and therefore achieved a greater peak muscle bulk and a larger muscle mass in old age. We can find no plausible explanation as to why a lower prior cognitive ability would favour a slower rate of muscle atrophy. We unfortunately do not have sufficiently detailed previous occupational history or socio-economic class data for the participants to be able to test this theory further at this point.

We found only one previous study which investigated the relationship between muscle size and brain size, and this study also found a positive relationship between muscle bulk and whole brain volume. Burns et al studied elderly people with early Alzheimer's disease (AD) (n=70) or normal cognition (n=70) and found that whole brain volume, normalized for head size, was predictive of lean mass as measured by DEXA (beta 0.20,  $p < .001$ ) in both groups (Burns et al., 2010). White matter volume was the primary driving factor for the relationship



(beta 0.19,  $p < .001$ ) while grey matter volume showed no association with lean mass. This indicates that the cause of loss of lean muscle mass in AD may be different to normal ageing as it is primarily grey matter that is lost in AD.

In the above study Burns et al also investigated the relationship between MMSE and a measure of global cognitive performance (a composite score made up of the results of a battery of tests, including the DSST and verbal fluency) with muscle mass (Burns et al., 2010). They found a significant positive association between both the global cognitive performance score (beta 0.12,  $p = .007$ ) and MMSE (beta 0.11,  $p = .009$ ) and muscle mass, controlling for age and sex but not for prior cognition which we have shown to correlate with both brain and muscle size (Table 5.3). Our study was able to investigate the relationship between cognitive decline, by adjusting for prior cognition using the NART score, and current cognition, whereas this study only looked at cross-sectional data from current cognition. This may explain why they found an association between current cognition and muscle mass and we did not.

Several large studies have also investigated the links between muscle size and cognition. In a large cross-sectional study of community dwelling women aged 75 or over ( $n = 7105$ ), Nourhashemi et al found that low cognitive function was associated with low fat free mass (Nourhashemi et al., 2002). However the cognitive test used was the Short Portable Mental Status Questionnaire (SPMSQ), which consists of only 10 questions and is mainly used as a screening test for cognitive impairment.

Conversely, Wirth et al studied 4095 consecutive geriatric hospital patients and found that fat-free mass was not associated with cognitive dysfunction, measured using MMSE, after adjusting for age, sex and Barthel index (Wirth et al., 2011). Also, Auyeung et al studied 2737 cognitively normal older people and found that appendicular skeletal muscle mass (ASM) was significantly predictive of MMSE 4 years later in men but not women (Auyeung et al., 2011). However, after adjustment for age, years of education and baseline MMSE score, the relationship in men was not significant either.

Our study has the benefit of including tests of both prior and current cognitive function. This allows us to look at cognitive decline rather than purely at current cognitive ability, and is the only study we could find that specifically tested the relationship between prior cognition and muscle size. Also, the three large studies mentioned above used cognitive tests which are

primarily designed to screen for cognitive impairment (ie SPMSQ and MMSE) rather than to detect the subtleties of change in cognition with age (Auyeung et al., 2011, Wirth et al., 2011, Nourhashemi et al., 2002), for which our cognitive tests were specifically chosen. Burns et al used more detailed cognitive tests; however the numbers involved in their study are much smaller compared to the other three studies. Our study is the first to measure muscle cross-sectional area and cognition or brain size; the above mentioned studies used either bioimpedance analysis or DEXA as the measure of muscle bulk.

The main limitations of our study are the lack of longitudinal data and the small sample size. In ageing studies longitudinal data are crucial as it is the rate of loss of muscle size or brain size that is of interest rather than measurements at a cross-sectional time point. With brain size we can partially correct for this using intracranial area, but with muscle size we are unsure if someone has lost 10% of their lean body mass in the previous decade or 50%, as clearly the peak muscle bulk obtained will affect the final outcome greatly. The study also contained mainly white males and this will affect the generalisability of our results.

## **5.5 Conclusion**

In healthy older men preservation of whole brain volume is associated with larger muscle size within a general linear model, however the relationship was not significant in the unadjusted correlation analyses therefore may represent residual confounding. Larger muscle size was associated with lower prior cognition, but not current cognition in both unadjusted correlations and general linear models. As we were unfortunately unable to use the data from wave 1 of this study due to the above mentioned technical issue, these data were again cross-sectional and not longitudinal. Therefore despite offering some limited evidence of associations between muscle structure and brain structure and function this study was not able to advance forward the concept of the common cause hypothesis as I was unable to look at how the trajectories of decline compared with each other for brain and muscle. I next planned to use the neck muscle measurement technique on a larger longitudinal cohort study (LBC 1936) which includes both men and women, to attempt to replicate these results and investigate the role of muscle function within these relationships along with other important covariates recorded within that study.

# **Chapter 6 Interrelationships between brain and muscle structure and function using data from wave 2 of the Lothian Birth Cohort 1936**

## **6.1 Introduction**

Thus far I have investigated the interrelationship between brain and muscle structure and function using a systematic review and analysis of an all-male dataset (n=51). In the systematic review I found evidence of a relationship between brain size and muscle size, and evidence of a relationship between both grip strength and gait speed and brain volume (ie white matter volume, regional grey matter volume and whole brain volume), brain atrophy and accumulation of WMHs (Kilgour et al., 2014). Cognitive function was not found to be associated with muscle size and I did not look at the relationship between cognitive function and physical function as this positive relationship has previously been well described. In the cohort study I found that in healthy older men, preservation of whole brain volume (i.e. less atrophy) was associated with larger muscle size in a general linear model, but not in unadjusted analyses. I also found an interesting negative relationship between NART score (a measure of childhood cognitive ability) and neck muscle size, although this dataset had no measure of muscle strength. However, in none of these studies had the interrelationship between all four measures (ie brain and muscle structure and function) been investigated.

Sarcopenia is described as both the loss of muscle size and function with age, with particular emphasis placed on the importance of grip strength and gait speed to aid diagnosis, which are the exact measures the LBC 1936 study contains (Roubenoff, 2001, Cruz-Jentoft et al., 2010, Muscaritoli et al., 2010). Within the field of cognitive ageing there is good evidence that brain structure (eg whole brain volume, markers of brain atrophy) and cognition are also related (McDaniel, 2005, Schmidt et al., 2005). Studying all four variables within one dataset allows us to understand the interrelationship between them which will allow a better understanding of how variations in one may affect another and the possible mediating role the other variables may play in this relationship. For example if we find that grip strength is associated with white matter (WM) volume, we can look to see if muscle size or cognition is the true mediator of this relationship or whether this is an independent effect. This should hopefully lead to a better understanding of potential mechanisms which may coexist within

brain and muscle ageing, and therefore potential therapies which may prove beneficial to both.

## **6.2 Methods**

### **6.2.1 LBC 1936**

For methods regarding the LBC 1936 study please refer to chapter 3: Methods for the Lothian Birth Cohort 1936 study.

### **6.2.2 Statistics**

All data were analysed using the SPSS 21.0 statistics package. Means, standard deviations and Pearson's correlations were calculated for parametric variables and median, interquartile range and Spearman's correlations for non-parametric variables. Multiple linear regression models were then tested, adjusting for important covariates (eg sex, age), before parameter estimates were calculated for significant relationships. Further testing for potential explanatory factors was then performed, searching for a meaningful change in partial eta squared in the chosen predictor variable (eg grip strength), which was taken to be a >1% change in partial eta squared. Multinomial logistic regression was then performed to test for associations between deep and periventricular Fazekas score (categorical variables) and measure of muscle size and function. Lastly Sobel's test was used to test whether variables exerted a significant mediating effect on certain associations. A significance of <0.05 was taken to be significant, as I had 8 primary outcomes to test: the association of neck muscle CSA with WM, GM and WML volume and cognition, and the association of physical function with WM, GM and WML volume and cognition. The effect of covariates and the relationship with Fazekas scores were planned secondary outcomes.

## 6.3 Results

### 6.3.1 Descriptive statistics and correlations

*Table 6-1: Descriptive statistics for neck muscle CSA, Mean (SD)*

|   | <b>Male<br/>(n=343)</b> | <b>Female<br/>(n=298)</b> | <b>Total<br/>(n=641)</b> |
|---|-------------------------|---------------------------|--------------------------|
| <b>Sternocleidomastoid CSA (cm<sup>2</sup>)</b> | 5.0 (1.1)               | 3.9 (0.8)                 | 4.5 (1.2)                |
| <b>Combined group CSA (cm<sup>2</sup>)</b>      | 20.8 (3.6)              | 14.3 (2.4)                | 17.7 (4.5)               |
| <b>Total neck muscle CSA (cm<sup>2</sup>)</b>   | 25.8 (4.2)              | 18.1 (2.8)                | 22.2 (5.3)               |

Independent t tests between male and female for all neck muscle CSA measurements were all  $p < 0.0001$

The Pearson correlation coefficient for grip strength in the dominant hand and non-dominant hand was 0.92 ( $p < 0.000001$ ), therefore grip strength for the dominant hand was used in all analyses. The dominant hand was: 94.2% right, 5.5% left and 0.4% ambidextrous (if ambidextrous, right hand measure was used for analyses).

*Table 6-2: Descriptive statistics for grip strength and 6 metre walk test, Mean (SD)*

|                               | <b>Male</b> | <b>Female</b> | <b>Total</b> |
|-------------------------------|-------------|---------------|--------------|
| <b>Grip strength (kg)</b>     | n=423       | n=395         | n=818        |
|                               | 35.4 (6.8)  | 21.2 (5.4)    | 28.5 (9.4)   |
| <b>6 metre walk (seconds)</b> | n=447       | n=413         | n=860        |
|                               | 4.13 (1.12) | 4.60 (1.46)   | 4.35 (1.32)  |

Independent t test comparing means for physical function variables between men and women were both  $p < 0.0001$

*Table 6-3: Descriptive statistics for brain structure variables*

|  | <b>Male<br/>n=361</b> | <b>Female<br/>n=324</b> | <b>Total<br/>n=685</b> |
|--|-----------------------|-------------------------|------------------------|
| <b>Normal appearing white matter volume (cm<sup>3</sup>)<sup>a</sup></b> | 521.7 (84.1)          | 465.3 (70.0)            | 495.1 (82.5)           |
| <b>Grey matter volume (cm<sup>3</sup>)<sup>a</sup></b>                   | 521.5 (70.8)          | 475.5 (62.8)            | 499.9 (70.9)           |
| <b>WML volume (cm<sup>3</sup>)*<sup>b</sup></b>                          | 7.9 (3.8-17.2)        | 7.3 (3.2-16.7)          | 7.7 (3.6-17.0)         |
| <b>WML score: Fazekas periventricular (0-3)<sup>a</sup></b>              | 1.35 (0.63)           | 1.36 (0.67)             | 1.36 (0.65)            |
| <b>WML score: Fazekas deep (0-3)<sup>a</sup></b>                         | 1.02 (0.63)           | 1.17 (0.71)             | 1.09 (0.67)            |

Mean (SD) given except for \* where median and interquartile range given

a Independent t test comparing means for men and women p<0.0001

b Independent samples Mann-Whitney U test comparing men and women p>0.05

*Table 6-4: Descriptive statistics for cognitive test scores*

|                   | <b>n</b> | <b>Median (interquartile range)</b> |
|-------------------|----------|-------------------------------------|
| <b>IQ age 11*</b> | 1028     | 101.7 (91.1-111.0)                  |

A Mann-Whitney U test found no significant difference between men and women for IQ age 11 (p>0.05)

The three factors measuring cognitive function were standardised to give a mean of 0 and a SD of 1.0. The sample sizes were as follows: G cognition (n=848); G processing speed (n=813); and G memory (n=823). Cognitive test scores did not significantly vary with sex (independent samples t test comparing men and women p>0.05).

Table 6-5: Descriptive statistics for covariates

|   | <b>n</b> | <b>Mean (SD)</b>  |
|---|----------|---|
| <b>Age at MR brain (years)</b>                    | 705      | 72.7 (0.7)  |
| <b>Sex</b>  | 1091     | Male 50.2%, Female 49.8%  |
| <b>Intracranial volume (cm<sup>2</sup>)</b>       | 679      | 1451.0 (140.6)  |
| <b>Systolic BP (sitting, mmHg)</b>                | 863      | 148.7 (18.8)  |
| <b>Diastolic BP (sitting, mmHg)</b>               | 863      | 78.0 (9.8)  |
| <b>ABPI</b>                                       | 756      | 1.09 (0.18)   |
| <b>Hypertension</b>                               | 866      | No 50.9%, Yes 49.1%   |
| <b>Hypercholesterolaemia</b>                      | 866      | No 58.9%, Yes 41.1%   |
| <b>Previous stroke</b>                            | 866      | No 93.6%, Yes 6.4%  |
| <b>Diabetes mellitus</b>                          | 866      | No 89.0%, Yes 11.0%   |
| <b>Cardiovascular disease</b>                     | 866      | No 71.1%, Yes 28.9%   |
| <b>Total chol (mmol/L)</b>                        | 832      | 5.15 (1.15)   |
| <b>chol:HDL ratio</b>                             | 832      | 3.75 (1.12)   |
| <b>HbA1c (%)</b>                                  | 826      | 5.75 (0.66)   |
| <b>CRP (mg/L)*</b>                                | 830      | 1.5 (1.5-6.0)   |
| <b>eGFR (ml/min)</b>                              | 820      | eGFR>60mL/min 87.1%<br>eGFR<60mL/min 12.9%  |
| <b>Salivary cortisol (waking) (nmol/L)</b>        | 89       | 24.0 (10.6)   |
| <b>Salivary cortisol (evening) (nmol/L)</b>       | 89       | 5.3 (9.1)   |
| <b>Salivary cortisol (diurnal slope) (nmol/L)</b> | 89       | -18.7 (14.6)  |
| <b>s100 (microg/L)</b>                            | 834      | 0.09 (0.05)   |
| <b>IL-6 (pg/mL)*</b>                              | 815      | 1.55 (1.04-2.35)  |
| <b>Years of education (years)</b>                 | 1091     | 10.7 (1.1)  |
| <b>Social class</b>                               | 1070     | I 17.8%<br>II 37.6%<br>III (skilled) 23.0%<br>III (unskilled)17.6%<br>IV 3.6%<br>V 0.6% |
| <b>Overcrowding index (people/room)*</b>          | 1088     | 1.25 (0.80-1.67)  |
| <b>Indoor vs. out toilet</b>                      | 1089     | Indoor 88.4%, Outdoor 11.6%   |
| <b>Alcohol consumption (yes/no)</b>               | 866      | No 12.0%, Yes 88.0%   |
| <b>Alcohol frequency (days/week)*</b>             | 759      | 2 (1-6)   |
| <b>Alcohol units/week*</b>                        | 764      | 7.0 (1.5-16.0)  |
| <b>Smoking status</b>                             | 866      | Never smoked 47.2%<br>Ex-smoker 44.3%<br>Current smoker 8.4%                            |
| <b>Days of exercise/month*</b>                    | 956      | 5.75 (0.0-12.0)   |
| <b>Telomere length (base pairs)</b>               | 844      | 3966.3 (737.8)  |
| <b>APOE4</b>                                      | 1028     | No E4 allele 70.2%, E4 allele 29.8%   |

\*median (interquartile range)

Table 6-6: Pearson's (white) and Spearman's (shaded) correlations for the main brain and muscle variables from LBC 1936 (n)

|                            |                        |                      |                     |                  |                  |                   |                    |                     |                  |                    |                            |                 |
|----------------------------|------------------------|----------------------|---------------------|------------------|------------------|-------------------|--------------------|---------------------|------------------|--------------------|----------------------------|-----------------|
|                            | <b>Neck muscle CSA</b> |                      |                     |                  |                  |                   |                    |                     |                  |                    |                            |                 |
| <b>Neck muscle CSA</b>     | 1<br>(641)             | <b>Grip strength</b> |                     |                  |                  |                   |                    |                     |                  |                    |                            |                 |
| <b>Grip strength</b>       | 0.62**<br>(607)        | 1<br>(820)           | <b>6 metre walk</b> |                  |                  |                   |                    |                     |                  |                    |                            |                 |
| <b>6 metre walk</b>        | -0.10*<br>(635)        | -0.30**<br>(815)     | 1<br>(860)          | <b>WM volume</b> |                  |                   |                    |                     |                  |                    |                            |                 |
| <b>WM volume</b>           | 0.18**<br>(636)        | 0.36**<br>(639)      | -0.20**<br>(669)    | 1<br>(673)       | <b>GM volume</b> |                   |                    |                     |                  |                    |                            |                 |
| <b>GM volume</b>           | 0.26**<br>(637)        | 0.28**<br>(639)      | -0.14**<br>(669)    | 0.03<br>(671)    | 1<br>(673)       | <b>WML volume</b> |                    |                     |                  |                    |                            |                 |
| <b>WML volume</b>          | 0.03<br>(637)          | -0.05<br>(639)       | 0.19**<br>(669)     | -0.06<br>(672)   | 0.00<br>(672)    | 1.00<br>(673)     | <b>Fazekas PVH</b> |                     |                  |                    |                            |                 |
| <b>Fazekas PVH</b>         | -0.01<br>(637)         | -0.06<br>(650)       | 0.12**<br>(680)     | -0.02<br>(673)   | -0.10*<br>(673)  | 0.69**<br>(673)   | 1.00<br>(685)      | <b>Fazekas Deep</b> |                  |                    |                            |                 |
| <b>Fazekas Deep</b>        | -0.10*<br>(637)        | -0.12**<br>(650)     | 0.16**<br>(680)     | -0.05<br>(673)   | -0.09*<br>(673)  | 0.64**<br>(673)   | 0.49**<br>(685)    | 1<br>(685)          | <b>IQ age 11</b> |                    |                            |                 |
| <b>IQ age 11</b>           | -0.09*<br>(607)        | 0.01<br>(773)        | -0.17**<br>(810)    | 0.12**<br>(637)  | 0.01<br>(637)    | -0.05<br>(637)    | -0.08*<br>(648)    | -0.03<br>(648)      | 1<br>(1028)      | <b>G cognition</b> |                            |                 |
| <b>G cognition</b>         | -0.02<br>(631)         | 0.15**<br>(804)      | -0.30**<br>(843)    | 0.23**<br>(664)  | 0.10**<br>(664)  | -0.14**<br>(664)  | -0.13**<br>(675)   | -0.07<br>(675)      | 0.57**<br>(800)  | 1<br>(847)         | <b>G process-ing speed</b> |                 |
| <b>G process-ing speed</b> | -0.07<br>(610)         | 0.12**<br>(774)      | -0.31**<br>(809)    | 0.23**<br>(643)  | 0.13**<br>(644)  | -0.21**<br>(643)  | -0.15**<br>(654)   | -0.12**<br>(654)    | 0.39**<br>(767)  | 0.75**<br>(806)    | 1<br>(813)                 | <b>G memory</b> |
| <b>G memory</b>            | -0.08<br>(607)         | 0.07<br>(779)        | -0.20**<br>(819)    | 0.11**<br>(639)  | 0.10*<br>(639)   | -0.11**<br>(639)  | -0.09*<br>(650)    | -0.03<br>(650)      | 0.52**<br>(777)  | 0.68**<br>(810)    | 0.46**<br>(781)            | 1<br>(823)      |

\*Correlation is significant at the 0.05 level (2-tailed), \*\*Correlation is significant at the 0.01 level (2-tailed)



### 6.3.2 Relationship between muscle size and cognition

Table 6-7: Multivariate General Linear Model (GLM) of neck muscle CSA predicting cognitive function

| Independent variables        | Dependent variables |        |                     |                    |        |                     |          |        |                     |
|------------------------------|---------------------|--------|---------------------|--------------------|--------|---------------------|----------|--------|---------------------|
|                              | G cognition         |        |                     | G processing speed |        |                     | G memory |        |                     |
|                              | t                   | sig    | Partial eta squared | t                  | sig    | Partial eta squared | t        | sig    | Partial eta squared |
| <b>Age</b>                   | -2.47               | 0.01   | 1.1%                | -3.24              | 0.001  | 1.9%                | -1.21    | >0.05  | 0.3%                |
| <b>Sex (M=1, F=2)</b>        | 1.22                | >0.05  | 0.3%                | 0.62               | >0.05  | 0.1%                | 0.04     | >0.05  | <0.1%               |
| <b>Age 11 IQ</b>             | 17.09               | <0.001 | 35.0%               | 10.20              | <0.001 | 16.1%               | 14.2     | <0.001 | 27.0%               |
| <b>Total neck muscle CSA</b> | -0.29               | >0.05  | <0.1%               | -1.09              | >0.05  | 0.2%                | -0.51    | >0.05  | <0.1%               |

Pillai's Trace for neck muscle CSA and overall model for three cognitive measures (F(3,541)=0.60, p>0.05, partial eta squared 0.3%)

As there were no significant relationships between total neck muscle CSA and cognitive function, further testing of the model with potential covariates was not performed.

### 6.3.3 Relationship between muscle function and cognition

Table 6-8: Multivariate GLM of physical function predicting cognitive function

| Independent variables | Dependent variables |                  |                     |                    |                  |                     |          |        |                     |
|-----------------------|---------------------|------------------|---------------------|--------------------|------------------|---------------------|----------|--------|---------------------|
|                       | G cognition         |                  |                     | G processing speed |                  |                     | G memory |        |                     |
|                       | t                   | sig              | Partial eta squared | t                  | sig              | Partial eta squared | t        | sig    | Partial eta squared |
| Age                   | -1.93               | >0.05            | 0.6%                | -2.33              | 0.02             | 0.9%                | -0.83    | >0.05  | 0.1%                |
| Sex (M=1, F=2)        | -2.36               | 0.019            | 0.9%                | -2.69              | 0.007            | 1.2%                | -2.02    | 0.044  | 0.7%                |
| Age 11 IQ             | 16.59               | <0.001           | 31.9%               | 9.16               | <0.001           | 12.5%               | 13.89    | <0.001 | 24.8%               |
| Grip strength         | <b>2.98</b>         | <b>0.003</b>     | <b>1.5%</b>         | <b>2.04</b>        | <b>0.042</b>     | <b>0.7%</b>         | 1.66     | >0.05  | 0.5%                |
| 6MWT                  | <b>-3.61</b>        | <b>&lt;0.001</b> | <b>2.2%</b>         | <b>-5.70</b>       | <b>&lt;0.001</b> | <b>5.3%</b>         | -1.93    | >0.05  | 0.6%                |

Pillai's Trace for grip strength and overall model for three cognitive measures (F(3,584)=2.97, p=0.03, partial eta squared 1.5%)

Pillai's Trace for 6MWT and overall model for three cognitive measures (F(3,584)=10.87, p<0.001, partial eta squared 5.3%)

Table 6-9: Parameter estimates for the effect size of grip strength and 6MWT on the individual cognitive measures

| Independent variables | Dependent variables |       |              |                    |       |              |          |       |            |
|-----------------------|---------------------|-------|--------------|--------------------|-------|--------------|----------|-------|------------|
|                       | G cognition         |       |              | G processing speed |       |              | G memory |       |            |
|                       | B                   | sig   | 95% CI       | B                  | sig   | 95% CI       | B        | sig   | 95% CI     |
| Grip strength (kg)    | 0.02                | <0.01 | 0.01-0.03    | 0.01               | 0.04  | 0.00-0.02    | 0.01     | >0.05 | 0.00-0.02  |
| 6MWT (seconds)        | -0.10               | <0.01 | -0.15- -0.05 | -0.18              | <0.01 | -0.24- -0.12 | -0.06    | >0.05 | -0.12-0.00 |

Potential covariates were added to the model to assess their role as a possible explanatory factor in the relationship. Potential covariates were identified through both hypothesis generated associations and identifying covariables through correlation matrices. The following covariates were investigated in each of the following models to see if they explained the association between the muscle and brain variables.

*Table 6-10: Table listing the covariates which were tested to investigate whether they explained any found association between the muscle and brain variables in each of the following models*

| <b>Social factors</b>             | <b>Physical measures</b>             | <b>Comorbidity</b>     | <b>Blood and salivary markers</b>                      | <b>Lifestyle factors</b>                            |
|-----------------------------------|--------------------------------------|------------------------|--|---|
| Years of education                | Systolic blood pressure              | Hypertension           | Cholesterol  | Drink alcohol yes/no                                |
| Social class                      | Diastolic blood pressure             | Diabetes mellitus      | Total cholesterol: high density lipoprotein ratio      | Number of days alcohol consumed per week            |
| Overcrowding index                | Ankle:brachial pressure index (abpi) | Hypercholesterolaemia  | Glycated haemoglobin (hba1c)                           | Number of units of alcohol consumed per week        |
| Indoor vs. Outdoor toilet age 11y |                                      | Cardiovascular disease | C reactive protein (crp),                              | Smoking status (current, ex, never)                 |
|                                   |                                      | Previous stroke        | Estimated glomerular filtration rate (egfr)            | Physical activity (number of days active per month) |
|                                   |                                      |                        | S100   |   |
|                                   |                                      |                        | Interleukin-6 (IL-6),                                  |   |
|                                   |                                      |                        | Telomere length  |   |
|                                   |                                      |                        | Apolipoprotein E4 (apoe4) carrier status               |   |
|                                   |                                      |                        | Salivary cortisol (waking, evening and diurnal slope)) |   |

From the above covariates (table 6-10), only the four included in the table below were found to significantly predict the three cognitive measures in the multivariate model investigating the association between cognitive function, grip strength and the 6MWT.

*Table 6-11: Effect of covariates added to Multivariate GLM of physical function predicting cognitive function*

| <b>Covariable</b>         | <b>n</b> | <b>Sig</b> | <b>Partial eta squared for covariate</b> | <b>Δ partial eta squared for grip strength</b> | <b>Δ partial eta squared for 6MWT</b> |
|---------------------------|----------|------------|--|--|---------------------------------------|
| <b>Years of education</b> | 592      | 0.008      | 2.0%                                     | -0.2%  | 0.0%                                  |
| <b>Social class</b>       | 582      | 0.004      | 1.9%                                     | 0.0%   | -0.5%                                 |
| <b>APOE4 carrier</b>      | 563      | 0.003      | 2.4%                                     | 0.0%   | 0.2%                                  |
| <b>Smoking status</b>     | 592      | 0.004      | 1.6%                                     | 0.0%   | 0.0%                                  |

However, these covariates do not act as explanatory variables for the relationship between cognitive function and grip strength or 6MWT as they did not modify the partial eta squared by more than 1% (which I took as the minimum change required to show a meaningful effect).

Evening salivary cortisol is not a significant predictor of overall cognitive function but it does act as an explanatory variable in the relationship between 6MWT and overall cognitive function (partial eta squared decreased from 11.7% to 9.0%). Within univariate models of cognitive function, evening salivary cortisol acts as explanatory variable between 6MWT and processing speed (partial eta squared 11.1% to 8.6%), but not for cognition or memory. The delta partial eta squared was calculated by first running the baseline model only including subjects where the covariable was measured and then running the model including the covariable. This was to ensure that any changes in partial eta squared were not just accounted for by changes in the sample size. However, evening cortisol does not mediate the effect of processing speed on 6MWT (Sobel's test statistic -1.29 (SE 0.02)  $p > 0.05$ ).

The other two measures of salivary cortisol did not act as explanatory variables for any of the other relationships between 6MWT or grip strength and either overall cognitive function or its univariate measures.

### 6.3.4 Relationship between muscle size and brain structure

Table 6-12: Multivariate GLM of neck muscle CSA predicting brain structure

| Independent variables        | Dependent variables                  |              |                     |                    |        |                     |
|------------------------------|--------------------------------------|--------------|---------------------|--------------------|--------|---------------------|
|                              | Normal appearing white matter volume |              |                     | Grey matter volume |        |                     |
|                              | t                                    | sig          | Partial eta squared | t                  | sig    | Partial eta squared |
| Age                          | -5.92                                | <0.001       | 5.3%                | 4.21               | <0.001 | 2.7%                |
| Sex (M=1, F=2)               | 0.38                                 | >0.05        | <0.1%               | -0.56              | >0.05  | <0.1%               |
| ICV                          | 15.28                                | <0.001       | 27.0%               | 10.72              | <0.001 | 15.4%               |
| <b>Total neck muscle CSA</b> | <b>-3.02</b>                         | <b>0.003</b> | <b>1.4%</b>         | 1.04               | >0.05  | 0.2%                |

Pillai's Trace for neck muscle CSA and overall model for two measures of brain structure (F(2,630)=4.56, p=0.01, partial eta squared 1.4%)

Table 6-13: Parameter estimates for the effect size of total neck muscle CSA on the individual brain structure measures

| Independent variables                         | Dependent variables                    |       |        |       |                                       |       |        |      |
|---|--|-------|--------|-------|---------------------------------------|-------|--------|------|
|   | White matter volume (cm <sup>3</sup> ) |       |        |       | Grey matter volume (cm <sup>3</sup> ) |       |        |      |
|   | B                                      | sig   | 95% CI |       | B                                     | sig   | 95% CI |      |
| <b>Total neck muscle CSA (cm<sup>2</sup>)</b> | -0.02                                  | 0.003 | -0.04- | -0.01 | 0.01                                  | >0.05 | -0.01- | 0.02 |

From the above listed covariates (table 6-10), only the six included in the table below were found to significantly predict the two measures of brain structure (ie white matter and grey matter volume) in the multivariate model.

Table 6-14: Effect of covariates added to Multivariate GLM of neck CSA predicting brain structure

| Covariable                              | n   | Sig   | Partial eta squared for covariate | Δ partial eta squared for neck CSA |
|---|-----|-------|-----------------------------------|------------------------------------|
| <b>Diagnosis of diabetes mellitus</b>   | 636 | 0.04  | 1.0%                              | -0.1%                              |
| <b>HbA1c</b>                            | 611 | 0.02  | 1.2%                              | -0.3%                              |
| <b>CRP</b>                              | 613 | <0.01 | 2.2%                              | -0.1%                              |
| <b>IL-6</b>                             | 603 | <0.01 | 2.1%                              | -0.2%                              |
| <b>Alcohol (days consumed per week)</b> | 498 | 0.04  | 1.3%                              | 0.2%                               |
| <b>Smoking status</b>                   | 636 | 0.02  | 1.0%                              | 0.1%                               |

However, none of these covariates act as explanatory variables for the relationship between overall brain structure and neck CSA or univariate measures of brain structure (ie WM or GM volume) and neck CSA.

### 6.3.5 Relationship between muscle function and brain structure

Table 6-15: Multivariate GLM of physical function predicting brain structure

| Independent variables | Dependent variables                  |              |                     |                    |              |                     |
|-----------------------|--------------------------------------|--------------|---------------------|--------------------|--------------|---------------------|
|                       | Normal appearing white matter volume |              |                     | Grey matter volume |              |                     |
|                       | t                                    | sig          | Partial eta squared | t                  | sig          | Partial eta squared |
| <b>Age</b>            | -4.97                                | <0.001       | 3.8%                | 3.97               | <0.001       | 2.4%                |
| <b>Sex (M=1, F=2)</b> | -3.09                                | 0.002        | 1.5%                | -0.55              | >0.05        | 0.0%                |
| <b>ICV</b>            | 14.36                                | <0.001       | 24.7%               | 11.12              | <0.001       | 16.4%               |
| <b>Grip strength</b>  | <b>2.62</b>                          | <b>0.009</b> | <b>1.1%</b>         | 0.89               | >0.05        | 0.1%                |
| <b>6MWT</b>           | <b>-2.01</b>                         | <b>0.045</b> | <b>0.6%</b>         | <b>-2.36</b>       | <b>0.018</b> | <b>0.9%</b>         |

Pillai's Trace for grip strength and overall model for two measures of brain structure (F(2,627)=5.40, p=0.005, partial eta squared 1.7%)

Pillai's Trace for 6MWT and overall model for two measures of brain structure (F(2,627)=7.58, p=0.001, partial eta squared 2.4%)

*Table 6-16: Parameter estimates for the effect size of grip strength and 6MWT on the individual brain structure measures*

| Independent variables     | Dependent variables                    |       |              |                                       |       |              |
|---------------------------|--|-------|--------------|---------------------------------------|-------|--------------|
|                           | White matter volume (cm <sup>3</sup> ) |       |              | Grey matter volume (cm <sup>3</sup> ) |       |              |
|                           | B                                      | sig   | 95% CI       | B                                     | sig   | 95% CI       |
| <b>Grip strength (kg)</b> | 1.16                                   | 0.009 | 0.29-2.04    | 0.37                                  | >0.05 | -0.45- 1.18  |
| <b>6MWT (seconds)</b>     | -4.51                                  | 0.045 | -8.92- -0.10 | -4.95                                 | 0.018 | -9.07- -0.84 |

From the listed covariates (table 6-10), only the four included in the table below were found to significantly predict the two measures of brain structure (ie white matter and grey matter volume) in the multivariate model.

*Table 6-17: Effect of covariates added to Multivariate GLM of physical function predicting brain structure*

| Covariable                              | n   | Sig  | Partial eta squared for covariate | Δ partial eta squared for grip strength | Δ partial eta squared for 6MWT |
|---|-----|------|-----------------------------------|---|--------------------------------|
| <b>HbA1c</b>                            | 610 | 0.02 | 1.3%                              | 0.0%                                    | -0.2%                          |
| <b>CRP</b>                              | 612 | 0.02 | 1.3%                              | -0.2%                                   | -0.4%                          |
| <b>Alcohol (days consumed per week)</b> | 563 | 0.02 | 1.5%                              | 0.0%                                    | 0.2%                           |
| <b>Smoking status</b>                   | 634 | 0.02 | 0.9%                              | -0.1%                                   | -0.2%                          |

However, none of the covariates act as explanatory variables for the relationship between brain structure and grip strength or 6MWT in the multivariate or univariate models.

Neither evening salivary or diurnal cortisol slope act as a significant predictor of brain volume, but they may act as explanatory variables in the relationship between 6MWT and brain structure (partial eta squared decreased from 5.5% to 4.1% for evening cortisol and from 5.4% to 3.8% for diurnal slope). However there was no univariate effect.

### 6.3.6 Relationship between muscle size and function and WML

Table 6-18: Univariate GLM of neck muscle CSA predicting WML volume

| Independent variables | Dependent variable            |       |                     |
|-----------------------|-------------------------------|-------|---------------------|
|                       | WML volume (mm <sup>3</sup> ) |       |                     |
|                       | t                             | sig   | Partial eta squared |
| Age                   | 3.36                          | 0.001 | 1.8%                |
| Sex (M=1, F=2)        | -2.20                         | 0.028 | 0.8%                |
| ICV                   | 2.43                          | 0.015 | 0.9%                |
| Total neck muscle CSA | 1.28                          | >0.05 | 0.3%                |

The partial eta squared for the corrected model is 2.7% (p 0.002).

As there were no significant relationships between total neck muscle CSA and WML volume, further testing of the model with potential covariates was not performed.

Table 6-19: Univariate GLM of physical function predicting WML volume

| Independent variables | Dependent variable            |              |                     |
|-----------------------|-------------------------------|--------------|---------------------|
|                       | WML volume (mm <sup>3</sup> ) |              |                     |
|                       | t                             | sig          | Partial eta squared |
| Age                   | 3.29                          | 0.001        | 1.7%                |
| Sex (M=1, F=2)        | -0.47                         | >0.05        | 0.0%                |
| ICV                   | 2.94                          | 0.003        | 1.4%                |
| Grip strength         | -0.90                         | >0.05        | 0.1%                |
| 6MWT                  | <b>2.88</b>                   | <b>0.004</b> | <b>1.3%</b>         |

The partial eta squared for the corrected model is 2.7% (p 0.002).



*Table 6-20: Parameter estimates for the effect size of grip strength and 6MWT on WML volume*

| Independent variables     | Dependent variables                           |       |              |
|---------------------------|---|-------|--------------|
|                           | White matter lesion volume (mm <sup>3</sup> ) |       |              |
|                           | B   | sig   | 95% CI       |
| <b>Grip strength (kg)</b> | -72.7   | >0.05 | -235.8- 90.4 |
| <b>6MWT (seconds)</b>     | 1161.2  | <0.01 | 366.7-1955.8 |

From the listed covariates (table 6-10), only the three included in the table below were found to significantly predict white matter lesion volume in the univariate model.

*Table 6-21: Effect of covariates added to Univariate GLM of physical function predicting WML volume*

| Covariable                                 | n   | Sig   | Partial eta squared for covariate | Δ partial eta squared for grip strength | Δ partial eta squared for 6MWT | Δ partial eta squared for the corrected model |
|--|-----|-------|-----------------------------------|---|--------------------------------|---|
| <b>Diagnosis of hypertension</b>           | 636 | <0.01 | 1.4%                              | 0.0%                                    | -0.3%                          | 1.3%  |
| <b>Total chol:HDL ratio</b>                | 614 | 0.02  | 0.9%                              | 0.0%                                    | 0.0%                           | 0.8%  |
| <b>Smoking status (current, ex, never)</b> | 636 | <0.01 | 1.7%                              | 0.0%                                    | -0.1%                          | 1.5%  |

However, none of these covariates act as explanatory variables for the relationship between brain structure and grip strength or 6MWT.

Interestingly adding waking salivary cortisol and diurnal cortisol slope to the model did increase the partial eta squared for the corrected model from 6.6% to 8.7% and from 6.6% to 7.7% respectively, despite neither measure being a significant predictor of WML volume.

*Table 6-22: Multinomial logistic regression for neck muscle CSA and PVH Fazekas score*

| PVH Fazekas Score | Predictor          | Male |       |            |               | Female |       |            |               |
|-------------------|--------------------|------|-------|------------|---------------|--------|-------|------------|---------------|
|                   |                    | n    | Sig   | Odds ratio | 95% CI for OR | n      | Sig   | Odds ratio | 95% CI for OR |
| 0                 | Reference category | 12   | -     | -          | -             | 8      | -     | -          | -             |
| 1                 | Neck muscle CSA    | 217  | <0.01 | 0.998      | 0.997-0.999   | 193    | >0.05 | 1.003      | 1.000-1.006   |
| 2                 | Neck muscle CSA    | 93   | <0.01 | 0.998      | 0.997-0.999   | 73     | >0.05 | 1.003      | 1.000-1.006   |
| 3                 | Neck muscle CSA    | 19   | >0.05 | 0.999      | 0.997-1.000   | 22     | >0.05 | 1.002      | 0.999-1.005   |

Adjusted for age at MRI

*Table 6-23: Multinomial logistic regression for neck muscle CSA and deep Fazekas score*

| Deep Fazekas Score | Predictor          | Male |       |            |               | Female |       |            |               |
|--------------------|--------------------|------|-------|------------|---------------|--------|-------|------------|---------------|
|                    |                    | n    | Sig   | Odds ratio | 95% CI for OR | n      | Sig   | Odds ratio | 95% CI for OR |
| 0                  | Reference category | 61   | -     | -          | -             | 37     | -     | -          | -             |
| 1                  | Neck muscle CSA    | 220  | >0.05 | 0.999      | 0.997-0.999   | 181    | >0.05 | 1.000      | 0.998-1.002   |
| 2                  | Neck muscle CSA    | 55   | >0.05 | 0.999      | 0.997-0.999   | 63     | >0.05 | 1.000      | 0.998-1.002   |
| 3                  | Neck muscle CSA    | 5    | >0.05 | 0.999      | 0.997-0.999   | 15     | >0.05 | 1.000      | 0.998-1.002   |

Adjusted for age at MRI

*Table 6-24: Multinomial logistic regression for grip strength and 6MWT with PVH Fazekas score*

| PVH Fazekas Score | Predictor          | Male |       |            |               | Female |             |             |                  |
|-------------------|--------------------|------|-------|------------|---------------|--------|-------------|-------------|------------------|
|                   |                    | n    | Sig   | Odds ratio | 95% CI for OR | n      | Sig         | Odds ratio  | 95% CI for OR    |
| <b>0</b>          | Reference category | 11   | -     | -          | -             | 9      | -           | -           | -                |
| <b>1</b>          | Grip strength      | 220  | >0.05 | 1.04       | 0.95-1.14     | 200    | >0.05       | 0.92        | 0.80-1.05        |
|                   | 6MWT               |      | >0.05 | 0.99       | 0.54-1.80     |        | >0.05       | 1.09        | 0.54-2.20        |
| <b>2</b>          | Grip strength      | 93   | >0.05 | 1.04       | 0.94-1.14     | 75     | >0.05       | 0.92        | 0.79-1.05        |
|                   | 6MWT               |      | >0.05 | 1.26       | 0.68-2.32     |        | >0.05       | 1.17        | 0.58-2.39        |
| <b>3</b>          | Grip strength      | 16   | >0.05 | 1.04       | 0.92-1.16     | 22     | <b>0.03</b> | <b>0.84</b> | <b>0.72-0.99</b> |
|                   | 6MWT               |      | >0.05 | 1.63       | 0.83-3.19     |        | >0.05       | 1.12        | 0.54-2.36        |

Adjusted for age at MRI

Results not altered when performing regression separately for grip strength and 6MWT

*Table 6-25: Multinomial logistic regression for grip strength and 6MWT with deep Fazekas score*

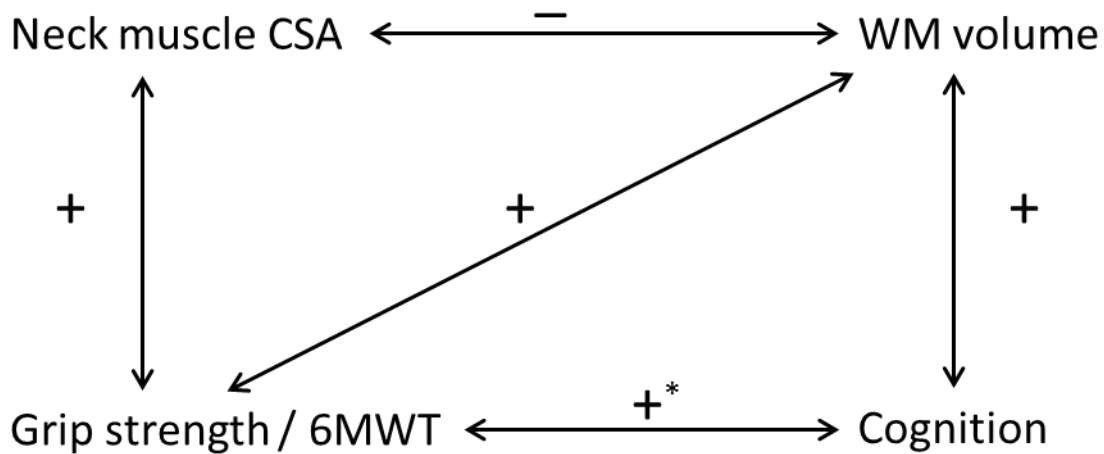
| Deep Fazekas Score | Grip/6MWT          | Male |             |             |                  | Female |       |            |               |
|--------------------|--------------------|------|-------------|-------------|------------------|--------|-------|------------|---------------|
|                    |                    | n    | Sig         | Odds ratio  | 95% CI for OR    | n      | Sig   | Odds ratio | 95% CI for OR |
| <b>0</b>           | Reference category | 59   | -           | -           | -                | 40     | -     | -          | -             |
| <b>1</b>           | Grip strength      | 221  | >0.05       | 1.04        | 0.99-1.08        | 191    | >0.05 | 1.02       | 0.95-1.09     |
|                    | 6MWT               |      | >0.05       | 1.35        | 0.97-1.87        |        | >0.05 | 1.03       | 0.77-1.37     |
| <b>2</b>           | Grip strength      | 55   | >0.05       | 1.02        | 0.96-1.08        | 61     | >0.05 | 0.95       | 0.88-1.03     |
|                    | 6MWT               |      | <b>0.01</b> | <b>1.62</b> | <b>1.11-2.35</b> |        | >0.05 | 0.97       | 0.70-1.34     |
| <b>3</b>           | Grip strength      | 5    | >0.05       | 1.01        | 0.88-1.16        | 14     | >0.05 | 0.91       | 0.81-1.02     |
|                    | 6MWT               |      | <b>0.03</b> | <b>2.02</b> | <b>1.09-3.73</b> |        | >0.05 | 0.93       | 0.59-1.46     |

Adjusted for age at MRI

Results not altered when performing regression separately for grip strength and 6MWT

### 6.3.7 Interrelationships between muscle structure and function and brain structure and function

Figure 6-1: Model representing significant interrelationships between muscle structure and function, WM volume and cognition

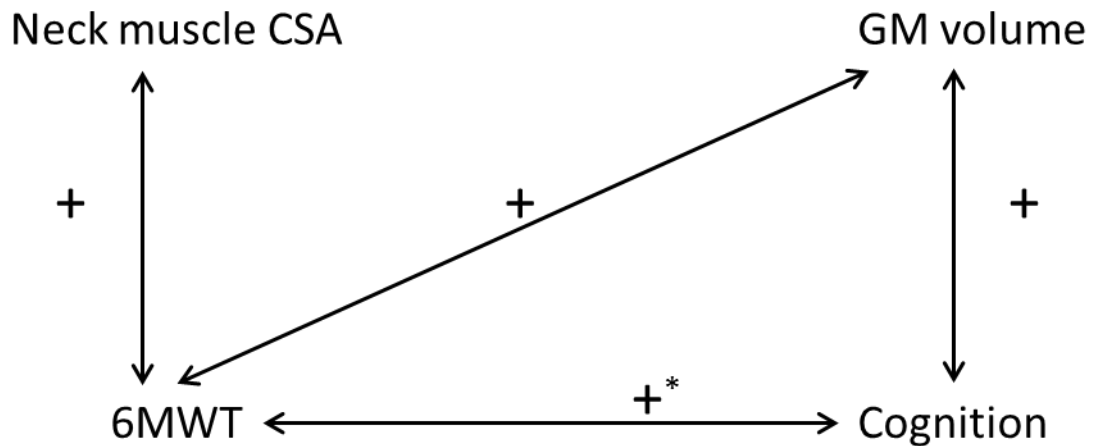


\*But no significant relationship between grip strength and 6MWT and G memory

Neck muscle CSA does not act as a mediator between grip strength and WM volume (Sobel test statistic -0.67, SE 269.47,  $p > 0.05$ ) but does act as a mediator between the 6MWT and WM volume (Sobel test statistic -2.17, SE 572.07,  $p = 0.03$ ).

WM volume acts as a mediator in the relationship between grip strength and G cognition (Sobel test statistic 2.46, SE 0.003,  $p = 0.01$ ) and G processing speed (Sobel test statistic 2.54, SE 0.003,  $p = 0.01$ ), and in the relationship between 6MWT and both G cognition (Sobel test statistic 3.56, SE 0.008,  $p = 0.0004$ ) and G processing speed (Sobel test statistic -3.55, SE 0.008,  $p = 0.0004$ ).

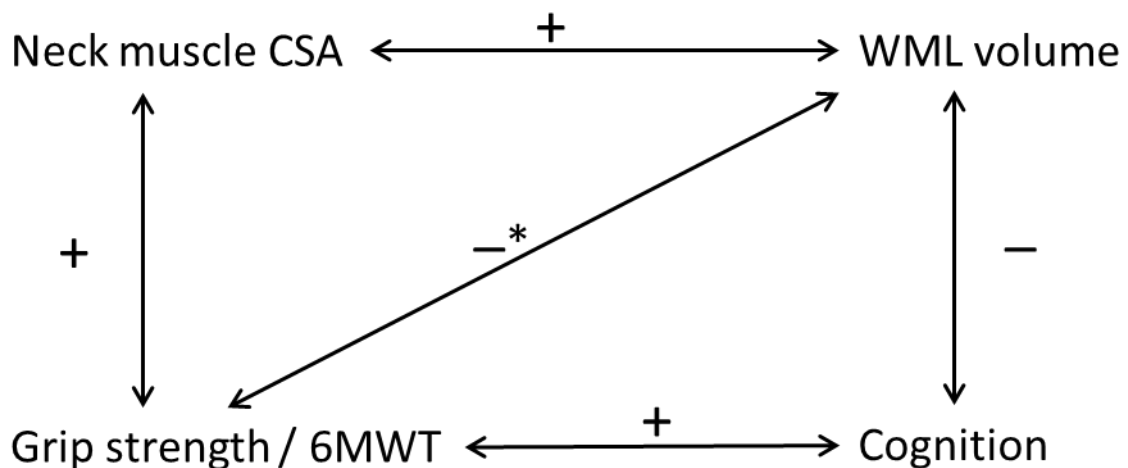
**Figure 6-2: Model representing significant interrelationships between muscle structure and function, GM volume and cognition**



\*But no significant relationship between grip strength and 6MWT and G memory

GM volume does not act as a mediator between 6MWT and G cognition (Sobel test statistic - 1.26, SE 0.008,  $p > 0.05$ ) or G processing speed (Sobel test statistic -0.96, SE 0.008,  $p > 0.05$ ).

**Figure 6-3: Model representing significant interrelationships between muscle structure and function, WML volume and cognition**



\* But no significant relationship with grip strength

Neck muscle CSA does not act as a mediator between the 6MWT and WML volume (Sobel test statistic -0.87, SE 47.62,  $p > 0.05$ ). WML volume does act as a mediator between 6MWT

and G cognition (Sobel test statistic -2.21, SE 0.005,  $p=0.03$ ) and G processing speed (Sobel test statistic -2.63, SE 0.006,  $p=0.009$ ).

## **6.4 Discussion**

### **6.4.1 Relationship between neck muscle CSA and cognition**

This analysis found no significant association between neck muscle CSA and cognition either as a whole or as individual measures of cognition (see table 6.7). This adds to previous work identified in our systematic review which found no association between muscle size and cognitive function (Kilgour et al., 2014). Interestingly I did find a negative correlation between age 11 IQ and neck muscle CSA. I previously found a negative association between NART, a measure of crystallised intelligence which is an indicator of childhood intelligence, and neck muscle CSA using data from the MHEM study, and I had postulated that perhaps those with a lower IQ were more likely to have a manual job and therefore develop larger neck muscles throughout their adult life (Kilgour et al., 2013). I was able to test that theory here as the LBC 1936 study contains data on social class. A Spearman's correlation does indeed find a significant association between social class and neck muscle CSA ( $\rho$  0.153,  $p < 0.001$ ), indicating that those from social class 5 have larger neck muscles than those from social class 1. Further detailed analyses of occupation may help explain this relationship further.

### **6.4.2 Relationship between physical function and cognition**

There was a significant association between markers of physical function (grip strength and 6MWT) and cognition as a whole and two of the individual measures. Those with stronger grip strength and faster 6MWT had better scores for G cognition and G processing speed, but there was no association with G memory. The relationships are stronger for 6MWT than grip strength, and whilst neither measure is a completely pure measure of muscle function, both being affected by joint problems, mood, comprehension and motivation, it may be considered that grip strength is a purer measure than 6MWT. Further analyses looking at predictors of 6MWT (eg comorbidity, HADS score) might further elucidate the differences between the two tests of physical function. In fact I found that a 1kg increase in grip strength would account for a 0.02 increase in G cognition score and a 0.01 increase in G processing speed, and that a 1 second faster 6MWT would equate to an increase of 0.10 in G cognition score and 0.18 in G processing speed.

Gait speed has previously been found to be associated with various markers of cognitive ability, including MMSE, DSST and a diagnosis of cognitive impairment/dementia (Abellan van Kan et al., 2009, Atkinson et al., 2007, Duff et al., 2008), but there is less evidence of the

relationship of gait speed to individual components of cognitive function. Similarly grip strength has previously been found to be associated with markers of cognitive ability including MMSE (Alfaro-Acha et al., 2006), the Community Screening Instrument of Dementia (Auyeung et al., 2008) and global cognition using multiple tests of cognitive function (Guerrero-Berroa et al., 2014). Interestingly the latter study found no association between episodic memory and grip strength, but did find an association with attention/working memory, language/semantic categorization and executive function. This supports our finding, that whilst certain components of cognition are associated with physical function, aspects of memory appear not to be. Studies of how cognition alters over the lifecourse have found that certain aspects decline over time whereas other aspects remain relatively robust. These studies are hampered by methodological issues; namely that either they are cross-sectional to allow researchers to look across the lifecourse or they are longitudinal and have mainly focused on older adults (Hedden and Gabrieli, 2004), therefore exact trajectories of individual cognitive functions across the lifecourse are yet to be firmly agreed upon. It is therefore interesting that we found a differential association of physical function with the different components of cognition, but difficult to speculate much further as to why this could be. It may be because the neural constructs underlying the variables overlap more for processing speed and cognition with walking and grip strength than they do with memory. Or it may be that factors which affect processing speed and cognition over the lifecourse, either intrinsic or extrinsic ageing factors, similarly affect gait speed and grip strength, whereas memory is more reliant on its trajectory of decline on factors which do not affect the other covariates. As larger longitudinal studies of cognition over the lifecourse emerge in the coming decades perhaps answers to these questions will become clearer.

Ogata et al looked at the relationship between grip strength and cognitive function using monozygotic and dizygotic twins and found that whilst there does appear to be certain common genetic factors which affect grip strength and tests of cognitive function, the heritability of grip strength is only 6% whereas cognition is much more heritable (47-71%) (Ogata et al., 2014). Therefore environmental factors must play a large role and previous studies have found that these factors tend to have specific effects on individual tests rather than the common effect genetic traits have on most cognitive tests (Deary et al., 2006b). None of the twenty six covariates I studied were explanatory factors in the relationship between cognitive and physical function. However evening cortisol, despite not being a significant predictor of cognitive function in the multivariate model did act as an explanatory



variable in the relationship between 6MWT and total cognitive score and the individual score for processing speed, but not for cognition or memory.

The sample size for the cortisol measures was much smaller than for the other covariates (table 6-10), which does make the effect of the evening cortisol less comparable with the effect of the other covariates. However the partial eta squared for evening cortisol, and the other cortisol measures, for brain volume was much bigger than for the other covariates. Previous studies have found links between evening cortisol and gait speed (Gardner et al., 2011) and waking cortisol and processing speed (Kuningas et al., 2007). These effects could be caused by direct tissue atrophy, as cortisol is known to cause both brain and muscle atrophy, or by brain and muscle cell dysfunction (MacLulich et al., 2005, Lupien et al., 1998, Falduto et al., 1990), however I found that evening cortisol does not act as a mediator between processing speed and 6MWT, which given the small delta partial eta squared is perhaps not surprising.

### **6.4.3 Relationship between brain structure and neck muscle CSA**

There was no association between grey matter volume and neck muscle CSA but interestingly there was a negative relationship between white matter volume and neck muscle CSA. This was the opposite of what I had hypothesized according to the common cause hypothesis. I found that a  $1\text{cm}^2$  increase in neck muscle CSA was associated with a  $0.02\text{cm}^3$  decrease in white matter volume; thereby the effect size is significant but small. One possible mechanism which might explain the direction of effect is that of postural control. Neck muscles are constantly in use even when sitting, unlike most peripheral muscles, and perhaps those with less healthy WM find it more difficult to maintain postural control and therefore their neck muscle have to work harder leading to larger neck muscles. It would be interesting to see if this relationship was replicated in other datasets. No covariates were found which acted as explanatory factors in this relationship. Previous studies looking at brain volume and muscle size again found no association with total GM volume, although regional grey matter volume may play a role, but there was evidence of a positive relationship between WM volume and muscle size (Kilgour et al., 2014). However these studies either looked at total muscle bulk (through DEXA or BIA) or muscle CSA of a limb (eg thigh CSA); no previous studies looking at neck or paraspinal muscles and brain volume which may have a different relationship.

#### **6.4.4 Relationship between brain structure and physical function**

White matter volume was significantly associated with grip strength and 6MWT but gray matter volume was only associated with 6MWT and not grip strength. A 1kg increase in grip strength was associated with a 1.2cm<sup>3</sup> increase in WM volume. A 1 second faster 6MWT was associated with a 4.5cm<sup>3</sup> increase in WM volume and a 5.0cm<sup>3</sup> increase in GM volume. Comparing partial eta squared results, whilst the 6MWT seems to be a more important predictor of cognition, grip strength has a more important role in predicting WM volume.

In the systematic review, chapter 2, I found no studies investigating the association between grip strength and whole brain, WM or GM volume other than a study using data from wave 1 and 2 of the LBC 1936 study (Aribisala et al., 2013), which found no association between WBV or GM volume and grip strength at either wave and a positive association with WM volume and grip strength only at wave 2, not at wave 1. This might indicate that the relationship between brain structure and grip strength changes with age and longitudinal studies would be useful in investigating this relationship further. Furthermore, studies have shown a relationship with brain atrophy (measured as an index of WBV against ICV, or as ventricular volume) and grip strength.

There is evidence that gait speed is positively associated with whole brain volume; this relationship may be driven by total WM volume or regional GM volumes, specifically the hippocampus. Previous studies have also shown a negative relationship with brain atrophy (measured as above) and gait speed (Kilgour et al., 2014). Longitudinal studies will be able to determine if those with smaller brains or regional brain volumes have a faster decline in gait speed or whether those with a slow gait speed have faster brain atrophy or whether the two decline in parallel. The answer to this question would then allow further exploration of the possible underlying mechanisms.

Testing for potential explanatory factors again led to no significant results in the multivariate model, but the univariate output found both evening cortisol and diurnal cortisol slope may act as explanatory factors in the relationship between 6MWT and brain volume, although there was no specific relationship with either white or grey matter volume found. Sample size again clouds this as the sample for the cortisol measures was much smaller than for the other covariates. As previously mentioned, cortisol has previously been shown to be associated with gait speed and brain volume (Gardner et al., 2011, Falduto et al., 1990), however further

larger studies are now needed to investigate the role of cortisol in the relationship between gait speed and brain volume, with the potential to lead on to possible therapeutic strategies.

#### **6.4.5 Relationship between white matter lesions and neck muscle CSA**

Our primary outcome for white matter disease was WML volume but as a secondary outcome I also looked at Fazekas score which gives an indication of WML burden location and extent. There was no significant association between neck muscle CSA and WML volume. There was a significant association between periventricular hyperintensity (PVH) Fazekas score and neck muscle CSA in men, with those scoring 1 or 2 having significantly smaller neck muscle CSA than those scoring 0. The comparison between group 3 and 0 was non-significant but both groups had a small n which may account for this. However there was no relationship between PVH score and neck muscle CSA in women and in both men and women there was no relationship between the deep WMH Fazekas score and neck muscle CSA. I found no previous studies looking at WMH and muscle size, which is interesting considering the large number of studies which have been published looking at muscle function and WMH (Kilgour et al., 2014). It may be that location of WMH is more important than absolute burden, but further longitudinal studies are required to investigate this area further.

#### **6.4.6 Relationship between white matter lesions and physical function**

Grip strength was not associated with WML volume but 6MWT was; a 1 second increase in 6MWT was associated with a  $1.16\text{cm}^2$  increase in WML volume. None of the potential covariates investigated were found to be significant explanatory factors in the multivariate model however the univariate analysis found that waking cortisol and diurnal cortisol slope may acts as explanatory variables. Along with gait speed (Gardner et al., 2011), white matter disease has also been found to be associated with cortisol (Cox et al., 2014). Elevated glucocorticoids may cause WMHs by impaired axonal sprouting in response to injury, decreased proliferation of oligodendrocytes, or reduced maintenance of axonal myelination following accumulated damage over time (Cox et al., 2014). However the direction of effect needs to be established as it could also be that WMHs develop leading to impaired supra-thalamic regulation of the HPA axis and therefore increased cortisol levels.

In women, a PVH Fazekas score of 3 was associated with significantly lower grip strength than a score of 0, but there was no significant relationship between PVH Fazekas score and

6MWT in either sex. In both men and women the deep Fazekas score was not associated with grip strength, however in men those with a score of 2 or 3 had a significantly slower 6MWT than those with a score of 0. The n for those who scored 0 or 3 was lower in the main than for those who scored 1 or 2, therefore it would be useful to repeat these associations in another cohort with a larger sample size. This may help to further explain the differences between the sexes and the importance of the location of WMHs to physical function.

The relationship between increased WMH and slower gait speed is well established; however it is less clear-cut whether PVH or deep WMHs are more important with studies showing conflicting results (Soumare et al., 2009, Moscufo et al., 2012, Wolfson et al., 2005, Rosano et al., 2006, Rosano et al., 2005, Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013, Rosano et al., 2010, Longstreth et al., 1996). Our study was the only one to look at the sexes separately and it may be that analysing other studies previous results by sex would alter outcomes. The relationship between grip strength and WMHs is less well studied, with one study showing a negative relationship between WMH volume and grip strength, with particular note that regional burden may play an important role (Sachdev et al., 2005), whilst two other studies found no significant relationship between WMH volume and grip strength, with one of these studies using the same data as here (ie LBC 1936) (Aribisala et al., 2013, Longstreth et al., 1996).

#### **6.4.7 The interrelationship between brain and muscle structure and function**

I identified previous studies which have looked at the relationship between 2 or 3 of the 4 variables (brain structure, cognitive function, muscle structure and physical function) in chapter 2 (Kilgour et al., 2014), but studies looking at the relationship between all 4 variables are much less common. Here I was able to look at all 4 variables and the interrelationship between them.

There are significant relationships between neck muscle CSA, WM volume and physical function (figure 6.1). Neck muscle CSA is a mediator between 6MWT and WM volume but is not a mediator between grip strength and WM volume. The relationship is complicated by the fact that there is a positive relationship between physical function and WM volume and physical function and neck muscle CSA but a negative relationship between neck muscle CSA and WM volume. However the effect size of the latter relationship was very small, therefore it seems that whilst a statistical effect exists for neck muscle CSA as a mediator, the

predominant association between physical function and WM volume is not through muscle size so must be mediated through other routes (eg motivation to perform the test well, maintaining good muscle quality, because strength and muscle size do not age in parallel) (Young et al., 1985, Skelton et al., 1994).

WM volume is a significant mediator in the relationship between G cognition and G processing speed and both grip strength and 6MWT, but not with G memory. White matter comprises the myelin ensheathed axonal tracts radiating from grey matter to the periphery, so it would make logical sense that it played a role in the relationship between physical function and cognition, as loss of white matter volume can lead to impairment in both areas (Kilgour et al., 2014).

I then looked at these same relationships but with grey matter volume (figure 6.2). As there was no association between neck muscle CSA and grey matter volume I did not look further at this relationship. GM does not act significantly as a mediator between 6MWT and cognition. Therefore other mechanisms must explain the relationship, including the role of WM volume as noted above and WML volume as discussed below.

Lastly I looked at the role of WML volume in these relationships (figure 6.3). Neck muscle CSA is not a mediator between WML volume and 6MWT, mirroring the results for WM volume. Therefore WML affects 6MWT independent of neck muscle CSA, which, as a proxy for generalized muscle bulk (Kilgour et al., 2012), again highlights the non-parallel ageing of muscle size and function. This could also be through balance impairment, reaction time or motivation. As such a large volume of literature focuses on the relationship between WMHs and gait speed, further studies looking at the associations between muscle size, gait speed and WMHs would be useful in understanding these important relationships further.

Interestingly WML volume was found to act as a mediator between 6MWT and both G cognition and G processing speed. WMHs have been found to be strongly associated with 6MWT and cognition in both this study and previous studies (Mosley et al., 2005, Soumare et al., 2009, Moscufo et al., 2012, Wolfson et al., 2005, Rosano et al., 2006, Rosano et al., 2005, Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013, Rosano et al., 2010, Longstreth et al., 1996), and are thought to cause these effects by impairing signaling between brain areas and consequently the periphery also.

Therefore, neck muscle CSA does not act as a mediator between grip strength and WM volume but does act as a mediator for 6MWT and WM volume (but not WML volume). This may be because grip strength and the 6MWT represent very different types of physical function and therefore the effect the ageing process has on them will be very different.

Both WM volume and WML volume are mediators of the relationship between 6MWT and cognition. It is plausible that as WML volume increases, so healthy WM volume decreases so perhaps it is not surprising that the majority of relationships are repeated but inverted between WM and WML volume, however this would also require that premorbid WM volume was not associated with risk of WML developing, which appears to be the case (Wen et al., 2006).

#### **6.4.8 Limitations and future directions**

Our study only included members of the LBC 1936, which means the population is very homogeneous. Whilst this means that comparisons between covariates are less likely to be affected by some outside influences, it affects the generalizability of our results, as the population is mainly white, all of the same age and all living in the same geographical location. Therefore it would be very interesting to repeat these associations in other studies which include measures of brain and muscle structure and function. Also, at the time of analysis I only had access to brain structure and neck muscle CSA from wave 2 of the study. A further wave has since been completed and it will be very interesting to look at these relationships over time. For example, neck muscle cross-sectional area is determined by peak muscle mass achieved and subsequent muscle loss, but the difference in neck muscle CSA between wave 2 and 3 will only relate to muscle loss, which is a purer measure of muscle ageing. The same will be true of the other measures and will allow us to look further at direction of effect within these relationships. This will allow us to look at sarcopenia within the LBC1936 cohort from the context of a lifecourse approach, by being able to separate out factors which only relate to ageing from those which contributed to peak muscle bulk achieved. This concept is an exact reflection of how osteoporosis and bone mineral density have been studied. There is now public health advice on how to reach a satisfactory peak bone mineral density (eg weight bearing exercise) and also medication and lifestyle advice to offer to those in older age groups who have developed low bone mineral density.

However, even with the longitudinal data from wave 3 of the study the LBC1936 study still only covers a very narrow age spectrum with regard to muscle mass and function. It will be

very interesting to look at alternate data sets which include subjects from a different age bracket and re-test the associations in these groups also. Conversely, whilst we do not have data on muscle mass and function from childhood within the LBC1936 study we do have data from childhood related to socioeconomic factors. Interestingly we did not find any of these factors to have a role as an explanatory variable in the associations found between brain and muscle. This may be because the LBC1936 cohort is unusual in its overall health and socioeconomic status. For example in the 2001 Scottish Household Survey of those born between 1937-1975 42.1% of respondents identified themselves as from a professional or managerial social class whereas at the time of wave 1 of the LBC1936 study 55.4% of the respondents identified themselves in this group (Iannelli and Paterson, 2005). Whilst these figures are not totally comparable in view of the fact that social class is increasing with subsequent generations it would be fair to conclude that the percentage of LBC1936 subjects in this group was over-represented compared to the general population.

Sarcopenia is defined as the loss of muscle mass and function with age (Cruz-Jentoft et al., 2010), therefore it is important to investigate both aspects with respect to ageing muscle. However there are confounding factors associated with measuring both of them. As muscle ages, the relative amount of non-contractile tissue, which comprises adipose and connective tissue, increases significantly (Taaffe et al., 2009, Inacio et al., 2014). In older adults this represents around 15% of the total muscle CSA in some muscle groups, which is around 2.5-fold greater than in young controls (~6%). Therefore measurements of muscle CSA or volume which are not corrected for the amount of non-contractile tissue present may underestimate the degree of sarcopenia present in an individual. Techniques used to measure the non-contractile component of muscle have mainly used CT but there are processes for measuring it using MR images (eg K means clustering) (Gray et al., 2011). However none of the techniques we applied to the neck muscles for measuring the non-contractile component worked adequately, mainly due to the small volumes of the neck muscles and the difficulties the software had in detecting the muscle boundaries.

Measurement of muscle function is also subject to confounding. For example in measuring gait speed multiple factors other than purely muscle function will contribute to the final score (eg joint disease, balance, vision). Even the purer measures of muscle strength measurement (eg isometric knee extensor strength) are subject to issues such as motivation, comprehension

and mood. Therefore whilst measurement of both muscle structure and function are subject to error, a combined measure is still the preferred method of detecting sarcopenia.

Finally the measurement of neck muscle CSA in this study was used as a proxy for general muscle bulk and not because the neck muscles were of specific interest in this cohort. That is why we used the well-established measures recorded for muscle function as part of LBC1936 (grip strength and 6MWT) as opposed to specific measures of neck muscle strength. However there are techniques for measuring specific neck muscle strength for 3 of the 4 muscles we studied. These include a technique to measure the strength of the sternocleidomastoid (Barton and Hayes, 1996), the semispinalis capitis (Rezasoltani et al., 2002) and the trapezius (Choudhari et al., 2012). No measurement technique was found to individually measure the strength of the splenius capitis muscle. It would be interesting to undertake a measure of neck muscle strength in a small sub-population to determine the relationship between neck muscle size and strength and also to correlate the other measures of muscle function with it to see if there is a consistent relationship or not, as it may be that neck muscles age in a different way to other muscle groups around the body in view of the fact they are in use for the vast majority of the waking day, even whilst sitting, unlike many other muscle groups.

## **Conclusion**

This study used data from a large population based cohort study to look at the interrelationships between brain and muscle structure and function. Neck muscle CSA was negatively associated with WM volume and PVH Fazekas score in men. Grip strength was positively associated with cognitive ability (specifically general cognition and processing speed, but not memory) and WM volume, and was negatively associated with PVH Fazekas score in women. 6MWT was also associated with the same cognitive ability measures as grip strength, and also WM, GM and WML volume. 6MWT was also positively associated with deep Fazekas score in men.

Neck muscle CSA was found to be a mediator between 6MWT and WM volume, and WM and WML volume both act as mediators between physical function and cognition. Brain ageing and muscle ageing therefore interact in a complex manner and understanding these interactions further could help reveal possible underlying mechanisms linking them; for example the role of cortisol, which we found to represent an explanatory factor between 6MWT and brain structure and cognition.



Again as these relationships are largely cross-sectional it is difficult to use these results to comment further on their reflection on the common cause hypothesis. However, this study has identified some evidence of complex relationships between brain and muscle structure and function which may lend some support to the hypothesis. Nonetheless as the associations are mostly small there are clearly other important driving factors leading to the magnitude of all four variables within this age group. It may be that a less healthy, more diverse, older age group would have displayed stronger associations as these individuals would have been more affected by the ageing process, however the best way to assess if the organs are ageing in parallel or not will be the future waves of this study and longitudinal analyses which are future work that I now plan to do.

.

# **Chapter 7 Investigating the relationship between markers of immunosenescence (CMV and IL-6) and grip strength and muscle size in an elderly cohort study**

## **7.1 Introduction**

Sarcopenia is an important cause of morbidity and mortality in older adults (Lauretani et al., 2003, Baumgartner et al., 1998, Rantanen, 2003), but despite its clinical importance, current understanding of the mechanisms underlying sarcopenia remains unclear. In the next two chapters I investigate two potential mechanisms underlying the development of sarcopenia: in this chapter the role of immunosenescence and inflammation and in the next chapter the role of glucocorticoids.

I chose these two areas following on from the literature review performed in chapter 1 in which I identified several possible mechanistic pathways which could underlie the process of sarcopenia. Following on from this when the data was collected from wave 2 of the LBC 1936 study I looked at the associations between these identified pathways and the markers of sarcopenia which LBC1936 contained (namely grip strength and neck muscle CSA). These preliminary analyses found that several of the pathways I had identified may be associated with the markers of sarcopenia, including some risk factors for vascular disease (eg total cholesterol, HDL cholesterol), the cortisol measures (as discussed in chapter 6) and the markers of immunosenescence (discussed in this chapter). Other markers which were looked at, for example markers of cell ageing (eg telomere length) and micronutrients (eg vitamin B12 and folate), did not show a significant association with the markers of sarcopenia and further analysis was not pursued.

Immunosenescence and inflammation have both been identified as possible contributing factors. Immunosenescence is the age-associated impairment of immune function due to changes in both the innate and adaptive immune response (Pawelec et al., 2010). These changes lead to a decreased ability to respond to pathogens, although an agreement on clinical biomarkers and associated clinical outcomes requires further research (Pawelec et al., 2010).

Seropositivity for cytomegalovirus (CMV, otherwise known as Human Herpes Virus 5), is common in older adults (Lubeck et al., 2010, Lopo et al., 2011). Most people are infected in childhood or young adulthood and become carriers of the virus in a latent state for the rest of their lives (Soderberg-Naucler and Nelson, 1999, Sinclair, 2008, Slobedman et al., 2010). The role of CMV status in immunosenescence is a topic of current research, and it remains unclear whether the relationship is causal or associative. However, latent CMV infection has been linked to several clinical outcomes, including frailty and increased mortality. Several studies have found an association between CMV seropositivity and/or CMV antibody titre and the presence of atherosclerosis and coronary heart disease, with some studies also demonstrating a correlation with survival time (Blankenberg et al., 2001, Muhlestein et al., 2000, Strandberg et al., 2009). Other studies have found associations between CMV and cognitive decline in older adults, (Aiello et al., 2006) and all-cause mortality (Roberts et al., 2010).

There is also evidence of an association between CMV infection and frailty. Aiello et al found that CMV antibody titre is negatively associated with the ability to carry out activities of daily living (ADLs) in elderly Latino subjects, after correcting for gender and age (Aiello et al., 2008). However, this relationship became non-significant after adjusting for the total number of health conditions, body mass index, and household income. Schmaltz et al found an association between frailty, defined using the Fried criteria, and CMV serostatus in older women (Schmaltz et al., 2005). Studies investigating frailty vary widely on the criteria used for diagnosis and not all frailty scores contain a measure of muscle mass (Fried et al., 2001, Rockwood and Mitnitski, 2007). Therefore, in order for clear conclusions to be drawn about the possible underlying mechanisms of sarcopenia, it is important to study it as an independent variable rather than as a component of a frailty score.

Interleukin 6 (IL-6) is a cytokine known to be part of the acute phase response, i.e. the initial immune system reaction to infection or trauma (Heinrich et al., 1990). Increasing age is associated with latent low grade inflammation; levels of IL-6 appear to increase with age, particularly following the andropause or menopause (Hager et al., 1994, Ershler and Keller, 2000). In a large cross-sectional study of septuagenarians, raised IL-6 levels were associated with reduced muscle mass and strength (Visser et al., 2002). However, in a further longitudinal cohort higher IL-6 levels were associated with loss of muscle strength, although no association was found with muscle mass (Schaap et al., 2006).

Several studies investigating the effect of CMV on frailty and functional ability have adjusted for IL-6 to assess its role as a mediator, as CMV is known to increase IL-6 gene expression and production in peripheral blood mononuclear cells (Geist and Dai, 1996). Indeed in the above mentioned study, Schmaltz et al found that CMV positive subjects with high IL-6 levels had a significantly higher prevalence of frailty than those with a low IL-6 level (Schmaltz et al., 2005). Also, data from the Women's Health and Aging Studies found that IL-6 appeared to modulate the effect of CMV antibody titre on frailty as an outcome (measured using the Fried criteria), although the effect did not reach statistical significance (Wang et al., 2010).

I could find no previous studies which have looked at the association between muscle mass and CMV serostatus or antibody titre. Furthermore I found only one study which addressed the relationship between CMV serostatus and handgrip strength, an important marker of muscle function in older age, and that study only looked at women (Schmaltz et al., 2005). As detailed above, IL-6 may play an important mediatory role in these relationships and it is therefore important to study this in tandem with CMV status. In this study I investigated the relationship between latent CMV infection, IL-6 level and markers of sarcopenia (muscle size and strength) in a healthy older cohort of community-dwelling men and women.

## **7.2 Methods**

### **7.2.1 Participants – The Lothian Birth Cohort 1936 (LBC 1936)**

As per chapter 3.

### **7.2.2 Neck muscle Cross-sectional Area**

As per chapter 4.

### **7.2.3 Grip strength**

Grip strength was measured with a Jamar Hydraulic Hand Dynamometer, with all participants performing 3 trials with their right and left hands; the best of the 3 trials was used for the following analyses.

### **7.2.4 CMV and IL-6 measures**

CMV was measured in plasma samples collected at age 70, using a CMV ELISA assay. Mock and viral-infected lysate was coated onto ELISA plates and incubated overnight. Standards (a mixture of three CMV positive plasma samples) and plasma samples were added to the plates and incubated for one hour before washing. An anti-IgG horseradish peroxidase conjugated secondary antibody was then added to the plate to incubate for one hour. After washing, TMB substrate was added and the reaction stopped by addition of 1M HCL. The sample was assessed using an ELISA reader at 450nm. To determine CMV titres, mock values were first subtracted from lysate values. The data were then analysed in PRISM, and CMV titres were calculated with reference to the standard curve. Values above 10 were considered to be seropositive. To ensure accuracy, all samples were tested in duplicate. IL-6 levels were analysed at the University of Glasgow using high sensitivity ELISA from R&D Systems. The minimum detectable dose ranged from 0.016-0.110 pg/mL (mean=0.039 pg/mL). The intra-assay CV ranged from 6.9 to 7.8%, while the inter-assay coefficient of variance ranged from 6.6 to 9.6%.

### **7.2.5 Childhood deprivation**

At the age 70 assessment, participants were asked to provide background demographic and environmental information about their childhood, specifically for when they were aged about 11 years. Participants reported the number of people they lived with and the number of rooms in the house, which was used to calculate an overcrowding index (people/room). Participants also reported: whether their household had indoor or outdoor toilet facilities; their father's

occupation to allow father's social class to be coded (categorised from I, professional, to V, unskilled); and the number of years they spent in full-time, formal education.

### **7.2.6 Statistical Analysis**

Descriptive statistics, exploratory analyses and general linear modelling (Analysis of Covariance; ANCOVA) were performed using SPSS version 18.0 for Windows (SPSS Inc., Chicago, Ill, USA). Missing values were excluded listwise for the ANCOVA analyses. For the ANCOVA, I constructed baseline models with the measures of neck muscle CSA and grip strength (right and left) as dependent (i.e. outcome) variables and CMV serostatus, CMV antibody titre and IL-6 level as independent variables, adjusting for age, gender and either height or weight, as a measure of body size, and the four measures of childhood deprivation. Total neck muscle CSA was found to correlate more strongly with weight ( $P < 0.001$ ), whereas both right and left grip strength correlated more strongly with height ( $p < 0.001$ ). Therefore I used the respective measures for adjustment in each of the analyses.

## 7.3 Results

There were 866 participants in wave 2 of the LBC 1936 study; 448 men (mean age 72.48 years, sd 0.70) and 418 women (mean age 72.51 years, sd 0.72). This represents 79.4% of the participants who attended at the first wave of testing aged 70 years (n=1091). Baseline data for neck muscle CSA, right and left grip strength, CMV serostatus, CMV antibody titre and IL-6 levels are shown in Table 7.1. Baseline data for measures of childhood deprivation are shown in Table 7.2.

*Table 7-1: Muscle size and strength, CMV and IL-6 baseline data*

| <b>Variable</b>                               | <b>Statistic/Group</b> | <b>Men</b>  | <b>Women</b> |
|---|------------------------|-------------|--------------|
| <b>Total Neck Muscle CSA (mm<sup>2</sup>)</b> | Mean                   | 2576.6      | 1814.5       |
|   | SD                     | 421.0       | 281.2        |
|   | n                      | 343         | 298          |
| <b>Right Grip Strength (kg)</b>               | Mean                   | 35.49       | 21.28        |
|   | SD                     | 6.82        | 5.54         |
|   | n                      | 448         | 416          |
| <b>Left Grip Strength (kg)</b>                | Mean                   | 34.69       | 19.93        |
|   | SD                     | 6.57        | 5.13         |
|   | n                      | 447         | 416          |
| <b>CMV Status</b>                             | Positive (%)           | 60.6        | 69.0         |
|   | Negative (%)           | 39.4        | 31.1         |
|   | n                      | 439         | 409          |
| <b>CMV Antibody Titre</b>                     | Median                 | 60.56       | 132.11       |
|   | IQ range               | 0.47-214.00 | 2.40-277.78  |
|   | n                      | 437         | 406          |
| <b>IL-6 Level</b>                             | Median                 | 1.60        | 1.48         |
|   | IQ range               | 1.05-2.42   | 1.01-2.29    |
|   | n                      | 425         | 390          |

*Table 7-2: Childhood deprivation baseline data*

| <b>Variable</b>                           | <b>Statistic/Group</b> | <b>Men</b> | <b>Women</b> |
|---|------------------------|------------|--------------|
| <b>Overcrowding index (age 11)</b>        | Median                 | 1.20       | 1.20         |
|   | IQ range               | 0.86-1.67  | 0.80-1.67    |
|   | n                      | 447        | 416          |
| <b>Indoor/outdoor toilet (age 11)</b>     | Indoor (%)             | 87.5       | 90.0         |
|   | Outdoor (%)            | 12.5       | 10.0         |
|   | n                      | 447        | 418          |
| <b>Father's social class</b>              | I (%)                  | 6.9        | 7.3          |
|   | II (%)                 | 21.5       | 17.2         |
|   | III (%)                | 56.7       | 54.7         |
|   | IV (%)                 | 6.9        | 13.3         |
|   | V (%)                  | 7.9        | 7.6          |
|   | n                      | 404        | 384          |
| <b>Years spent in full time education</b> | Median                 | 10         | 10           |
|   | IQ range               | 10-12      | 10-12        |
|   | n                      | 448        | 418          |

I assessed associations between IL-6 and CMV antibody titre (using Spearman's rho correlations) and CMV serostatus (using the Wilcoxon independent samples test) with age, height, weight, the muscle variables and the measures of childhood deprivation (Table 7.3). The association between CMV serostatus and indoor/outdoor toilet was analysed using the chi-square test. In men, being CMV seropositive or having a high CMV titre is associated with all the markers of childhood deprivation. In women, being CMV seropositive or having a high CMV titre is associated with a higher overcrowding index and lower social class of their father, and in addition a high CMV titre is associated with fewer years of formal education. In men, IL-6 levels only significantly correlate with the number of years of full time education (i.e. the more years of education the lower the IL-6 level), whereas in women all the markers of childhood deprivation correlate with a higher IL-6 level, except for the indoor/outdoor toilet question.

If IL-6 was acting as mediator for CMV infection (i.e. CMV infection causes inflammation, raising IL-6 levels, which causes increased sarcopenia), I would expect a correlation between the two variables. However, the Spearman's rho correlation between IL-6 and CMV antibody titre was non-significant ( $\rho=0.06$ ,  $p=0.07$ ), while a Wilcoxon independent samples test for IL-6 and CMV serostatus was also non-significant (test statistic 1.28,  $p=0.20$ ).



**Table 7-3: Wilcoxon independent samples test (CMV serostatus) and Spearman's rho correlations (CMV antibody titre and IL-6 level) (p values)**

|   | Male                               |                                    |                                    | Female                             |                                  |                                   |
|---|------------------------------------|------------------------------------|------------------------------------|------------------------------------|----------------------------------|-----------------------------------|
|   | CMV status (pos/neg) <sup>a</sup>  | CMV titre <sup>b</sup>             | IL-6 titre <sup>b</sup>            | CMV status (pos/neg) <sup>a</sup>  | CMV titre <sup>b</sup>           | IL-6 titre <sup>b</sup>           |
| <b>Age in days at Wave 2</b>                  | .68<br>(.50)                       | .09<br>(.06)                       | .09<br>(.08)                       | <b>2.32</b><br>(.02)               | <b>.17</b><br>( <b>&lt;.01</b> ) | .08<br>(.14)                      |
| <b>Height in cm</b>                           | <b>-2.08</b><br>(.04)              | <b>-.11</b><br>(.02)               | <b>-.12</b><br>(.01)               | <b>-2.50</b><br>(.01)              | -.09<br>(.07)                    | .00<br>(.95)                      |
| <b>Weight in kg</b>                           | 1.44<br>(.15)                      | .07<br>(.15)                       | <b>.16</b><br>( <b>&lt;.01</b> )   | -1.06<br>(.29)                     | .02<br>(.71)                     | <b>.30</b><br>( <b>&lt;.001</b> ) |
| <b>Total neck muscle CSA (mm<sup>2</sup>)</b> | -1.60<br>(.11)                     | -.06<br>(.26)                      | .09<br>(.10)                       | 1.02<br>(.31)                      | .09<br>(.12)                     | <b>.21</b><br>( <b>&lt;.001</b> ) |
| <b>Grip strength right hand (kg)</b>          | -.62<br>(.54)                      | -.05<br>(.34)                      | <b>-.25</b><br>( <b>&lt;.001</b> ) | <b>-2.81</b><br>(.01)              | <b>-.10</b><br>(.04)             | -.07<br>(.16)                     |
| <b>Grip strength left hand (kg)</b>           | -1.95<br>(.05)                     | <b>-.10</b><br>(.03)               | <b>-.25</b><br>( <b>&lt;.001</b> ) | <b>-2.04</b><br>(.04)              | -.08<br>(.12)                    | -.08<br>(.11)                     |
| <b>Overcrowding index age 11</b>              | <b>4.53</b><br>( <b>&lt;.001</b> ) | <b>.17</b><br>( <b>&lt;.001</b> )  | .03<br>(.56)                       | <b>3.56</b><br>( <b>&lt;.001</b> ) | <b>.16</b><br>( <b>&lt;.01</b> ) | <b>.10</b><br>(.04)               |
| <b>Father's job class as a number</b>         | <b>3.82</b><br>( <b>&lt;.001</b> ) | <b>.19</b><br>( <b>&lt;.001</b> )  | .03<br>(.55)                       | <b>2.51</b><br>(.01)               | <b>.14</b><br>( <b>&lt;.01</b> ) | <b>.16</b><br>( <b>&lt;.01</b> )  |
| <b>No. of years of full-time education</b>    | <b>-3.07</b><br>( <b>&lt;.01</b> ) | <b>-.17</b><br>( <b>&lt;.001</b> ) | <b>-.13</b><br>(.01)               | -1.57<br>(.12)                     | <b>-.11</b><br>(.01)             | <b>-.10</b><br>(.04)              |
| <b>Indoor=1 or outdoor=2 toilet at age 11</b> | <b>0.47*</b><br>(.02)              | <b>.10</b><br>(.03)                | .04<br>(.37)                       | <b>0.69*</b><br>(.33)              | .06<br>(.18)                     | .05<br>(.30)                      |

<sup>a</sup> Wilcoxon independent samples test statistic (and associated p values) for CMV status and all predictor variables except indoor/outdoor toilet age 11, which was analysed using the chi-square test and shows \*the odds ratio for being CMV seropositive if indoor toilet age 11

<sup>b</sup> The columns for CMV titre and IL-6 titre show the Spearman's rho correlation with the predictor variables (and the associated p value)

General linear models (ANCOVAs) were then created for each muscle variable separately with each measure of immune status (ie CMV status, CMV titre and IL-6 level) before rerunning the models of CMV status and antibody titre adjusting for IL-6 status also. Tables 7.4 and 7.5 present the results for the ANCOVA for CMV serostatus and neck muscle CSA, and IL-6 and grip strength. The p value gives the significance of the independent variable's association and the partial eta squared gives a measure of effect size, for which Cohen (Cohen, 1988) indicates the following: small  $\geq 0.0099$ ; medium  $\geq 0.0588$ ; large  $\geq 0.1379$ .

I found that CMV seropositivity was associated with a smaller neck muscle CSA in men but not in women ( $p = 0.028$ , partial eta squared = 0.01) (Table 7.4). In this model, lower weight and female sex were also associated with smaller neck muscle CSA. When this model was corrected for IL-6 level the effect remained significant ( $p = 0.047$ ). The model for neck muscle CSA and CMV antibody titre showed no significant association, both with and without adjustment for IL-6 level, nor did the model with IL-6 as predictor variable, without adjusting for CMV infection.

*Table 7-4: ANCOVA for CMV status and Total Neck Muscle CSA*

| <b>Source</b>   | <b>Sig.</b> | <b>Partial<br/>Eta<br/>Squared</b> |
|---|-------------|------------------------------------|
| <b>Age in days at Wave<br/>2</b>                      | .38         | <.01                               |
| <b>Weight in kg</b>                                   | <.001       | .22                                |
| <b>Sex<br/>(Male=1, Female=2)</b>                     | <.001       | .42                                |
| <b>Overcrowding index<br/>age 11</b>                  | .29         | <.01                               |
| <b>Indoor=1 or<br/>outdoor=2 toilet at<br/>age 11</b> | .88         | <.01                               |
| <b>Father's job class as<br/>a number</b>             | .70         | <.01                               |
| <b>No. of years of full-<br/>time education</b>       | .43         | <.01                               |
| <b>CMV serostatus<br/>(neg=1, pos=2)</b>              | .10         | .01                                |
| <b>Sex * CMV<br/>serostatus</b>                       | .028        | .01                                |

Weaker grip strength in both right and left hands was found to be associated with higher IL-6 level; right grip strength  $p < 0.00001$ , partial eta squared = 0.032 and left grip strength  $p < 0.00001$ , partial eta squared = 0.027 (Table 7.5). The associations remain strongly positive even after adjustment for CMV status ( $p < 0.0001$ ) and CMV antibody titre ( $p < 0.0001$ ). In these models (shown in table 7.5), older age, shorter stature and female sex were also significantly associated with weaker grip strength in both hands. The models using CMV status and antibody titre alone were not significantly associated with grip strength in either hand.

*Table 7-5: ANCOVA for IL-6 titre and Grip Strength Right and Left Hands*

| Source                                 | Right Hand |                     | Left Hand |                     |
|--|------------|---------------------|-----------|---------------------|
|  | Sig.       | Partial Eta Squared | Sig.      | Partial Eta Squared |
| Age in days at Wave 2                  | .005       | .01                 | .004      | .01                 |
| Height                                 | <.001      | .08                 | <.001     | .07                 |
| Sex (Male=1, Female=2)                 | <.001      | .28                 | <.001     | .34                 |
| Overcrowding index age 11              | .72        | .00                 | .25       | .00                 |
| Indoor=1 or outdoor=2 toilet at age 11 | .75        | .00                 | .64       | .00                 |
| Father's job class as a number         | .95        | .00                 | .65       | .00                 |
| No. of years of full-time education    | .051       | .01                 | .10       | .00                 |
| IL-6 Level                             | <.001      | .03                 | <.001     | .03                 |

## 7.4 Discussion

This report used data from waves 1 and 2 of a population-based elderly cohort study, to investigate the relationship between latent CMV infection, IL-6 levels and sarcopenia, measured using neck muscle CSA and grip strength in both hands. There was no significant group difference for sex, age or CMV status and titre between those who participated in wave 1 but not wave 2 (n=225) and those who participated in both waves (n=866) (independent t tests,  $p>0.05$ ). I found that men who were seropositive for CMV antibody at age 70 years had a neck muscle CSA on average 4% smaller at age 73 than men who were seronegative. This effect remained positive whether adjusting for IL-6 level or not. It is well documented that muscle mass is lost at roughly 1% per year (Goodpaster et al., 2006, Visser et al., 2003), therefore being a man who is CMV seropositive in your 70s confers the same risks of low muscle bulk as being 4 years older. I did not detect a significant association in women between CMV serostatus and neck muscle CSA, or between CMV serostatus and grip strength in either hand. This may reflect the restricted range of social class seen within the LBC1936 cohort study, as the vast majority of participants in the study were from social class I to III in childhood (see table 7-2) and as mentioned previously CMV serostatus is associated with social class. Other studies have postulated that as CMV seropositivity is so common in older adults it may be more important to measure CMV antibody titre itself. However, I found no association between CMV antibody titre and either neck muscle CSA or grip strength. This result may indicate that latent CMV infection leads to increased muscle loss over an extended period, as CMV is commonly acquired in childhood, and the titre reflects the current situation, which may have less impact on muscle bulk. Longitudinal studies will be able to explore these relationships further.

IL-6 levels were found to strongly predict grip strength in both right and left hands in men and women. IL-6 predicted 3.2% of the variance in right-sided grip strength and 2.7% of the variance in left-sided grip strength. These associations remained significant when adjusting for CMV serostatus or antibody titre. I found no significant association between IL-6 levels and neck muscle CSA. Therefore our findings do not support previous work that has found that IL-6 may act as a mediator by which latent CMV infection causes frailty (Schmaltz et al., 2005, Wang et al., 2010).

It is widely accepted that muscle size and strength do not decline in a parallel manner (Young et al., 1985, Skelton et al., 1994), therefore they are not purely a function of each other and it

may be that different factors cause decline in one parameter more than the other, as our results have shown. Similarly, a study looking at the effect of IL-6 levels on muscle found a significant association with decline in grip strength but not muscle mass (Schaap et al., 2006), again indicating parameter-specific effects. Also, these results are based on cross-sectional data and therefore do not necessarily reflect changes with age. Therefore it could be that individuals with latent CMV infection or lifelong raised IL-6 levels have always had smaller/weaker muscles, rather than an increased rate of decline in muscle mass or function with age. However, as sarcopenia is currently diagnosed using reference to peers or a healthy young population, having a lower peak muscle mass and function should still be considered risk factors for sarcopenia. Again, longitudinal studies may help elucidate these relationships further.

The sole previous study I found that investigated the relationship between CMV status and grip strength only included women (Schmaltz et al., 2005). They found no significant difference in grip strength between seropositive and seronegative women. I have replicated this finding, but found a sex specific effect between male sex and CMV serostatus. There is evidence that men lose more muscle mass than women with age even after correction for body stature (Gallagher et al., 1997), therefore differing factors may play more or less of a role between the genders. Additionally, their muscles are larger to start with and therefore a reduction may be easier to detect.

It is unclear how CMV might directly influence physical functioning, however latent CMV infection has been identified as an important component of an immune risk phenotype that is associated with immunosenescence, inflammation, and several latent health conditions observed with ageing (Pawelec et al., 2006). All these may represent possible contributory mechanisms to sarcopenia. For example, if CMV infection predisposes to cardiovascular disease this, in turn, might limit exercise and hence loss of muscle strength and size. Should our findings be confirmed, exploration of potential causal pathways will be warranted.

Raised plasma IL-6 levels are known to increase proteolysis within muscle, by upregulating the proteolytic UPP pathway (Skipworth et al., 2006), however it is not known if the degree of increase seen with ageing is enough to cause atrophy and it is thought that proteolysis is not a major factor in normal ageing muscle (Tsujinaka et al., 1996). However, IL-6 may exert its effect through less direct routes. It is known that IL-6 causes anorexia, which would lead to decreased protein substrate. Also, IL-6 can both activate cortisol secretion and induce

11beta-hydroxysteroid type 1 expression, so it may exert its effect via the steroid pathway (Weber et al., 1997, Tomlinson et al., 2001). Furthermore, animal studies have demonstrated that inflammatory cytokines can induce muscle apoptosis by DNA fragmentation (Skipworth et al., 2006), though such models may not represent low level inflammatory changes occurring over a prolonged period.

The narrow geographic, age and ethnic mix within the LBC 1936 cohort means this study may not prove generalisable. However, the narrow age range helps to reduce the powerful effect of advancing age on many of the parameters measured in this and other studies and which may have led to the impression of stronger direct relationships between co-associated variables than is actually the case. Additionally the size of the sample studied and the fact that I replicated results in some of our analyses found in other work is reassuring. The high correlation between the markers of childhood deprivation and CMV status were as predicted and raise the possibility that other correlates of childhood socioeconomic deprivation may be mediated by CMV infection, although this relationship may be less strong in other populations. Also, when studying sarcopenia it is important to consider rate of decline rather than solely cross-sectional measures. Therefore in the future, longitudinal studies will be crucial in developing an understanding of these relationships. Finally, the concept of a homogeneous model of sarcopenia, whereby all muscle throughout the body ages at the same rate, is proving increasingly unlikely to be valid with studies showing varying rates of muscle ageing around the body (Janssen et al., 2000, Gallagher et al., 1997, Gallagher and Heymsfield, 1998). Therefore whilst our measure of neck muscle CSA has previously been shown to correlate strongly to mid-thigh muscle CSA, it is important to consider that different factors may worsen or ameliorate muscle ageing in different muscle groups throughout the body.

## **7.5 Conclusion**

In a large population-based elderly cohort study I found that men who were seropositive for CMV had smaller neck muscle CSA than men who were seronegative, and this effect was independent of IL-6 level. I also found that higher IL-6 levels, but not CMV levels, were strongly associated with lower grip strength in both hands in men and women. These associations were not attenuated when the model was adjusted for CMV serostatus or antibody titre. These findings support the hypothesis that there may be a relationship between

immunosenesence and muscle size and longitudinal studies are now required to investigate this relationship further.

# **Chapter 8 Investigating the relationship between plasma cortisol, urinary glucocorticoid metabolites, GR and 11 $\beta$ HSD1 mRNA expression in skeletal muscle, and muscle size and strength**

## **8.1 Introduction**

After investigating the role of immunosenescence and inflammation in the development of sarcopenia in the previous chapter, I next went on to research another possible underlying mechanism: glucocorticoid dysregulation. It is well known that glucocorticoids at pharmacological levels or in spontaneous Cushing's syndrome cause myopathy, with a combination of muscle atrophy and dysfunction. However, it is believed that corticosteroids may cause greater atrophy of type 1 muscle fibres and a relative increase in number of type 2b muscle fibres (Rebuffe-Scrive et al., 1988, Krotkiewski and Bjorntorp, 1986, Danneskiold-Samsøe and Grimby, 1986), whereas in sarcopenia there is no change in the ratio between type 1:type2b muscle fibre number with age (Sato et al., 1984, Mitchell et al., 2012). However, these studies all looked at the effect of cortisol within specific conditions (eg obesity, Cushing's disease or pharmacological studies of the effects of corticosteroids), therefore the role of minor elevations in endogenous corticosteroids in the development of sarcopenia out with these conditions now requires further study.

Glucocorticoids are believed to cause myopathy through a combination of increased protein breakdown (particularly through the ubiquitin-proteasome system) (Schakman et al., 2008), decreased protein synthesis (by inhibiting transport of amino acids into muscle and inhibiting the action of insulin and IGF-1) (Shah et al., 2000) and decreasing production of IGF-1 and myostatin (Schakman et al., 2008). In the context of sarcopenia, this mechanism could occur via elevated circulating glucocorticoids due to age-related hypothalamic-pituitary-adrenal (HPA) axis dysregulation. Alternatively, it could occur selectively within the muscle, by increased activity of the glucocorticoid receptor (GR) or the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1). 11 $\beta$ HSD1 converts inactive cortisone to active cortisol and is known to be present and biologically active in human muscle as well as many other tissues (Jang et al., 2006, Whorwood et al., 2001, Hughes et al., 2012). Indeed a recent study by Tiganescu et al found that elevated 11 $\beta$ HSD1 activity was increased in skin biopsies from older adults compared to younger adults and that this increased activity was associated with



markers of skin ageing (eg dermal atrophy and deranged collagen structural organization) (Tiganescu et al., 2013). Establishing links between GC and sarcopenia could lead to novel therapies, as several 11 $\beta$ HSD1 inhibitors are currently in clinical development for type 2 diabetes and other degenerative diseases, including cognitive dysfunction (Hughes et al., 2008).

There is some evidence of an association between increased plasma and salivary cortisol and lower muscle mass and strength but these data are inconsistent (Waters et al., 2008, Peeters et al., 2008, Izquierdo et al., 2001, Gardner et al., 2011). Glucocorticoid metabolites in a 24 hour urine sample may be more informative than plasma cortisol levels since they reflect glucocorticoid status over the diurnal cycle. Additionally ratios of the metabolites can be used as an index of peripheral 11 $\beta$ HSD activity (Best and Walker, 1997). However, no studies to date have examined the relationship between urinary glucocorticoid metabolites and sarcopenia. Similarly, there are no published data examining the relationship between GR and 11 $\beta$ HSD1 expression and muscle loss and function in older adults. Importantly, expression of 11 $\beta$ HSD1 and GR mRNA has been shown to reflect glucocorticoid activity, for example there is a correlation between 11 $\beta$ HSD1 mRNA expression and enzyme function (Wake et al., 2003).

The aim of this study was to investigate the relationship between plasma and urinary glucocorticoid metabolites and levels of mRNA encoding GR and 11 $\beta$ HSD1 in skeletal muscle, with muscle size and strength. I hypothesized that increased glucocorticoid signalling in skeletal muscle acting through GR by, (a) elevated circulating cortisol, (b) increased expression of 11 $\beta$ HSD1 or (c) increased expression of GR, is associated with reduced muscle size and strength.

## **8.2 Methods**

### **8.2.1 Participants**

Participants were healthy volunteers recruited at two sites in Scotland: young and older men were recruited in Aberdeen (Group 1) and older men and women were recruited in Edinburgh (Group 2). This allowed us to test for possible age and gender effects. Participants were defined as healthy after applying previously published health selection criteria to the responses to a questionnaire (Greig et al., 1994). Existing samples were available from two nearby cities in Scotland so these were used for analysis, rather than beginning a new de novo cohort collection. No comparisons were made between these two independent cohorts.

### **8.2.2 Ethics Statement**

Written informed consent was obtained and all procedures received local ethical committee approval. In Edinburgh this was by the Lothian Local Research Ethics Committee 02 and in Aberdeen this was by the North of Scotland Research Ethics Committees. The study conformed to the standards set by the Declaration of Helsinki.

### **8.2.3 Anthropometry**

Body weight was measured with participants in light clothing using a beam scale (Seca, UK). Height was measured using a wall mounted stadiometer.

### **8.2.4 Muscle function**

Maximum voluntary isometric knee extensor strength was measured using an established method (Edwards and Hyde, 1977). Following instruction, the participant made a maximum voluntary contraction (Newtons) which was held for 5 seconds. Three separate measurements were obtained and the highest value was used in subsequent analysis.

### **8.2.5 Muscle size**

Mid-thigh quadriceps cross-sectional area (CSA) was measured using a 1.5T MR scanner (Phillips Gyroscan Intera). T1-weighted axial images were taken with the isocentre of the magnetic field located at the mid-femur point which was landmarked prior to the scan according to International Standards of Anthropometric Assessment (ISAK) guidelines 2001. Imaging parameters were: slice thickness 10 mm; acquisition matrix 512 x 512; echo time (TE) 15 ms; repetition time (TR) 425 ms; and flip angle 90°. The CSA of the quadriceps was quantified using Analyze 8.0 (Mayo Clinic, Rochester, USA) according to a previously

published technique (Gray et al., 2011). Two of the subjects from Group 2 did not undergo MRI due to claustrophobic symptoms.

### **8.2.6 Plasma cortisol**

Blood samples were obtained from participants in the morning after overnight fast (mean time 0945h, range 0915-1030h). Plasma cortisol was measured by competitive immunoassay with direct chemiluminescent technology using the Bayer Advia Centaur method (see [http://labmed.ucsf.edu/labmanual/db/resource/Centaur\\_Cortisol.pdf](http://labmed.ucsf.edu/labmanual/db/resource/Centaur_Cortisol.pdf)).

### **8.2.7 Quadriceps muscle biopsy**

Quadriceps femoris samples were obtained from the region of vastus lateralis via percutaneous needle biopsy using a Bergstrom needle (Bergström, 1975). The biopsy was obtained in a sterile environment by sharp dissection under local anaesthetic using 1% lidocaine. The samples were then snap frozen in liquid nitrogen and stored at -80 °C before analysis (Stephens et al., 2010).

### **8.2.8 RNA Isolation**

Total RNA was isolated from quadriceps muscle biopsies using the Qiazol reagent (Qiagen, Crawley, UK) and miRNeasy RNA isolation columns (Qiagen, Crawley, UK). Briefly biopsies were homogenised in 1400ul or 700ul Qiazol depending on the size of the tissue sample using a Polytron PT1200E (Kinematica AG). Total RNA was isolated from the homogenised muscle using miRNeasy columns with an on column DNase treatment step using the RNase-Free DNase Set (Qiagen, Crawley, UK). After elution from the column into 30ul nuclease free H<sub>2</sub>O, RNA was quantified using the Nanodrop instrument (Labtech, UK) and quality assessed using the Bioanalyzer (Agilent, UK). All samples had 260/280 ratios above 1.8, and RIN scores above 7.5.

### **8.2.9 cDNA preparation and qPCR**

RNA samples were converted to cDNA using the Ovation RNA Amplification kit (Nugen, Netherlands). RNA was diluted to 10ng/ul and 50ng total RNA was used in the amplification reaction carried out according to the manufacturer's instructions, yielding between 3ug and 11ug cDNA. For qPCR, cDNA was diluted to ~50ng/ul. Quantitative RT-PCR reactions were run, in triplicate, on an Applied Biosystems Step One Plus system. The reaction mix was POWER SYBR Green x2 Master mix 12.5ul, forward primer (10uM) 1ul, reverse primer (10uM) 1ul, H<sub>2</sub>O 9.5ul and cDNA 1ul. Reaction conditions were 95°C for 10 mins, 95°C for

15s, 60°C for 60s (40 cycles) followed by melting curve generation from 60°C to 95°C. Ct values were examined and within triplicates any value greater than 0.3 Ct were removed before means were calculated. Data were then analysed using the delta Ct method with HPRT as a normaliser. After normalisation data were inverted and scaled such that the largest value for each gene was set to 100.

Primer sequences used were NR3C1 FP – CTGTCGCTTCTCAATCAGACTC; RP – GCATTGCTTACTGAGCCTTTTG; 11 $\beta$ HSD1 FP – AGGCTGCTGCCTGCTTAGGA; RP – AGCCCCAGAATGGGGAGGAGA; HPRT FP – TGACACTGGCAAAACAATGCA; RP- GGTCCTTTTCACCAGCAAGCT. HPRT was chosen as a normaliser as preliminary analysis of housekeeping gene performance showed HPRT to be stable across samples and expressed at a similar level to genes of interest compared to b-actin, GAPDH, b2M and 18S.

### **8.2.10 Urinary glucocorticoid metabolism**

24 hour urine samples were collected to quantify urinary glucocorticoid metabolites using gas chromatography electron impact mass spectrometry following solid phase extraction, hydrolysis of conjugates and formation of their methoxime-trimethylsilyl derivatives, as described previously (Best and Walker, 1997).

Two composites of the data were used in subsequent analyses. Firstly, total urinary steroids, comprising the sum of 5 $\beta$ -tetrahydrocortisol (5 $\beta$ THF), 5 $\alpha$ -tetrahydrocortisol (5 $\alpha$ THF), the main urinary metabolites of cortisol, and tetrahydrocortisone (THE), the main urinary metabolite of cortisone (total urinary GC = 5 $\beta$ THF + 5 $\alpha$ THF + THE). Secondly, an indirect indicator of systemic 11 $\beta$ HSD activity, comprising the ratio of 5 $\beta$ THF and 5 $\alpha$ THF to THE (ratio of cortisol to cortisone metabolites = (5 $\beta$ THF + 5 $\alpha$ THF)/THE).

### **8.2.11 Statistical Analysis**

Statistical analysis was performed using SPSS version 18.0. Bivariate correlations were performed using Spearman's rho to allow analysis of the non-parametric variables. Forced entry multiple linear regression was performed and the data from the two groups were analysed separately, when possible, to test reproducibility. Due to the large difference in age between the older and younger groups, age was analysed as a binary variable in the multivariate regression. Group 1 (n=52) had 80% power at the p=0.05 level to detect a correlation of r=0.38 and Group 2 (n=30) had 80% power at the p=0.05 level to detect a

correlation of  $r=0.49$ . In view of the power calculations and the exploratory nature of the study, adjusting for multiple hypotheses testing was not deemed to be appropriate.

## 8.3 Results

In total, 82 participants were recruited. Table 8.1 shows numbers of participants and their age, height, BMI, and the main outcome variables for each group. Independent t tests found significant sex and age related differences for height, muscle size and muscle strength but not for BMI or any measure of glucocorticoid status (Table 8.1).

*Table 8-1: Group characteristics*

|  | <b>Group 1<br/>younger<br/>men (n=19)</b> | <b>Group 1<br/>older men<br/>(n=33)</b> | <b>p-<br/>value<sup>a</sup></b> | <b>Group 2<br/>older men<br/>(n=16)</b> | <b>Group 2<br/>older<br/>women<br/>(n=14)</b> | <b>p-<br/>value<sup>b</sup></b> |
|--|---|---|---------------------------------|---|---|---------------------------------|
| <b>Age (years)</b>   | 22.2 (1.7)                                | 70.2 (4.4)                              | <0.001                          | 79.1 (3.4)                              | 80.1 (3.7)                                    | n/s                             |
| <b>Height (cm)</b>   | 177.6 (6.7)                               | 171.9 (5.4)                             | 0.001                           | 171.3 (6.1)                             | 157.6 (5.9)                                   | <0.001                          |
| <b>BMI (kg/m<sup>2</sup>)</b>                                    | 24.0 (2.5)                                | 25.2 (2.5)                              | n/s                             | 25.3 (3.9)                              | 24.1 (3.1)                                    | n/s                             |
| <b>Muscle Size<br/>(cm<sup>2</sup>)</b>                          | 92.7 (11.5)                               | 67.3 (7.4)                              | <0.001                          | 63.5 (7.3)                              | 43.8 (6.8)                                    | <0.001                          |
| <b>Muscle<br/>Strength<br/>(Newton)</b>                          | 774.9 (136.6)                             | 525.2 (73.6)                            | <0.001                          | 364.7 (79.7)                            | 273.4 (73.4)                                  | 0.003                           |
| <b>Total Urinary<br/>GC*<br/>(microg/day)</b>                    | 9887 (7721-<br>18372)                     | 10224 (7841-<br>17000)                  | n/s                             | 8192 (5534-<br>12506)                   | 4925 (3699-<br>6806)                          | n/s                             |
| <b>11<math>\beta</math>HSD<br/>activity (urine<br/>THFs:THE)</b> | 1.12 (0.37)                               | 1.15 (0.45)                             | n/s                             | 1.28 (0.79)                             | 0.81 (0.49)                                   | n/s                             |
| <b>Plasma cortisol<br/>(nmol/litre)</b>                          | -   | -                                       |                                 | 349 (106)                               | 321 (65)                                      | n/s                             |
| <b>GR mRNA</b>   | -   | -                                       |                                 | 59.4 (24.5)                             | 58.3 (18.8)                                   | n/s                             |
| <b>11<math>\beta</math>HSD1<br/>mRNA</b>                         | -   | -                                       |                                 | 25.3 (19.7)                             | 32.2 (31.8)                                   | n/s                             |

Data are mean (SD) except \*non-parametric data therefore median and IQ range shown

a. Independent t test between younger and older men in Group 1

b. Independent t test between men and women in Group 2

n/s = not significant

Table 8.2 shows bivariate correlations, which confirmed that measures of body size (height and BMI) were significantly associated with muscle size and strength.

*Table 8-2: Bivariate correlations including muscle size and strength*

|   | Group 1 muscle size | Group 1 muscle strength | Group 2 muscle size | Group 2 muscle strength |
|---|---------------------|-------------------------|---------------------|-------------------------|
| <b>Height</b>                           | .34*                | .22                     | .68**               | .42*                    |
| <b>BMI</b>                              | -.04                | -.07                    | .32                 | .38*                    |
| <b>Total urinary GC</b>                 | -.04                | .01                     | .61**               | .45*                    |
| <b>11<math>\beta</math>HSD activity</b> | -.09                | -.05                    | .35                 | .16                     |
| <b>Plasma cortisol</b>                  | -                   | -                       | -.20                | -.31                    |
| <b>GR mRNA</b>                          | -                   | -                       | .11                 | .04                     |
| <b>11<math>\beta</math>HSD1 mRNA</b>    | -                   | -                       | -.15                | -.29                    |

Data are Spearman's Rho Correlation Coefficients

\*\*p<0.01 (2-tailed)

\*p<0.05 (2-tailed)

Therefore in constructing multivariate models I adjusted for body size as well as age and gender, which had been selected *a priori* due to their accepted relationships with muscle size and strength. BMI correlated with total urinary GC (rho=0.60, p=0.0005) and plasma cortisol (rho= -0.52, p=0.006), whereas height did not significantly correlate with total urinary GC or plasma cortisol (p>0.05 for both). Therefore in multivariate analyses with urinary GC and plasma cortisol as predictor variables I adjusted for potential confounding by BMI, gender and age (see Tables 8.3 & 8.4). Neither BMI nor height correlated with the muscle GR or 11 $\beta$ HSD1 mRNA expression levels. Therefore because height correlated more significantly with muscle size and strength than BMI (Table 8.1), I adjusted for height and gender for the multivariate analyses with muscle GR and 11 $\beta$ HSD1 mRNA as predictor variables (Table 8.4).

Plasma cortisol was measured in Group 2 only. There were no significant association between fasting morning plasma cortisol and muscle size and a non-significant negative trend with muscle strength ( $\beta$  -0.35, p=0.08) (Table 8.4). In both groups neither total urinary glucocorticoids nor the ratio of cortisol:cortisone metabolites were associated with muscle size or strength (Tables 8.3 & 8.4).

I used muscle biopsies from a subset of Group 2 to examine the relationships between GR and 11 $\beta$ HSD1 mRNA levels and muscle size and strength. Increased 11 $\beta$ HSD1 mRNA was

significantly associated with lower muscle strength after adjustment for sex and height ( $\beta$  - 0.35,  $p=0.039$ ,  $n=22$ : 12 men mean age 79.8 (sd 3.6) and 10 women, mean age 80.5 (sd 4.1)). There were no significant relationships between GR mRNA and muscle size or strength, or between 11 $\beta$ HSD1 mRNA and muscle size (Table 8.4).

**Table 8-3: Regression coefficients for the glucocorticoid measures in models predicting muscle size/strength (Group 1)**

| <b>Glucocorticoid Measure</b>       | <b>Muscle Size<br/>Beta (sig, n)</b> | <b>Muscle Strength<br/>Beta (sig, n)</b> |
|-------------------------------------|--------------------------------------|--|
| <b>Total Urinary GC<sup>a</sup></b> | -0.10 (p=0.24, 52)                   | <-0.01 (p=0.97, 52)                      |
| <b>THFs:THE<sup>a</sup></b>         | <0.01 (p=0.95, 52)                   | 0.04 (p=0.66, 52)                        |

<sup>a</sup> adjusting for age and BMI

**Table 8-4: Regression coefficients for the glucocorticoid measures in models predicting muscle size/strength (Group 2)**

| <b>Glucocorticoid Measure</b>                    | <b>Muscle Size<br/>Beta (sig, n)</b> | <b>Muscle Strength<br/>Beta (sig, n)</b> |
|--|--------------------------------------|--|
| <b>Plasma Cortisol<sup>a</sup></b>               | -0.12 (p=0.38, 25)                   | -0.35 (p=0.08, 27)                       |
| <b>Total Urinary GC<sup>a</sup></b>              | 0.23 (p=0.10, 28)                    | 0.18 (p=0.38, 30)                        |
| <b>THFs:THE<sup>a</sup></b>                      | 0.08 (p=0.47, 28)                    | 0.10 (p=0.55, 30)                        |
| <b>GR mRNA<sup>b</sup></b>                       | 0.03 (p=0.84, 20)                    | 0.04 (p=0.81, 22)                        |
| <b>11<math>\beta</math>HSD1 mRNA<sup>b</sup></b> | -0.17 (p=0.17, 20)                   | <b>-0.35 (p=0.04, 22)</b>                |

<sup>a</sup> adjusting for gender and BMI

<sup>b</sup> adjusting for gender and height



## 8.4 Discussion

This study investigated the relationship between circulating and tissue indices of glucocorticoid status and muscle size and strength in two groups. Group 1 allowed comparison of older with younger men. There were no age differences in urinary cortisol metabolites, although muscle biopsies were not obtained in this group so I did not test the effect of ageing *per se* on muscle mRNA levels. Group 2 allowed comparison of older men with older women. There were no differences in plasma cortisol, urinary glucocorticoid metabolites or muscle GR or 11 $\beta$ HSD1 mRNA levels between the sexes in this relatively small sample. Within each group I explored associations between glucocorticoid variables and muscle size and strength after adjustment for potential confounding effects of age, gender and body size as appropriate. In these analyses, indices of HPA axis function, including morning plasma cortisol and 24 h urinary cortisol metabolite excretion, were not associated with muscle strength or size. Additionally urinary cortisol:cortisone metabolite ratios, which principally reflect 11 $\beta$ HSD activity in the major organs of liver and kidney, were not associated with muscle strength or size. However, in muscle itself, higher levels of mRNA encoding the cortisol-amplifying enzyme 11 $\beta$ HSD1 were associated with reduced muscle strength. This finding is consistent with the hypothesis that enhanced glucocorticoid signalling within muscle contributes to sarcopenia.

To our knowledge there have been no previous investigations of muscle glucocorticoid signalling in human sarcopenia. I hypothesized that because 11 $\beta$ HSD1 and GR regulate the exposure of target tissues to glucocorticoids, increased expression of 11 $\beta$ HSD1 and GR could therefore contribute to sarcopenia in the absence of an increase in circulating GCs. I found that increased 11 $\beta$ HSD1 mRNA expression in muscle is associated with lower muscle strength. This is consistent with this hypothesis. I did not find a relationship between 11 $\beta$ HSD1 mRNA expression and muscle size, but in normal ageing, muscle strength is reported to deteriorate more rapidly than muscle size; suggesting a decline in force generating capacity with age (Goodpaster et al., 2006, Young et al., 1985). A number of contributory mechanisms have been proposed to explain this (eg increased muscle fibre stiffness); our data suggest a possible role for increased GC action at the muscle level. GC may affect strength more than muscle mass by exacerbating glycation of the myosin molecule, which appears to: slow the intrinsic shortening velocity of the muscle fibre; decrease force per cross-sectional area; and increase intramuscular collagen cross-linking which can cause muscle stiffness (Hook et al., 2001, Haus et al., 2007). In addition, GC may cause mitochondrial dysfunction

and reduced oxidative capacity, which would similarly result in a decrease in force generating capacity (Mitsui et al., 2002). Furthermore an association between insulin resistance and sarcopenia has been found (Haus et al., 2007, Janssen and Ross, 2005) and there is evidence that the morphological changes seen with steroid induced myopathy may be associated with insulin resistance, as indicated by low glycogen synthase activity, indicating a further possible pathway (Rebuffe-Scrive et al., 1988). 11 $\beta$ HSD1 is known to act locally within muscle, resulting in measurable production of cortisol in samples from veins draining human muscle, and therefore increased 11 $\beta$ HSD1 mRNA expression is likely to increase myocellular cortisol levels thereby mediating these effects (Hughes et al., 2012). More research with larger samples and with a wider range of severity of sarcopenia is required to investigate the relationship between 11 $\beta$ HSD1 expression and activity and muscle ageing.

I found no relationship between GR mRNA expression and muscle mass or strength. It is possible that polymorphisms of GR modulate the effect of GC on muscle, and that level of expression is less important than genotype. For example male carriers of the ER22/23EK polymorphism in GR, which is associated with relative GC resistance, have greater muscle mass and strength than non-carriers (van Rossum et al., 2004).

There are no published studies investigating the association between urinary GC and muscle size or strength. However, there are studies reporting associations between salivary and plasma GCs and muscle size and function. In a previous study of men and women >75 years higher salivary, but not serum, cortisol was associated with lower appendicular skeletal mass (ASM) measured using DEXA (Waters et al., 2008). Similarly, in a large longitudinal ageing study higher salivary but not serum cortisol predicted loss of grip strength over 6 years, but there was no association of cortisol with baseline grip strength or ASM (Peeters et al., 2008). A smaller study including both young and older men found that increased serum cortisol correlated with lower knee extensor strength in both age groups and with quadriceps cross-sectional area only in the older group (Izquierdo et al., 2001). The Caerphilly Prospective Study, which included measurements of cortisol status and physical performance over 20 years, found that higher mid-life plasma cortisol predicted faster walking speeds in older age, although salivary cortisol did not correlate with walking speed or balance in older age (Gardner et al., 2011). Collectively, these studies provide contradictory evidence relating salivary or plasma cortisol to muscle strength and mass. Taken with our data, there does not

appear to be a consistent association between activation of the HPA axis and age-associated sarcopenia. These negative findings are important in excluding this plausible hypothesis.

Some limitations of this study should be acknowledged. I examined the effect of GC on ageing muscle using a younger and older group of volunteers separated in age by nearly 50 years and by many lifestyle factors; a problem inherent to cross-sectional studies. Longitudinal studies investigating rate of decline of muscle mass and function and measures of GC would be more informative but are difficult to conduct due to the slow decline of muscle mass and strength during ageing. The sample sizes were relatively modest, particularly with respect to muscle GC data which were obtained from only a subset of Group 2 who underwent muscle biopsy. It has also been shown that sarcopenia affects the upper and lower limbs differently and our study investigated only the lower limbs (Janssen et al., 2000, Aniansson et al., 1983, Aniansson et al., 1986, Aniansson et al., 1992). Also, our healthy older volunteers constituted a sample which may be not fully representative of the ageing population; this may influence the generalisability of our results. Finally, I used mRNA expression as a marker of activity rather than a direct measure of 11 $\beta$ HSD1 activity, however several studies have found correlations between mRNA expression and enzyme activity in rodents and humans, so I regard mRNA as an appropriate indicator of 11 $\beta$ -HSD1 activity (Wake et al., 2003).

## **8.5 Conclusion**

Sarcopenia is one of the major causes of frailty and disability in older people. It is associated with greatly increased risk of loss of independence and institutionalization. In this novel investigation of healthy old and young people I found a significant association between increased muscle 11 $\beta$ HSD1 expression and lower quadriceps strength. I found no significant associations between plasma cortisol, urinary GC metabolites or GR expression and muscle mass or strength. Longitudinal studies are now required to investigate these relationships and to further explore the possibility of 11 $\beta$ HSD1 inhibitors as a novel treatment for sarcopenia.

# Chapter 9 A novel technique to measure 11 $\beta$ -hydroxysteroid dehydrogenase activity in human brain in vivo

## 9.1 Introduction

In the last two chapters I investigated potential underlying mechanisms for the development and progression of sarcopenia. In the latter chapter the role of glucocorticoid dysregulation was researched. Within the field of cognitive ageing there has been considerable interest in the role of glucocorticoid dysregulation, particularly with regard to 11beta-hydroxysteroid dehydrogenase type 1. The 11beta-hydroxysteroid dehydrogenases (11 $\beta$ HSDs) are intracellular enzymes that catalyse interconversion of inactive cortisone and active cortisol; 11 $\beta$ HSD type 1 is a predominant reductase, regenerating cortisol from cortisone, while 11 $\beta$ HSD type 2 is an exclusive dehydrogenase, inactivating cortisol to cortisone. An extensive literature documents the role of the 11 $\beta$ HSDs in regulating intracellular glucocorticoid concentrations and hence modulating tissue-specific activation of corticosteroid receptors (reviewed in (Seckl and Walker, 2001, Stewart and Krozowski, 1999)). 11 $\beta$ HSD1 amplifies glucocorticoid action in liver, adipose tissue, inflammatory cells and vasculature, providing a therapeutic target for inhibition in type 2 diabetes. 11 $\beta$ HSD2 limits cortisol action, and thereby facilitates aldosterone action in the distal nephron and a few other sites, explaining the mineralocorticoid excess state which ensues when 11 $\beta$ HSD2 is inhibited by liquorice.

Both 11 $\beta$ HSD isozymes also contribute to systemic turnover of cortisol. Activity of the two isozymes has been quantified in humans using stable isotope (deuterated) glucocorticoid tracers (figure 9.1). Production of cortisone can be measured by the rate of dilution of 1,2- $^2\text{H}$ <sub>2</sub>-cortisone (d2-cortisone) by cortisone, and is inhibited by liquorice (Hughes et al., 2012). A more complex tracer is used to measure regeneration of cortisol by 11 $\beta$ HSD1: 9,11,12,12- $^2\text{H}$ <sub>4</sub>-cortisol (d4-cortisol) is infused and production of cortisol is measured by the rate of dilution of d4-cortisol by cortisol (an index of net cortisol production from all sources, including the adrenal gland) and by 9,12,12- $^2\text{H}$ <sub>3</sub>-cortisol (d3-cortisol, a specific measure of cortisol regeneration by 11 $\beta$ HSD1) (Andrew et al., 2002). Using these tracers in combination with selective venous catheterisation and measurement of blood flow has allowed quantification in humans of cortisol-cortisone interconversion in splanchnic (Hughes

et al., 2012, Basu et al., 2004, Andrew et al., 2005), subcutaneous adipose (Hughes et al., 2012, Stimson et al., 2009) and skeletal muscle (Hughes et al., 2012) circulations, and exclusion of significant 11 $\beta$ HSD activity in the myocardium (Iqbal et al., 2014). These studies reveal rapid shuttling between cortisol and cortisone such that in healthy men at rest, remarkably, the magnitude of extra-adrenal regeneration of cortisol by 11 $\beta$ HSD1 is greater than the magnitude of adrenal cortisol secretion. They also suggest, surprisingly, that 11 $\beta$ HSD1 may catalyse both reductase and dehydrogenase activity in vivo, resulting in 'recycling' between cortisol and cortisone even in tissues where 11 $\beta$ HSD2 is not expressed (Hughes et al., 2012).

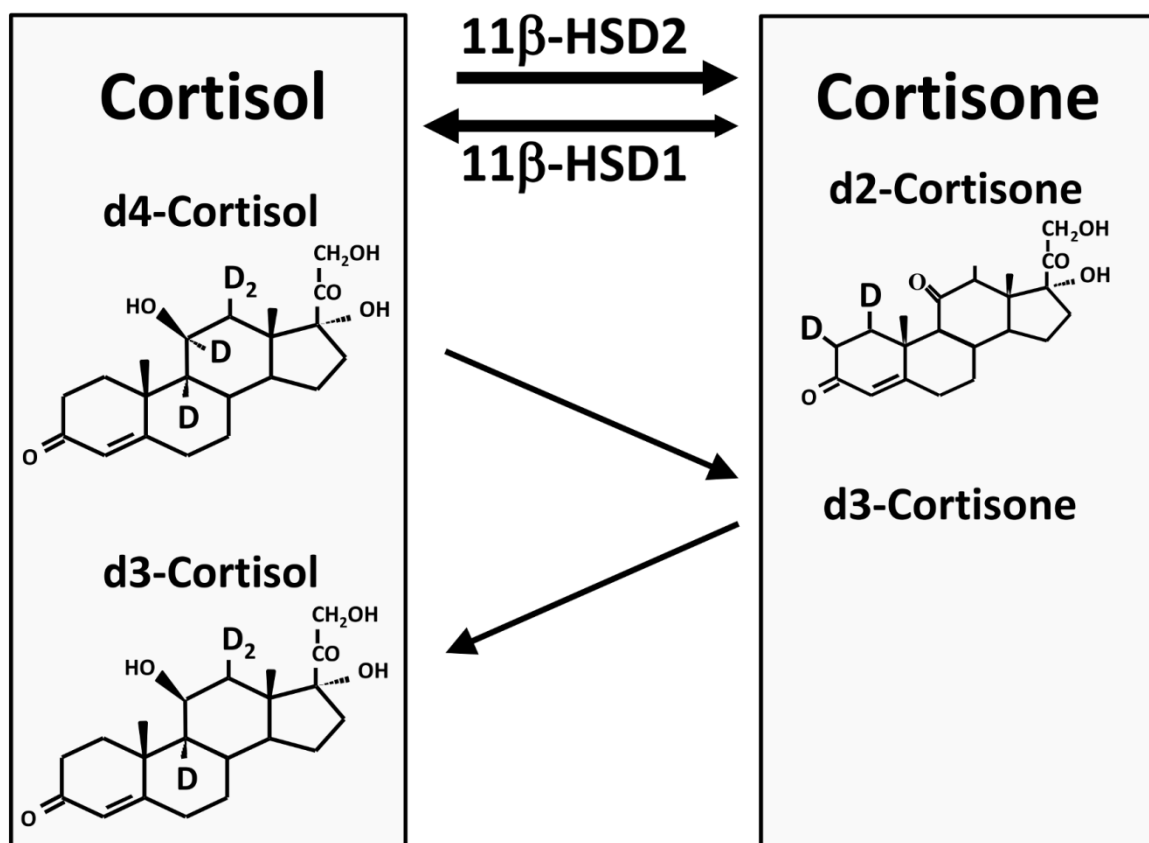
11 $\beta$ HSDs may also play key roles in the brain (Yau and Seckl, 2012). 11 $\beta$ HSD2 is expressed in the developing but not the adult brain (Brown et al., 1996). 11 $\beta$ HSD1 is expressed more widely in the adult brain, and notably in the prefrontal cortex, hippocampus and cerebellum, a distribution confirmed in humans by a small study (n=4) (Sandeep et al., 2004). Larger post mortem studies on human brain would now be useful to validate this finding. Increased local regeneration of cortisol by 11 $\beta$ HSD1 may cause glucocorticoid-dependent neurotoxicity and hence contribute to cognitive ageing and dementia, while inhibition of 11 $\beta$ HSD1 has been proposed as a therapeutic strategy to treat age- and dementia-associated cognitive dysfunction. In mice, 11 $\beta$ HSD1 levels in the hippocampus and parietal cortex rise with age and correlate with impaired cognitive performance, while transgenic overexpression of 11 $\beta$ HSD1 in the forebrain accelerates age-associated cognitive decline (Holmes et al., 2010). Conversely, 11 $\beta$ HSD1 knockout mice are protected from age-related learning impairment (Yau et al., 2001, Sooy et al., 2010). Moreover, selective 11 $\beta$ HSD1 inhibitors, after either systemic or intracerebroventricular administration, improve cognitive function in aged mice (Sooy et al., 2010). Indeed in humans, the non-selective 11 $\beta$ HSD inhibitor carbenoxolone improved cognitive performance in healthy elderly and diabetic men (Sandeep et al., 2004). However, a recent Phase II clinical trial of a selective 11 $\beta$ HSD1 inhibitor, ABT384, in patients with mild-moderate Alzheimer's disease was halted prematurely because of lack of efficacy (Marek et al., 2014). It is uncertain whether this reflected selection of patients whose cognitive dysfunction is no longer responsive to reducing cortisol action, or inadequate inhibition of brain 11 $\beta$ HSD1 by ABT384.

Against this background, quantification of brain 11 $\beta$ HSD1 activity in vivo in humans would be highly desirable. Here, we aimed: (i) to determine whether, as a major organ by mass,

brain 11 $\beta$ HSD1 contributes to cortisol/cortisone turnover in vivo; (ii) to establish whether 11 $\beta$ HSD1, as the only 11 $\beta$ HSD isozyme expressed in adult brain (Sandeep et al., 2004), catalyses only regeneration of cortisol or also recycling between cortisol and cortisone; (iii) to provide a tool with which to quantify changes in human brain 11 $\beta$ HSD1 activity with dementia, and to use as a pharmacodynamics biomarker to quantify enzyme inhibition during clinical development of selective 11 $\beta$ HSD1 inhibitors for treating dementia. We therefore extended the previous studies using arteriovenous sampling with stable isotope tracer infusions in vivo to quantify 11 $\beta$ HSD activities in human brain.

Figure 9-1: Stable isotope tracers for measuring cortisol-cortisone interconversion by 11 $\beta$ -HSDs

11 $\beta$ -HSD2 is a unidirectional enzyme catalysing the dehydrogenase conversion of cortisol to cortisone. 11 $\beta$ -HSD1 is a potentially reversible enzyme catalysing interconversion of cortisol and cortisone, predominantly in the reductase (cortisone to cortisol) direction. The shaded boxes on left and right represent the circulating pools of cortisol and cortisone, respectively. Production of cortisone can be measured by infusing a tracer, d2-cortisone, into the cortisone pool and measuring its dilution by cortisone. Similarly, production of cortisol can be measured by infusing a tracer, d4-cortisol, into the cortisol pool. When d4-cortisol is metabolised by 11 $\beta$ -HSD2, the deuterium in the 11 $\alpha$  position is removed, producing d3-cortisone; when this d3-cortisone is converted back to cortisol by 11 $\beta$ -HSD1 it is highly unlikely that a deuterium rather than a proton will be reincorporated, so that d3-cortisol is produced. Dilution of d4-cortisol with d3-cortisol therefore indicates 11 $\beta$ -HSD1 reductase activity.



## 9.2 Materials and Methods

### 9.2.1 Participants

Participants were recruited through advertisements in the local press and around the University campus. Participants were healthy male volunteers between 18-70 years old. Exclusion criteria were: glucocorticoid medication (by any route of administration) within the past three months; diabetes mellitus, cerebrovascular disease or other significant chronic illness; history of recent heavy alcohol or illegal drug use; current use of any immunosuppressive medication; abnormal screening liver, thyroid, renal or coagulation function (ie INR>1.5 or platelets <50 x 10<sup>9</sup>/L) or abnormal full blood count or random blood glucose; research participant in the previous 3 months; any contraindication to magnetic resonance (MR) imaging. The study complied with the Declaration of Helsinki; ethical approval was obtained from the local Research Ethics Committee (Scotland A REC, reference 12/SS/0079) and all participants gave written informed consent.

### 9.2.2 Reagents

Reagents were obtained from Sigma (Poole, U.K.), Steraloids (Newport, RI), or VWR (Lutterworth, UK). 1,2 [<sup>2</sup>H]<sub>2</sub>-cortisone (d2-cortisone) and 9,11,12,12 [<sup>2</sup>H]<sub>4</sub>-cortisol (d4-cortisol) were from Cambridge Isotope Laboratories (Andover, MA). Solvents were high-performance liquid chromatography grade from Fisher Scientific (Loughborough, UK).

### 9.2.3 Clinical protocol

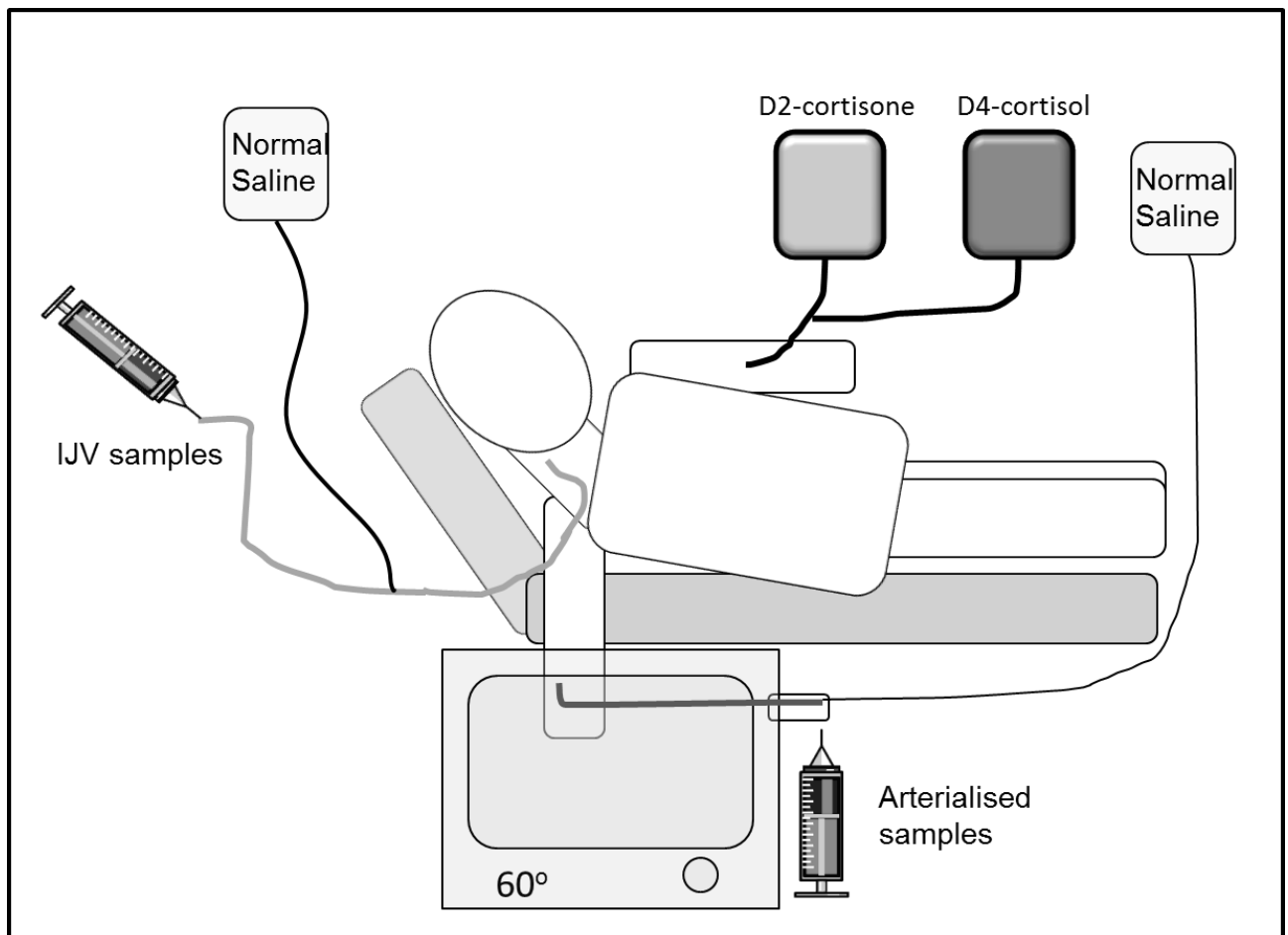
Screening tests were performed within the 2 weeks prior to the procedure. On the day of the study subjects attended the Clinical Research Facility around 0830h. They were allowed to eat a light breakfast at home but were only allowed water after their arrival at the research facility. They first underwent an ECG-gated phase contrast MR neck scan, which lasted 20-30 minutes. After this, a cannula was inserted into the left antecubital fossa and blood was taken to measure baseline endogenous steroids and their background isotopomers. At t=-5mins a 0.7mg loading bolus of d4-cortisol was given intravenously, followed by an infusion at 0.35mg/hour (15.94 nmol/min) at t=0mins. A cannula was inserted into a vein in the dorsum of the right hand in a retrograde direction and when required the hand was placed in a hot box at 60°C to arterialise the blood. Arterialisation of the blood was accepted if the oxygen saturation was >98%.



A jugular bulb cannula was inserted in the dominant internal jugular vein (assessed by MR venography) under ultrasound guidance. Placement was checked using a plain lateral C-spine x-ray: the tip of the catheter was visualised to ensure it was above the second cervical vertebra (Ali et al., 2001). The oxygen saturation of the blood was checked to ensure it was <85%.

At t=145 min a 76.0µg bolus of d2-cortisone was administered intravenously, followed by an infusion at 105.3µg/hour (4.88 nmol/min). From t=180 min four sets of simultaneous blood samples were taken from the arterialized and jugular bulb cannulae at ten minute intervals (t=180, 190, 200 and 210 mins) into Lithium heparin and plasma separated and stored at -80°C until analysis.

*Figure 9-2: Schematic diagram depicting set up of: subject, tracers and arteriovenous sampling with hot box*



### 9.2.4 Magnetic resonance scanning

The MR imaging was performed with participants in the supine position on a 1.5 Tesla MR imaging unit (Signa HDxt, GE Healthcare, Milwaukee, USA) at the Brain Research Imaging

Centre ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)). An eight channel neurovascular array coil was used. A non-contrast MR angiogram of the carotids from the level of the exterior auditory meatus down to the angle of the mandible was performed, followed by an MR venogram over the same area with a saturation band over the inferior aspect of the slices. The MR venogram was used to select the level for the ECG-gated phase-contrast MR scan by measuring a quarter of the way down from the jugular bulb to where the facial vein enters the internal jugular vein; axial images were taken perpendicular to the table. This level was chosen to ensure blood flow was measured in the vessel before any of the tributaries entered, the facial vein being the first, and to avoid measuring flow dynamics in the bulb itself, which may not be representative of the rest of the vessel as there may be some pooling of blood where it first enters the bulb. Sixteen phase and magnitude images were taken at this level over one cardiac cycle. The flip angle was 25°, bandwidth 15.63 KHz, echo time (TE) 6ms, repetition time (TR) 25 ms and encoding velocity (Venc) 100cm/s. Data were checked at the point of acquisition for any aliasing artefact. The field of view (FOV) was 25.6 cm×25.6 cm and slice thickness 5 mm. These images took approximately 8 minutes to acquire per patient, depending on heart rate.

The image analysis software Medis Q flow (Medis medical imaging systems; Leiden, the Netherlands) was used to calculate jugular venous blood flow in mL/min on the side which was cannulated.

### 9.2.5 Laboratory analysis

Steroids (cortisol, d4-cortisol, 9,12,12 [<sup>2</sup>H]<sub>3</sub>-cortisol (d3-cortisol), cortisone and d2-cortisone) were quantified using liquid chromatography–tandem mass spectrometry (LC-MS). An internal standard solution (0.5microg epicortisol (Steraloids; Newport, RI), 0.25microg 2,2,4,6,6,9,12,12 [<sup>2</sup>H]<sub>8</sub>-cortisone (d8-cortisone, Santa Cruz Biotechnology; Heidelberg, Germany) and 0.25microg 2,2,4,6,6,17A,21,21 [<sup>2</sup>H]<sub>8</sub>-corticosterone (d8-corticosterone, Cambridge Isotope Laboratories; Andover, MA) with 9µL methanol) was added to 1.5 mL of plasma, before 15 mL of chloroform was added for extraction of the steroids. The organic phase was then reduced to dryness under oxygen free nitrogen at 60°C before being reconstituted in mobile phase (acetonitrile: water (35:65) with 0.1% formic acid). Samples were injected on to a Sunfire C18 column (150mm x 4.6mm x 5µm), with a column temperature of 10<sup>0</sup>C and a mobile phase flow rate of 1.5 mL/min using an Acquity Ultra Performance Liquid Chromatograph (Waters, Manchester, UK) coupled to a Qtrap 5500 mass spectrometer (AB Sciex, Warrington, UK). Ionization was achieved in positive electrospray

mode. The following transitions (precursor→product mass-to-charge ratios) used were as follows: cortisol (363→121), d2-cortisol (365→121), d3-cortisol (366→121), d4-cortisol (367→121), cortisone (361→77), d3-cortisone (364→164) and d2-cortisone (363→165). Steroid concentrations and tracer/tracee ratios were calculated from calibration curves and corrected for background isotopomer enrichments as described previously (Boonen et al., 2013).

### 9.2.6 Data analysis and kinetic calculations

Whole-body rate of appearance (Ra) of cortisol, d3-cortisol and cortisone were calculated by dividing the rate of tracer infusion by the relevant tracer/tracee ratio (d4-cortisol/cortisol, d4-cortisol/d3-cortisol and d2-cortisone/cortisone, respectively) (Hughes et al., 2012, Andrew et al., 2002). Brain tissue production of cortisol was calculated using data from arterial (A) and internal jugular vein (V) samples and corrected for cerebral blood flow using equation 1. Brain tissue production of d3-cortisol and cortisone were also calculated with equation 1, substituting arterial concentrations of d3-cortisol or cortisone and the relevant arterial and venous tracer:tracee ratios, as appropriate (Hughes et al., 2012). Net release or uptake of cortisol across the brain was calculated using equation 2. Net release or uptake of d4-cortisol or cortisone were also calculated with equation 2, substituting arterial and venous concentrations of d4-cortisol and cortisone, respectively.

*Equation 1:*

$$\text{Tissue cortisol production} = \left( (\text{blood flow} \times [\text{cortisol}_A]) \times \left( \frac{\text{d4-cortisol: cortisol}_A}{\text{d4-cortisol: cortisol}_V} \right) \right) - (\text{blood flow} \times [\text{cortisol}_A])$$

*Equation 2:*

$$\text{Net cortisol release} = ([\text{cortisol}_V] - [\text{cortisol}_A]) \times (\text{blood flow})$$

### 9.2.7 Statistical analysis

There were no prior data for cortisol or cortisone production or uptake across the brain on which to base a power calculation, therefore we used previous data from a study on cortisol and cortisone production in skeletal muscle in healthy men (Hughes et al., 2012). We calculated that in order to detect the same magnitude of difference from zero for net uptake or release across brain of cortisol, d3-cortisol or cortisone, with a similar variance to that in skeletal muscle, would require sample sizes of 3, 5 and 7, respectively (for alpha 0.05, power 80% in a two-tailed test). We therefore considered a sample size of 8 to be reasonable.

Using SPSS, Student's t test was used to detect a difference from zero in brain production of cortisol, d3-cortisol and cortisone. The small n in the study required the use of parametric tests despite being unable to fully check the assumptions because it has been shown that in small study samples non-parametric tests are not sensitive enough to pick up even a large effect size (Bland and Altman, 2009). However, on visual inspection there were no obvious outliers and the data appeared to meet the assumptions of normality.

### 9.3 Results

Eight healthy men were recruited with a mean age of 38.1years (sd 16.5) and mean BMI of 24.9kg/m<sup>2</sup> (sd 3.7).

*Table 9-1: Demographics for the subjects*

| <b>Subject 1</b> | <b>Age on day of study (years)</b> | <b>Height (m)</b>  | <b>Weight (kg)</b> | <b>BMI (kg/m<sup>2</sup>)</b> |
|------------------|------------------------------------|--------------------|--------------------|-------------------------------|
| <b>1</b>         | 46.6                               | 1.76               | 62.5               | 20.3                          |
| <b>2</b>         | 31.8                               | 1.82               | 77.9               | 23.5                          |
| <b>3</b>         | 45.1                               | 1.87               | 97.4               | 28.0                          |
| <b>4</b>         | 25.0                               | 1.79               | 81.4               | 25.4                          |
| <b>5</b>         | 48.8                               | 1.74               | 95.2               | 31.4                          |
| <b>6</b>         | 21.5                               | 1.71               | 65.0               | 22.2                          |
| <b>7</b>         | 67.0                               | 1.78               | 85.0               | 26.8                          |
| <b>8</b>         | 19.2                               | 1.79               | 69.4               | 21.7                          |
| <b>Mean (SD)</b> | <b>38.1 (16.5)</b>                 | <b>1.78 (0.05)</b> | <b>79.2 (13.1)</b> | <b>24.9 (3.7)</b>             |

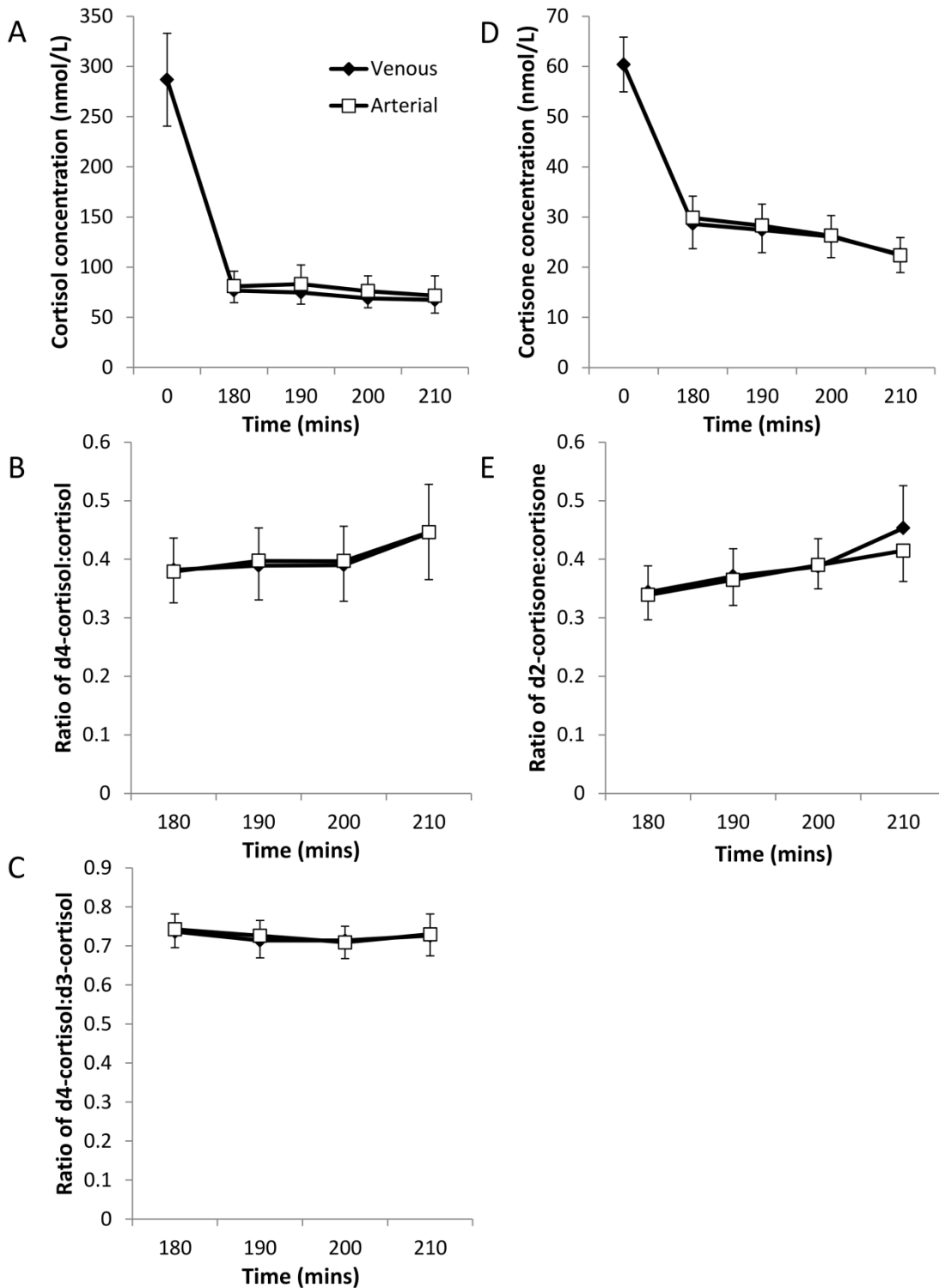
In 7 of the 8 subjects, the right internal jugular was found to be dominant or co-dominant on the MR scan and was used for cannulation and measurement of blood flow; in one subject the left internal jugular vein (IJV) was dominant and was cannulated instead. Mean blood flow in the selected IJV for all subjects was 481 mL/min (sd 232).

Endogenous cortisol and cortisone concentrations in both arterialized and jugular venous samples reduced from baseline to 180 mins of infusion, when the first set of arteriovenous samples were obtained, which is consistent with diurnal variation (figure 9.3A, D). In both arterialized and jugular venous samples, concentrations of cortisol and cortisone and tracer/tracee ratios were similar at all four sampling time points between 180 and 210 mins of infusion, consistent with steady state being achieved (figure 9.3).

**Figure 9-3: Arterialized and jugular venous steroid concentrations and tracer/tracee ratios during steady state stable isotope tracer infusion.**

Data are mean  $\pm$  SEM for n=8. A) Cortisol concentration; B) cortisone concentration; C) d4-cortisol/cortisol ratio; D) d4-cortisol/d3-cortisol ratio; E) d2-cortisone/cortisone ratio.

Statistical comparisons were made for the kinetic parameters derived from these 'raw' data (table 9.2).



Mean values in arterialized samples in steady state were used to calculate whole body rates of appearance of cortisol, d3-cortisol and cortisone (table 9.1), all of which were readily detectable, confirming technical success of the tracer infusions. Mean steady state data from arterialized and jugular venous samples were combined with blood flow measurements to calculate net release (or uptake) of cortisol and cortisone (equation 2) and to estimate cortisol, d3-cortisol and cortisone production across the brain (equation 1) (table 9.2). Surprisingly, d4-cortisol concentrations were higher in jugular vein than arterialized samples, so that there was net release of d4-cortisol across the brain in steady state. No other indices of brain steroid release / uptake or production were significantly different from zero.

*Table 9-2: Calculated steady state kinetics for cortisol and cortisone for whole body and brain*

|                                     | Whole body                       | Brain                            |
|-------------------------------------|----------------------------------|----------------------------------|
| <b>Cortisol:</b>                    |                                  |                                  |
| Rate of appearance of cortisol      | 47.5 (29.9 to 65.1) <sup>a</sup> | 0.43 (-0.27 to 1.12)             |
| Rate of appearance of d3-cortisol   | 22.5 (19.8 to 25.3) <sup>a</sup> | 0.21 (-0.20 to 0.62)             |
| Net brain release of cortisol       | -                                | -0.54 (-3.75 to 2.66)            |
| Net brain release of d4-cortisol    | -                                | 0.46 (0.22 to 0.70) <sup>b</sup> |
| Net brain release of d3-cortisol    | -                                | 0.12 (-0.36 to 0.60)             |
| <b>Cortisone:</b>                   |                                  |                                  |
| Net rate of appearance of cortisone | 14.4 (10.8 to 17.9) <sup>a</sup> | -0.02 (-0.35 to 0.30)            |
| Net brain release of cortisone      | -                                | -0.23 (-0.69 to 0.23)            |
| Net brain release of d2-cortisone   | -                                | -0.01 (-0.05 to 0.02)            |

Data are nmol/min shown as mean (95% CI) for n=8, except for net brain release of d2-cortisone, where reliable data could only be gained for n=5.

<sup>a</sup> p<0.001, <sup>b</sup> p<0.005 vs. zero using Student's one-sample t test.

All other associations p>0.05.

## 9.4 Discussion

We found no detectable interconversion of cortisol and cortisone across the human brain in eight healthy male volunteers, using deuterated glucocorticoid tracers and arteriovenous sampling. Previous studies using this approach have detected interconversion of cortisol plus or minus cortisone within liver, adipose tissue and skeletal muscle (Hughes et al., 2012, Basu et al., 2004, Andrew et al., 2005, Stimson et al., 2009). Systemic cortisol/cortisone turnover values were similar in the subjects reported here to those observed in previous studies, so the approach was technically successful. The lack of statistically significant net production or release of cortisol, d3-cortisol or cortisone across the brain could not be attributed to variability in blood flow, either methodological due to the magnetic resonance method used or biological due to asymmetry of internal jugular blood flow, since we did not observe expected gradients in relevant steroid concentrations from arterialized to jugular venous blood, and we sampled from the dominant jugular vein and therefore from venous drainage of a substantial proportion of brain tissue. Any contribution of 11 $\beta$ HSD in brain to whole body turnover between cortisol and cortisone is, therefore, negligible. It remains possible that, had we sampled from discreet brain subregions, 11 $\beta$ HSD activity might have been measurable, but this remains a speculation. The consequences of brain 11 $\beta$ HSD1 activity are likely to be confined to the sub-regions in which the enzyme is highly expressed (Sandeep et al., 2004).

In the absence of arteriovenous gradients in cortisol or cortisone concentrations, we did find a net release of d4-cortisol across the brain. It is unclear why there was release of tracer from the brain but it may indicate over-priming and higher circulating d4-cortisol levels earlier in the infusion, with resulting re-release from brain during steady state; however, this speculation cannot be tested in the absence of earlier samples.

One previous study has evaluated *in vivo* 11 $\beta$ HSD1 activity in brain, by measuring peripheral venous plasma and cerebrospinal fluid (CSF) steroid concentrations during d4-cortisol infusion (Katz et al., 2013). Unfortunately, data were presented for only two subjects without administration of a potent 11 $\beta$ HSD1 inhibitor; strangely, these subjects did not appear to reach steady state of tracer enrichment after 4 hours of infusion and tracer/tracee ratios in plasma were not adjusted for d4-cortisol infusion to calculate rate of appearance of cortisol and d3-cortisol and establish if the results were comparable with other published studies. CSF d3-cortisol concentrations were shown to be higher, relative to d4-cortisol, than plasma d3-cortisol concentrations, but only by comparing CSF data with plasma obtained 60 minutes



earlier; this adjustment was applied on the grounds of closer correlation between plasma and CSF steroid concentrations separated by 60 minutes than those separated by shorter or longer intervals, but again indicates that steady state was not achieved in CSF. The authors attributed the apparent excess of d3-cortisol in CSF to brain 11 $\beta$ HSD1, partly on the basis that it was abolished in a dose-dependent fashion by administration of the 11 $\beta$ HSD1 inhibitor ABT384. However, ABT384 dramatically lowered plasma d3-cortisol concentrations, consistent with potent inhibition of systemic 11 $\beta$ HSD1 activity; the associated fall in CSF d3-cortisol concentrations, which fell below the limit of quantification in most samples, can be explained by the dramatic fall in plasma d3-cortisol without invoking any contribution of brain 11 $\beta$ HSD1. Against this background, it has not been established whether activity of 11 $\beta$ HSD1 in brain is sufficient in magnitude to affect CSF cortisol concentrations.

We took a different approach, measuring steroids in jugular venous blood and calculating arteriovenous differences during steady state tracer infusion. With measurement of blood flow, this allows absolute quantification of 11 $\beta$ HSD activities for all tissue draining to the cannulated internal jugular vein. 11 $\beta$ HSD1 is known to be expressed in specific neuronal subregions of the human brain: hippocampus (in particular the dentate gyrus and the cornu ammonis), prefrontal cortex and, the area of highest expression, the granule cell layer of the cerebellum (Sandeep et al., 2004). The venous drainage of the human brain is complex and appears to display considerable inter-individual differences. Cerebellar venous blood drains to the superior and inferior cerebellar veins and usually ultimately into the internal jugular veins. However, posture affects cerebrovenous drainage such that in the supine position the internal jugular veins drain around 95% of blood flow from the intracerebral structures, whilst in the erect position this can drop to as little as 25%, with the remainder draining through the vertebral venous plexus (Valdúeza et al., 2000). Our subjects were reclining at an angle of 45 degrees for the arteriovenous sampling, but the measure of internal jugular vein blood flow was performed supine within a magnetic resonance imaging machine. It could be that during blood sampling more of the cerebellar venous drainage was to the vertebral venous system rather than to the internal jugular veins, and that brain 11 $\beta$ HSD1 was underestimated as a result. Moreover, the forebrain subregions where 11 $\beta$ HSD1 is expressed represent a minority, by mass, of forebrain tissue and hence any contribution of these subregions to the plasma steroid pool may be diluted by blood from elsewhere. More

selective venous cannulation might therefore detect 11 $\beta$ HSD1 activity which was not measurable here, but this is unlikely to be feasible in healthy volunteers.

As there were no previous data on brain release or uptake of cortisol or cortisone, sample size was calculated using data from a previous study on skeletal muscle (Hughes et al., 2012). With the current novel results from brain in hand, we have performed new power calculations which show that to demonstrate that the observed mean differences are statistically significantly different from zero for Ra cortisol, Ra d3-cortisol and net Ra cortisone we would need sample sizes of 40, 57 and 4006 respectively (for alpha 0.05, power 90% in a two-tailed test). We do not consider it justified to undertake invasive studies in this large number of subjects in order to more precisely quantify any small amount of cortisol production in brain, having shown its mean magnitude to be negligible relative to other tissues.

If 11 $\beta$ HSD activity in brain had been detectable with the approach used here, this could have provided a useful pharmacodynamic tool to quantify brain enzyme inhibition by selective 11 $\beta$ HSD1 inhibitors in development for treatment of dementia (Katz et al., 2013, Sooy et al., 2010). As things stand, neither this approach nor the CSF approach attempted by Katz et al. appear well suited for this purpose in healthy volunteers. Given that 11 $\beta$ HSD1 expression increases with age in mice and is predictive of cognitive decline (Holmes et al., 2010), it remains possible that arteriovenous sampling could be used to detect cortisol regeneration by 11 $\beta$ HSD1 in the brains of patients with dementia or with risk factors for cognitive decline (eg diabetes, older age).

# **Chapter 10 Assessing asymmetry and prominence of the internal jugular venous and internal carotid artery blood flow in healthy adult men**

## **10.1 Background**

Chapter 9 used magnetic resonance (MR) phase contrast imaging to quantify internal jugular vein and internal carotid artery blood flow. Within this study marked asymmetry was noted within the venous system along with large variations in the prominence of each system. In this chapter I describe these findings and their importance for future research and clinical practice.

Advances in imaging technology have greatly improved current understanding of the cerebral vascular system. MR phase contrast imaging allows accurate quantification of blood flow in even extremely narrow vessels, and as there is no radiation exposure, imaging can be undertaken at multiple levels and therefore within multiple vessels. Prior to the development of this technology, studies had to rely on either Doppler ultrasound, methods using indocyanine green or thermodilution techniques, in all of which it is almost impossible to measure arterial and venous flow simultaneously or to measure more than one vessel at a time (Griffiths et al., 2001). Also as MR phase contrast imaging measures flow throughout a cardiac cycle it generates a mean measurement rather than instantaneous values provided by other techniques. Improved understanding of the cerebral vasculature is not only interesting from an anatomical and physiological point of view but also has clinical implications for: choice of placement for catheterisation of the internal jugular vein; studying the effect changes in anatomy can have on jugular venous oxygen saturation ( $S_jO_2$ ); and planning of neurosurgical procedures (Beards et al., 1998, Samy Modeliar et al., 2008).

Whilst studies using MR phase contrast imaging of the cerebrovascular system have been performed on patients (eg in multiple sclerosis) the flow of the main arterial and venous vessels supplying and draining the brain in a healthy population has been much less researched (ElSankari et al., 2013, Feng et al., 2012). I therefore undertook to measure blood flow in the internal jugular veins and internal carotid arteries in healthy men and to assess their asymmetry and inter-relationship.

## **10.2 Methods**

### **10.2.1 Participants**

Participants were recruited as per chapter 9. The study complies with the Declaration of Helsinki; ethical approval was obtained from the local Research Ethics Committee (Scotland A REC, reference 12/SS/0079) and all participants gave written informed consent.

### **10.2.2 MR protocol**

The MR imaging protocol is described in chapter 9.

### **10.2.3 Image analysis**

The image analysis software Medis Q flow (Medis medical imaging systems; Leiden, the Netherlands) was used to calculate jugular venous blood flow on the side which was cannulated. The outline of the internal jugular vein was traced on each of the magnitude images from the cardiac cycle and blood flow calculated using data from the corresponding phase images. For completeness the blood flow for the opposite internal jugular vein and both common carotid arteries were also measured.

### **10.2.4 Statistical analysis**

All statistical analysis was performed on SPSS version 21.0. Non-parametric tests were used to make comparison across groups as the assumptions for parametric tests were violated for some of the variables.

### 10.3 Results

Eight healthy men were recruited to the study with a mean age of 38.1 years (sd 16.5) and a mean BMI of 24.9kg/m<sup>2</sup> (sd 3.7). Cross-sectional area and velocity for all four vessels are shown in tables 10.1 & 10.2.

*Table 10-1: Cross sectional area for all subjects for ICA and IJV with median and interquartile range (mm<sup>2</sup>)*

| <b>Subject</b>                      | <b>Right ICA<br/>CSA</b> | <b>Left ICA<br/>CSA</b> | <b>Total ICA<br/>CSA</b> | <b>Right IJV<br/>CSA</b> | <b>Left IJV<br/>CSA</b> | <b>Total IJV<br/>CSA</b> |
|-------------------------------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| <b>1</b>                            | 32.0                     | 34.7                    | 66.7                     | 97.9                     | 71.5                    | 169.4                    |
| <b>2</b>                            | 39.7                     | 38.4                    | 78.1                     | 72.9                     | 11.1                    | 83.9                     |
| <b>3</b>                            | 56.3                     | 39.1                    | 95.4                     | 60.3                     | 18.2                    | 78.5                     |
| <b>4</b>                            | 32.1                     | 41.0                    | 73.0                     | 66.2                     | 38.2                    | 104.4                    |
| <b>5</b>                            | 35.6                     | 34.2                    | 69.8                     | 70.2                     | 17.1                    | 87.4                     |
| <b>6</b>                            | 33.1                     | 39.7                    | 72.8                     | 42.2                     | 150.0                   | 192.2                    |
| <b>7</b>                            | 31.4                     | 40.1                    | 71.5                     | 88.8                     | 9.0                     | 97.7                     |
| <b>8</b>                            | 25.7                     | 37.7                    | 63.5                     | 104.2                    | 6.1                     | 110.3                    |
| <b>Median</b>                       | 32.6                     | 38.7                    | 72.2                     | 71.5                     | 17.7                    | 101.1                    |
| <b>IQ range</b>                     | 31.5-38.7                | 35.4-40.0               | 67.5-76.8                | 61.7-95.6                | 9.5-63.2                | 84.8-154.6               |
| <b>Coefficient<br/>of variation</b> | 0.26                     | 0.06                    | 0.13                     | 0.27                     | 1.23                    | 0.36                     |

Right and left IJV CSA were significantly different (p=0.038, Mann-Whitney U Test), but right and left ICA CSA were not significantly different (p>0.05, Mann-Whitney U Test).

*Table 10-2: Mean velocity for all subjects for ICA and IJV with median and interquartile range (cm/second)*

| <b>Subject</b>                  | <b>Right ICA velocity</b> | <b>Left ICA velocity</b> | <b>Mean ICA velocity</b> | <b>Right IJV velocity</b> | <b>Left IJV velocity</b> | <b>Mean IJV velocity</b> |
|---------------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| <b>1</b>                        | 17.4                      | 15.4                     | 16.4                     | 6.7                       | 4.8                      | 5.8                      |
| <b>2</b>                        | 13.1                      | 12.4                     | 12.7                     | 6.4                       | 7.3                      | 6.8                      |
| <b>3</b>                        | 10.6                      | 10.5                     | 10.5                     | 19.8                      | 0.3                      | 10.1                     |
| <b>4</b>                        | 14.2                      | 17.2                     | 15.7                     | 18.7                      | 9.3                      | 14.0                     |
| <b>5</b>                        | 16.4                      | 17.1                     | 16.8                     | 7.1                       | 7.5                      | 7.3                      |
| <b>6</b>                        | 18.8                      | 14.5                     | 16.7                     | 2.1                       | 8.2                      | 5.1                      |
| <b>7</b>                        | 16.3                      | 13.4                     | 14.8                     | 2.6                       | 2.1                      | 2.3                      |
| <b>8</b>                        | 12.9                      | 12.4                     | 12.6                     | 5.3                       | 1.1                      | 3.2                      |
| <b>Median</b>                   | 15.2                      | 13.9                     | 15.2                     | 6.5                       | 6.1                      | 6.3                      |
| <b>IQ range</b>                 | 12.9-17.1                 | 12.4-16.7                | 12.6-16.6                | 3.3-15.8                  | 1.4-8.0                  | 3.7-9.4                  |
| <b>Coefficient of variation</b> | 0.18                      | 0.17                     | 0.16                     | 0.80                      | 0.69                     | 0.55                     |

Neither right and left IJV velocity nor right and left ICA velocity were significantly different ( $p > 0.05$ , Mann-Whitney U Test). The IQ range for the right IJV velocity was wider than for the left IJV velocity.

Spearman's correlation coefficients were calculated for each vessel between the CSA and the velocity; there were no significant correlations between CSA and velocity for any of the vessels.

*Table 10-3: Spearman's correlation coefficients between each vessel's CSA and velocity*

|            | <b>Right ICA</b> | <b>Left ICA</b> | <b>Total ICA</b> | <b>Right IJV</b> | <b>Left IJV</b> | <b>Total IJV</b> |
|------------|------------------|-----------------|------------------|------------------|-----------------|------------------|
| <b>rho</b> | 0.67             | -0.10           | -0.33            | -0.21            | 0.52            | 0.38             |
| <b>sig</b> | >0.05            | >0.05           | >0.05            | >0.05            | >0.05           | >0.05            |

Table 10.4 shows median blood flow for the internal jugular veins and internal carotid arteries. Right and left IJV blood flows were significantly different ( $p=0.028$ , Mann-Whitney U Test), whereas right and left ICA blood flows were not significantly different ( $p>0.05$ , Mann-Whitney U Test).

*Table 10-4: Blood flow for all subjects for ICA and IJV with median and interquartile range (ml/min)*

| <b>Subject</b>                  | <b>Right ICA flow</b> | <b>Left ICA flow</b> | <b>Total ICA flow</b> | <b>Right IJV flow</b> | <b>Left IJV flow</b> | <b>Total IJV flow</b> |
|---------------------------------|-----------------------|----------------------|-----------------------|-----------------------|----------------------|-----------------------|
| <b>1</b>                        | 327                   | 315                  | 642                   | 385                   | 203                  | 587                   |
| <b>2</b>                        | 308                   | 289                  | 596                   | 277                   | 48                   | 324                   |
| <b>3</b>                        | 353                   | 241                  | 593                   | 709                   | 5                    | 714                   |
| <b>4</b>                        | 274                   | 420                  | 694                   | 742                   | 215                  | 957                   |
| <b>5</b>                        | 299                   | 304                  | 603                   | 534                   | 32                   | 565                   |
| <b>6</b>                        | 369                   | 349                  | 718                   | 54                    | 732                  | 785                   |
| <b>7</b>                        | 307                   | 322                  | 629                   | 137                   | 11                   | 148                   |
| <b>8</b>                        | 199                   | 284                  | 483                   | 333                   | 4                    | 337                   |
| <b>Median</b>                   | 307                   | 309                  | 616                   | 359                   | 40                   | 576                   |
| <b>IQ range</b>                 | 286-340               | 287-336              | 594-681               | 207-621               | 8-209                | 331-750               |
| <b>Coefficient of variation</b> | 0.17                  | 0.17                 | 0.12                  | 0.63                  | 1.59                 | 0.49                  |

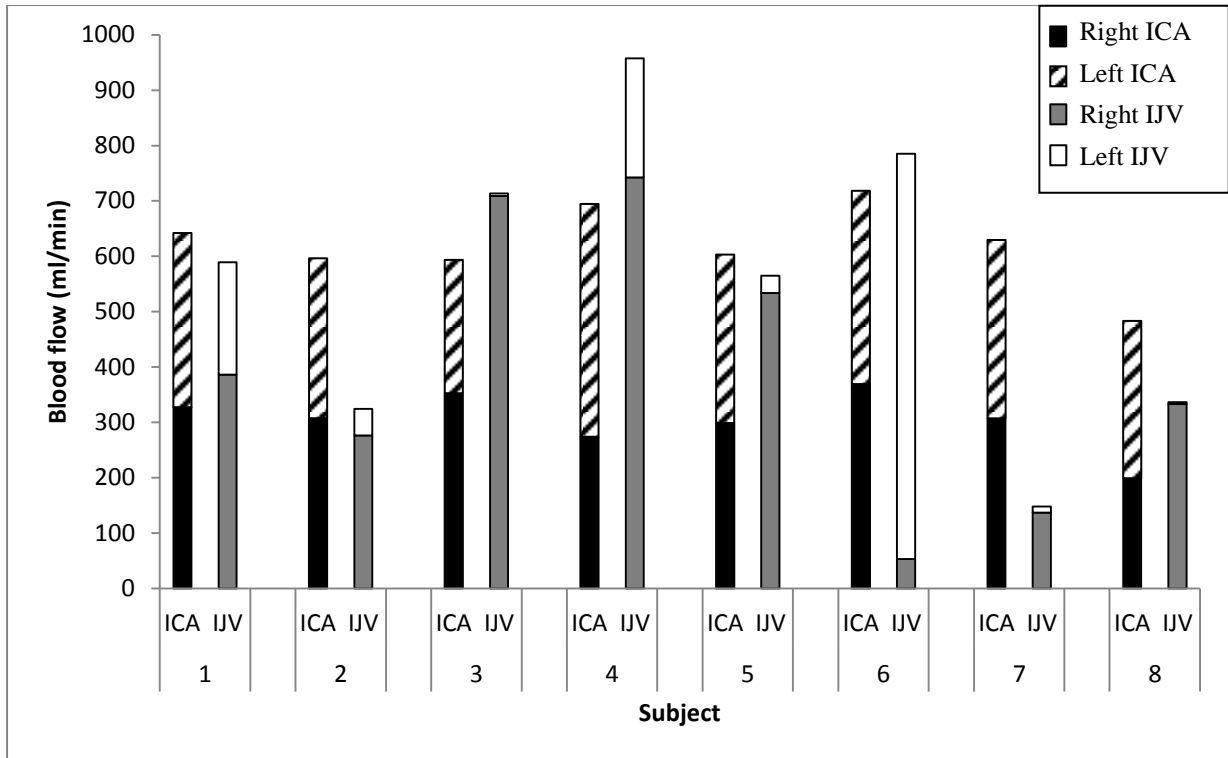
In all but one of our subjects, the right internal jugular vein was dominant. In the only subject who was left IJV dominant, blood flow in the right IJV was only 7.3% of total IJV blood flow. The dominant IJV blood flow had a median value of 93.0% of total IJV blood flow (IQ range 81.4-96.7%, coefficient of variation 0.13). The dominant IJV had a blood flow around double or more the non-dominant IJV in all our subjects (ie ratio of dominant to non-dominant IJV blood flow was  $\geq 0.66$ ).

The ICA blood flow was more symmetric. In four of the subjects the right ICA had a faster blood flow and in the other four the left ICA had a faster blood flow. The dominant ICA had a median blood flow of 51.5% total ICA blood flow (IQ range 51.0-59.3%, coefficient of variation 0.08). Also, the inter-quartile range for total ICA was narrower, showing less inter-individual variation than IJV blood flow.

The ratio of total IJV blood flow to total ICA blood flow also showed considerable variation (see figure 10.1). In three of the subjects the IJVs had a combined larger blood flow than the

ICAs and in the other 5 subjects the ICAs had a larger blood flow than the IJVs. Median total IJV blood flow as a percentage of total ICA blood flow was 92.6% (IQ range 62.1-114.8; minimum 23.5%; maximum 137.9%, coefficient of variation 0.42).

**Figure 10-1: Bar chart of total IJV and total ICA blood flow split into right and left for each subject**





## 10.4 Discussion

Eight healthy men underwent phase contrast ECG-gated MR scans to calculate blood flow in the internal jugular veins and internal carotid arteries. I used this data to look at the asymmetry of the IJVs and ICAs and the ratio of IJV:ICA blood flow. I found a significant difference between right and left sided IJV blood flow, with the right IJV being dominant in 7 of our 8 subjects. The dominant IJV had a blood flow at least double the non-dominant IJV in all our subjects; significant asymmetry in IJV blood flow should therefore be considered normal.

Asymmetry in the internal jugular veins has been inferred from cross sectional areas (CSA) of vessels in previous studies using different imaging modalities. We found median left IJV CSA to be significantly bigger than median right IJV CSA (table 10.1). Using ultrasound, Modeliar et al measured the CSA of the IJVs in 60 subjects with a mean age of 62 years (sd 19) and found that in more than 65% of subjects the right sided IJV was larger (Samy Modeliar et al., 2008). In a similar study of 80 ICU patients with a mean age of 59 years Lichtenstein et al. defined a dominant IJV as more than twice the CSA of the contralateral IJV; they found that 62.5% of subjects had a dominant IJV and of these, 68% were right side dominant and 32% left. They also noted that in 21% of their subjects, the right IJV was  $0.4\text{cm}^2$  or less, making cannulation difficult or impossible (Lichtenstein et al., 2001). In another study MR angiography was used to measure IJV CSA in 64 participants with a wide age range (mean 48.4 years, range 16-76) and found that 64% of subjects had a larger right IJV than left (Kopelman et al., 2013).

However, vessel CSA does not always accurately reflect blood flow and we found this here also. Vessel CSA was not significantly correlated with velocity in any of the four blood vessels we studied (table 10.3), the two parameters used to calculate blood flow. More recent studies have used phase contrast MR imaging (PC-MRI) and two such studies have examined asymmetry in IJV blood flow. Beards et al examined 25 healthy subjects with an age range of 22-34 years, showing a significant difference between right (mean 302ml/min) and left (mean 210ml/min) IJV blood flow ( $p=0.0036$ ) (Beards et al., 1998). They also measured blood flow within the transverse sinus and found that in 4 subjects there was complete atresia of one transverse sinus and in 13 there was significant asymmetry (52%). ElSankari et al used PC-MRI to measure blood flow in the transverse sinus, IJV, ICAs and vertebral arteries in subjects with multiple sclerosis and in 21 healthy controls, who had a median age of 33

(ElSankari et al., 2013). In the healthy controls the dominant vessel was on the right in 12 subjects (ie 60%) and left in 4 subjects (20%), with equal flow in 4 subjects (20%).

Interestingly, they found that the dominant transverse sinus did not correspond with IJV dominance, with transverse sinus dominant side being the right in 9 subjects (ie 47%), left in 4 subjects (21%) and equal in 6 subjects (32%).

Our study confirms this previous work that significant asymmetry of the IJV is common even in a healthy population. This has implications for both clinical and research practice. In the clinical setting, ultrasound visualisation of the IJV prior to cannulation is now recommended, as it decreases failure rate and rates of complication (eg arterial puncture) (8), however our study highlights the value of imaging both IJVs prior to cannulation. Cannulation of the left IJV has been found to be more time consuming and associated with a higher rate of complications than right IJV cannulation, and it is likely that asymmetry in the vessels contributes to this (9). Therefore we recommend ultrasound imaging of both right and left IJVs prior to cannulation to assess asymmetry and aid selection of which vessel to cannulate.

I found no significant difference between right and left ICA blood flow. Also, the interquartile range and coefficients of variation were much smaller for ICA blood flow than IJV (table 10.4), indicating that there is less anatomical variation in the arterial blood supply to the brain than there is in the venous drainage. This is consistent with one previous paper which reported PC-MRI of ICA blood flow and found no significant difference between right and left sided ICA blood flow in any of their healthy subjects (ElSankari et al., 2013). Interestingly, they did find asymmetry in vertebral artery blood flow, with a dominant vertebral artery being on the right in 20%, left in 40% and co-dominant in 40%. So whilst ICA blood flow seems symmetrical, vertebral artery blood flow may not be.

I also found that the IJVs drain a variable amount of the cerebral arterial blood supply, with the IJVs having a blood flow between 24 and 138% of the total ICA blood flow in our study. The arterial blood supply to the brain is largely made up of the right and left internal carotid arteries and the right and left vertebral arteries. It is thought that the vertebral arteries contribute around 27% to global cerebral blood flow and the internal carotid arteries contribute around 73% (Sato et al., 2011). Cerebrovenous drainage is more complex and our data show that non-jugular venous systems must be of differing importance to total cerebral venous drainage between individuals, however detailed studies of these non-jugular venous systems are lacking. Kopelman et al named all non-jugular venous flow seen on a PC-MRI as

vertebral venous plexus if it lay behind a line intersecting the two IJVs in the sagittal plane at the level of the jugular bulb, and pterygopalatine plexus if the vessel lay anterior to this line (Kopelman et al., 2013). These plexi interconnect with the IJV venous system too, so that depending on blood flow and position (ie supine or erect) each system may drain a varying proportion of the total cerebral arterial blood flow. They measured IJV and non-jugular venous vessels CSA and found that there was an inverse correlation between the two sets of CSAs ( $r^2$  -0.25,  $p < 0.0001$ ), meaning that in subjects with larger IJVs the non-jugular venous systems were less important to total cerebrovenous drainage (Kopelman et al., 2013). This finding is consistent with our data where the subjects with the biggest total IJV blood flow also have the smallest ratio of IJV:ICA blood flow and vice versa in the subjects with the largest total IJV blood flow.

I found only one other study which looked at the proportion of ICA blood flow drained by the cerebrovenous system. Stoquart-ElSankari et al measured cerebral blood flow using PC-MRI in 12 healthy elderly subjects (mean age 71.9 years) and 19 young healthy volunteers (mean age 27.5 years) (Stoquart-ElSankari et al., 2007). The IJVs drained 60.7% of the total arterial (ICA and vertebral arterial) blood flow in the elderly group and 60.3%, in the young group; which is remarkably stable across the lifespan. Coefficients of variation in this study, total arterial blood flow 0.19 elderly, 0.14 young and IJV blood flow 0.48 elderly, 0.52 young, are also comparable with ours and confirm a greater variability in IJV blood flow than cerebral arterial blood flow between subjects. These findings in combination with ours have implications for both clinical and research practice. In the clinical setting, when measuring  $SjO_2$  as a marker for brain oxygen delivery, depending on the degree of blood drained by the jugular venous system, results could vary widely (Andrews et al., 1991, Chan et al., 1992, Andrews and Colquhoun, 1994, McKeating et al., 1998, Grune et al., 2014). Also, in rare cases where a double resection of the internal jugular veins is required, different subjects may respond very differently depending on the capacity of their non-jugular venous system. In the research setting, it will be important to consider when sampling from the jugular veins that the side chosen and the degree of non-jugular venous drainage present may affect results.

In our study I did not measure vertebral arterial blood flow or non-jugular venous drainage, so I am not able to give a full picture of the cerebral arterial and venous relationships. As yet, no study has done this, mainly due to difficulty measuring blood flow in the non-jugular venous system. However with improving image analysis tools this may be possible in the

future. Also, due to the constraints of our MR scanner, all measurements were performed on subjects whilst they were supine. It may well be that measurements taken at an angle of 45 or 90 degrees to the horizontal would give different results which may be more physiologically important. With advances in MR scanning, including vertical MR scanners, this may be possible.

## **10.5 Conclusion**

I used phase contrast ECG-gated MR scans to calculate blood flow in the internal jugular veins and internal carotid arteries in eight healthy men. There is significant asymmetry of blood flow within the healthy jugular venous system, and considerable inter-individual variation in the proportion of ICA blood flow drained by the IJVs. This is likely to be due to variations in the contribution of the vertebral venous plexus to cerebrovenous drainage between individuals. This may have important implications for both intensive care and neurosurgery and future research studies which use a measure of cerebrovascular blood flow, like the technique we developed in chapter 9.

# Chapter 11 Summary and Conclusions

This thesis set out to investigate the relationship between sarcopenia and age-related cognitive decline (ARCD) and their potential biological correlates, with particular focus on the role of glucocorticoids. The field of cognitive ageing is well established with many advanced longitudinal studies looking at potential mechanisms and covariates; sarcopenia by contrast is a relatively new field, although there is a rapidly expanding body of literature examining this important area.

I started the thesis by discussing current diagnostic criteria for both conditions, detailing what is known about the structural changes associated with them and performing a literature review of current evidence for possible underlying mechanistic processes. This was to enable me to identify possible common areas which might explain any relationships I identified between brain and muscle ageing. This literature review helped form the hypotheses that then directed further studies within the thesis by identifying gaps in the current literature and possible common mechanistic pathways. Key areas identified were: further research into the relationship between sarcopenia and ARCD given the potential multiple common causative areas identified; the role of glucocorticoids and their intracellular amplifier  $11\beta$ HSD1 in sarcopenia and ARCD; and the contribution and relative importance of inflammation, immunosenescence, genetic factors, environmental and lifestyle factors in the aetiology and progression of sarcopenia and ARCD.

This led on to a systematic review of the literature on the relationship between brain and muscle structure and/or function. The review did not include the relationship between physical (ie muscle) function and cognition (ie brain function) because there are already reviews of this area, whereas much less has been published on the role of structure in the relationship between brain and muscle (Abellan van Kan et al., 2009, Atkinson et al., 2007, Scherder et al., 2007, Drago et al., 2011, Moran et al., 2013).

The review found that the literature supported a positive association between whole brain volume (WBV) and total white matter volume with muscle size, and also evidence that some areas of regional grey matter volume (right temporal pole and bilateral ventromedial prefrontal cortex) may be negatively associated with muscle size (Honea et al., 2009, Burns et al., 2010, Wetmore et al., 2011, Heymsfield et al., 2012, Kilgour et al., 2013, Weise et al., 2013). However, only six papers (from four studies) were found to look at brain and muscle

structure. This is most likely because studies either involved a brain scan or a measure of muscle structure. Clearly one scan which measured both would be extremely useful for this emerging field. Longitudinal studies would allow this relationship to be further investigated, to determine whether those who have lost more brain tissue have a greater reduction in muscle mass, or whether brain atrophy leads to decreased muscle mass, or if sarcopenia leads on to greater brain atrophy.

There was no association between grip strength and WBV, but there was an association with brain atrophy. None of the papers contained a longitudinal measure of muscle mass, so whilst brain atrophy can estimate degree of loss of brain tissue from peak size, there is no corresponding measure for muscle. Greater white matter (WM) volume and lower white matter hyperintensity (WMH) volume were also both associated with grip strength (Doi et al., 2012, Aribisala et al., 2013, Sachdev et al., 2005).

Gait speed was found to be positively associated with whole brain volume; this relationship may be driven by total WM volume or regional grey matter (GM) volumes, specifically the hippocampus (Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013). Like grip strength, gait speed is also associated with markers of brain ageing; WMH accumulation, brain atrophy and WM atrophy all show evidence of either a temporal association with gait speed or change in gait speed with time, with periventricular hyperintensities (PVH) and brainstem WMHs playing a particularly important role, but not subcortical WMH (Rosano et al., 2005, Soumare et al., 2009, Callisaya et al., 2013). However, only one of these studies looked at brain structure change over time and further longitudinal studies like this will prove very informative but are expensive to conduct (Callisaya et al., 2013).

There was no evidence found for an association between cognition and muscle size, except in studies where MMSE was used as the cognitive test, with evidence both for and against a significant association (Kilgour et al., 2013, Wetmore et al., 2011, Pedersen et al., 2012, Berryman et al., 2013, Bites et al., 2013, Auyeung et al., 2013, Magri et al., 2006, Liu et al., 2014). Whilst the MMSE is used as a screening tool for dementia it has both a ceiling and a floor effect and is not considered a useful test of cognitive function other than for its primary function (Franco-Marina et al., 2010).

The systematic review identified a relative paucity of evidence for a relationship between muscle and brain structure, particularly in longitudinal studies, and the possibility of a

technique to measure both variables on one scan was recognised as being extremely useful within the field. I next went on to describe the process of developing such a technique.

The technique I developed uses volumetric MR brain scans, which are a good method for measuring brain volume and other markers of brain ageing (eg WMH accumulation), to measure neck muscle cross-sectional area (CSA) (Kilgour et al., 2012). I demonstrated through a series of studies that the technique is feasible and repeatable with good inter-rater reliability. I also demonstrated that neck muscle CSA can be used as a proxy for general muscle bulk by showing it has a strong correlation with thigh muscle CSA. I have organised for wave 3 from the LBC 1936 study to undergo neck muscle CSA measurement on its MR brain scans and in this wave I have also arranged for the subjects to undergo estimation of lean mass using a BIA machine. I plan to use this data to further test the validity of neck muscle CSA as a useful indicator of general muscle bulk, in addition to using it for longitudinal analysis of the measured variables.

It is interesting to note that not all muscles appear to age at the same rate. For example more muscle appears to be lost from the lower than the upper limbs (Janssen et al., 2000) and even within a limb, deep muscles may be less affected than superficial muscles (Ikezoe et al., 2011). It would be informative to look at the longitudinal decrease in size of the neck muscles, which are used even in seated postural control, in relation to peripheral muscles (eg quadriceps or the calf muscles). Wave 4 of the LBC 1936 study is shortly to be commenced and the subjects are undergoing volumetric MR brain scanning and BIA measurement again and using the wave 3 plus this data I will be able to look at the longitudinal relationship between neck muscle CSA and total lean mass, and estimate whether or not they are declining in a proportionate manner.

After developing the neck muscle CSA measurement technique it was first utilised on a cohort of healthy older men (Kilgour et al., 2013). Data on brain volume and cognitive function had already been collected as part of the original study and I measured the neck muscle CSA on the volumetric MR brain scans. These data showed that in healthy older men preservation of whole brain volume (ie the ratio of WBV corrected for intracranial area) is associated with larger muscle size. Interestingly I also found that larger muscle size was associated with lower prior cognition but not current cognition. I postulated that this may be a reflection of an association between occupation and cognitive ability; that is that subjects with a lower cognitive ability may be more likely to have had a manual occupation and

therefore would have built up a larger peak muscle mass. Unfortunately this study did not have data on occupation or social class, so I was not able to test that theory here, but on looking at the wave 2 LBC 1936 data I was able to show a positive correlation between social class and neck muscle size (ie those from social class I had a smaller neck muscle CSA than those from social class V).

Next I performed neck muscle CSA measurement on the wave 2 volumetric MR brain scans from LBC 1936 (n=641), a large population based cohort study with data on cognition age 11 years old, to look at the interrelationships between brain and muscle structure and function. In this larger study I also had measures of muscle function: grip strength and six metre walk time.

I found that neck muscle CSA was not associated with cognition, GM volume or WML volume, but that it was negatively associated with normal WM volume and PVH Fazekas score in men. I had postulated that larger brain volumes would be associated with larger neck muscle CSA (after controlling for ICV as a proxy for skull size) under the common cause hypothesis. So finding that smaller WM volume was associated with larger neck muscle CSA contradicted this. One possible explanation could be that those with a smaller WM volume find it more difficult to maintain their postural control and therefore are required to make constant small postural adjustments thus increasing their neck muscle CSA. However it is worth noting that the effect size was small. It will be very interesting to look at the wave 3 LBC 1936 data and see if there is the same or opposite association with total lean mass (which will include central and peripheral muscle bulk) and WM volume.

There is a well-established association between gait speed and WMHs, but in my systematic review I found no previous studies looking at the association between WMHs and muscle size (Soumare et al., 2009, Moscufo et al., 2012, Wolfson et al., 2005, Rosano et al., 2006, Rosano et al., 2005, Silbert et al., 2008, Marquis et al., 2002, Aribisala et al., 2013, Rosano et al., 2010, Longstreth et al., 1996). Therefore there was no evidence addressing the question of whether muscle size mediates the relationship between WMHs and gait speed. I found a relationship between PVH Fazekas score and neck muscle CSA in men and not in women, and I found no association between deep WMH Fazekas score and neck muscle CSA in either sex. Location of WMH may be important in determining their impact on muscle function (Kilgour et al., 2014), therefore it makes sense that location may also affect muscle size. The LBC 1936 cohort is particularly healthy for their age and of a higher social class and better



cognition than the average population of that age, partly predetermined by their willingness to undergo multiple tests and have ongoing input with a longitudinal study. Therefore there were relatively low numbers of subjects for men and women with Fazekas scores of 2 or 3. It may be that in an older or less healthy cohort a stronger relationship would be found between WMH and muscle size. It will again be interesting to look at the lean muscle mass results in wave 3 and 4 of LBC 1936 and its association with WMH volume and location.

Grip strength was positively associated with cognitive ability (general cognition and processing speed, but not memory) and WM volume and was negatively associated with PVH Fazekas score in women, but was not associated with GM or WML volume. In this case PVH Fazekas score again proved more important than deep WMH score, but in women this time and not in men. The same caveat of a low number of subjects scoring 2 or 3 hold true for this data also. However the only previous study I found which looked at WMH location and grip strength also found that regional burden may play an important role (Sachdev et al., 2005). Previous studies have also found an association between grip strength and some aspects of cognition (Guerrero-Berroa et al., 2014), but the only study I found looking at the relationship between grip strength and brain volume also used data from wave 2 of LBC 1936 (Aribisala et al., 2013).

The six metre walk test (6MWT) was associated with the same cognitive ability measures as grip strength (general cognition and processing speed but not memory), and also WM, GM and WML volume. 6MWT was also positively associated with deep Fazekas score in men. Whilst gait speed has previously been shown to be associated with cognition (eg MMSE, DSST and cognitive impairment) (Abellan van Kan et al., 2009, Atkinson et al., 2007, Duff et al., 2008), studies looking at its association with other specific cognitive domains are currently lacking. Our results show that WMH location may prove important in the relationship with 6MWT, but further studies are now needed from other datasets to assess this relationship further.

With measures from all four variables in this dataset (brain structure and function, and muscle structure and function) I was able to look at the inter-relationships between them and test for possible explanatory factors. I found that neck muscle CSA is a mediator between 6MWT and WM volume. This means that neck muscle CSA partially explains the relationship between 6MWT and WM volume, being both predicted by WM volume and a

predictor of 6MWT independent of WM volume. Neck muscle CSA is not a mediator between 6MWT and WML volume, or grip strength and WM volume.

WM, GM and WML volume all act as mediators between physical function and cognition, so brain structure clearly plays a key part in this relationship. I found only one significant explanatory factor in all the relationships that were looked at in this study, which was salivary cortisol. Evening salivary cortisol was found to be an explanatory factor between 6MWT and cognition, with a particular role in the relationship between 6MWT and processing speed. Also, evening salivary cortisol and the diurnal slope between morning and evening salivary cortisol were found to be explanatory variables in the relationship between 6MWT and total brain volume. This would indicate that cortisol may be one of the factors underlying the common cause hypothesis; that ageing progresses in all organs at a similar rate due to common underlying mechanisms. Salivary cortisol was only assessed on 89 of the subjects in the LBC 1936 study and they were all male, so further confirmatory studies are now required.

In the first chapter of the thesis I described the theory of the common cause hypothesis. This theory postulates that the same intrinsic factors driving ageing in one organ will similarly be acting in all the other organs, meaning that in those with a faster rate of intrinsic ageing (secondary to free radical generation, mitochondrial ageing etc) the trajectory of decline facing each organ will be similarly steep, only being altered by extrinsic factors having differential effects on the separate organs (eg smoking and lung function). Conversely in those subjects with a slower intrinsic rate of ageing the trajectory of decline with ageing affecting each organ should be similarly slow. If this theory was proven to be correct then preventative advice or novel treatments found to slow down the intrinsic rate of ageing in one organ should similarly improve ageing within other organs also, which has exciting ramifications for treating age-related conditions. In this thesis I studied evidence which would support or refute this interesting concept both within the systematic review and within the studies I performed on the longitudinal cohorts I had access to. The systematic review identified mainly studies using cross-sectional analyses but some of the longitudinal data it looked at, and similarly markers for brain ageing within the cross-sectional studies (eg WMH accumulation and brain atrophy), were found to have associations with muscle function. Due to a technical issue with regard to the MHEM study I was unable to use wave 1 of its data, as we were unable to measure the neck muscle CSA on these scans and data completion of wave 3 from the LBC1936 was not completed within the timescale of my PhD for all covariates I

needed, therefore in neither of the studies I had access to was I able to analyse the relationships between muscle and brain longitudinally. However, again there was evidence of a relationship between markers of brain ageing and muscle variables, but the findings were not fully consistent across the datasets and in fact one finding was the converse to what we had predicted with the common cause hypothesis (ie we found that smaller WM volume was associated with larger neck muscle CSA). Ultimately without the benefit of having longitudinal data with which to study the ageing trajectories of muscle and brain it is difficult to draw any firm conclusions on the validity of the common cause hypothesis at present, although using the limited data I had access to (ie the few longitudinal studies in the systematic review and markers of brain ageing in the cohort studies) it appears that even if there is a common rate of ageing shared between muscle and brain, there is a larger component of individual ageing within each organ, caused by either the relative importance or presence of particular intrinsic or extrinsic ageing factors.

I next went on to look at some of these possible factors and the role they may play in muscle and brain ageing. I looked at the role of immunosenescence and inflammation in sarcopenia within the LBC 1936 study and found that men who were seropositive for CMV had smaller neck muscle CSA than men who were seronegative and this effect was independent of IL-6 level, however there was no significant relationship between CMV titre and neck muscle CSA. This may be because the effect of chronic CMV infection (which usually initially occurs in childhood) may affect muscle size over a long period, so that increasing titres in older age play less of a role. I also found that higher IL-6 levels, but not CMV levels, were strongly associated with lower grip strength in both hands in men and women; these associations were not attenuated when the model was adjusted for CMV serostatus or antibody titre. All the findings were independent of markers of childhood deprivation, which is known to be associated with CMV infection. These findings support the hypothesis that there is a relationship between immunosenescence and markers of sarcopenia.

I then analysed data from a cohort study of elderly and young subjects from two Scottish cities to investigate further the relationship between glucocorticoid status and sarcopenia. I found a significant association between increased muscle 11 $\beta$ HSD1 expression (which converts inactive cortisone to active cortisol) and lower quadriceps strength in older men and women (Kilgour et al., 2012). However I found no significant associations between plasma cortisol, urinary GC metabolites or GR expression and muscle mass or strength. Therefore it

seems that whilst circulating levels of glucocorticoids may not play an important role in the aetiology of sarcopenia in this dataset, tissue specific levels do play a role. This study raises the possibility of 11 $\beta$ HSD1 inhibitors as a novel treatment for sarcopenia, but further larger confirmatory studies of the association are now required.

I then performed a clinical study on healthy men to determine if it is possible to detect 11 $\beta$ HSD1 activity in the human brain in vivo. This technique would be very useful in further development of 11 $\beta$ HSD1 inhibitors as a potential treatment in cognitive impairment. Unfortunately I found no measurable release or production of cortisol, d3-cortisol or cortisone across the human brain, using arterial and venous blood samples and deuterated glucocorticoid tracers. If I had included subjects with cognitive impairment or risk factors for cognitive impairment (eg type 2 DM or older age) I may have found a significant result due to increased 11 $\beta$ HSD1 activity in these subjects, however in view of the invasive nature of this technique alternative approaches will be required to quantify the effects of selective 11 $\beta$ HSD1 inhibitors on enzyme activity in human brain. Furthermore it is known that 11 $\beta$ HSD1 activity is limited to particular subregions (Sandeep et al., 2004), for example the hippocampus, therefore any technique which allowed measurement of the enzyme activity within specific areas of the brain would be an extremely useful development in the future.

As part of the above study I decided to sample venous blood from just below the jugular venous bulb on the right side of each subject. However, as the study progressed it became increasingly apparent how asymmetric jugular venous drainage is, unlike carotid arterial supply which was not statistically different between the right and left side. Also after measuring blood flow in both carotid arteries and both internal jugular veins on the ECG-gated phase magnitude MR brain scan, I noticed that the percentage of blood supplied by the carotid arteries which was drained by the internal jugular veins was again remarkably variable. This means that in the different individuals in our study (some of whom showed production of cortisol across the brain), more or less of the brain tissue known to express 11 $\beta$ HSD1 activity may have drained to the IJV we were sampling from. This has implications for future studies which use blood sampled from the jugular venous bulb as a proxy for cerebral venous blood and also has clinical implications for neurosurgery and intensive care monitoring.

## 11.1 Chapter order within the thesis

In this thesis the chapter order largely represents the work in the chronological manner it was performed, however some of the work in the chapters was completed non-chronologically but it was felt that the flow of results from general to specific still suited the chosen chapter order. For example, chapter 1 was performed at the outset of the PhD, whilst chapter 2 was commenced at the outset of the PhD but not finally completed until halfway through the third year of my PhD. This was partly because: the number of studies which were screened as part of the review was very large ( $n > 22,000$ ); the researcher who was helping me screen the titles was working full time in a clinical role, therefore there was some delay in completing longlisting and shortlisting; and also because the journal where we published the review asked for an update on the review prior to publication as there had been a significant time lapse between the original search and submission to the journal. This meant that the systematic review subsequently included a publication which formed the basis for chapter 5 (Kilgour et al., 2013), despite the results from the original version of the review being available to me earlier than the publication of this chapter, and these early results had informed the chosen analyses particularly with regard to the LBC1936 data. Chapters 4, 5, 6 and 7 are presented in the chronological order they were performed. Chapters 8 and 9 were performed concurrently with the preceding chapters and were grouped at the end of the thesis as they both looked at the role of glucocorticoids. Chapter 10 is based on the analysis of data gained from chapter 9 and therefore appears after it.

## 11.2 Limitations

I described limitations in each of the studies within the specific chapters; however certain themes are apparent across the chapters. Current evidence for whether an association exists between brain structure and muscle structure is limited, and is largely reliant on cross-sectional studies. Therefore conclusions drawn within the systematic review are sometimes based on only a small selection of studies. Longitudinal studies would prove very useful as the trajectory of how the ageing process is affecting the two organs (brain and muscle) could then be examined. These data could be used to assess whether ARCD predicts sarcopenia or vice versa, or whether the two decline in parallel.

The technique I developed to measure neck muscle CSA on volumetric brain scans will be useful for such studies but it has a number of limitations. Firstly it can only be used on volumetric scans, and this precludes its use in most scans collected in clinical practice. Also, I

found it could not be used on volumetric CT scans either after accessing a stroke study with such scans. This is because as radiation is used in these scans the field of view is kept as small as possible and therefore extension into the neck is inadequate. Furthermore the technique is relatively time consuming and requires a degree of operator knowledge; however I am currently investigating the possibility of making the technique semi-automated using computer based learning algorithms.

Within all the cohort studies I used, subjects were screened for certain conditions (eg dementia) and there might also be a degree of subject self-selection (ie it is likely that subjects with certain conditions would be less likely to take part in cohort studies). This means that whilst the studies are less affected by confounding comorbidities, they may not be truly reflective of the general population. In fact by the time subjects are in their eight decade it would be assumed that certain levels of obesity, dementia and mobility problems would be present, all of which may be under-represented in these studies. This is reflected in certain aspects of the LBC 1936 study where the number of subjects with Fazekas scores of 2 or 3 was lower than might be expected for this age group.

### **11.3 Summary**

The interrelationship between muscle and brain structure and function was investigated using a systematic review and analysis of data from cohort studies in healthy elderly people (MHEM and LBC 1936). I consistently found a relationship between: some measures of brain structure and muscle size; markers of brain structure and muscle function, mostly grip strength and gait speed; and cognition and muscle function. However I found no relationship between current cognition and muscle size in any of the above studies. These data therefore partly support the common cause hypothesis.

Cortisol was identified as a possible explanatory factor in the relationship between gait speed and cognition, and also gait speed and brain volume. I found an association between markers of immunosenescence and markers of sarcopenia (neck muscle CSA and grip strength) and also an association between expression of the cortisol amplifying enzyme  $11\beta$ HSD1 and quadriceps strength. Therefore both glucocorticoid dysfunction and immunosenescence may be possible mechanisms underlying the common cause hypothesis, but other pathways must also be involved as the effect sizes that were found are modest.

I developed a technique to measure  $11\beta$ HSD1 activity in the whole human brain; however the amount of cortisol produced within the brain was not detectable, indicating that specific regional testing may be required. This study also generated novel data on asymmetries within the IJV system and variability in the cerebrovascular venous system with implications for research and clinical practice.

Further longitudinal studies investigating the association between sarcopenia and ARCD are now required to improve understanding of these important relationships further. I plan to use the data collected from LBC 1936 wave 3 and the data to be collected from wave 4 to explore these relationships in longitudinal analyses. Hopefully this will generate research hypotheses for future studies investigating potential underlying mechanisms and ultimately improve treatment options for these two important age related conditions. As the population of the world ages, increasing attention is paid to improving healthy life expectancy, which includes delaying two of the most feared consequences of growing older: poor mobility and falls, and cognitive decline. I hope to contribute to this existing body of research in the hope that significant progress in these areas will translate to tenable quality of life improvements for older adults.

## References

- ABBASI, A., DUTHIE, E. H., SHELDAHL, L., WILSON, C., SASSE, E., RUDMAN, I. & MATTSON, D. E. 1998. Association of dehydroepiandrosterone sulfate, body composition, and physical fitness in independent community-dwelling older men and women. *Journal of the American Geriatrics Society*, 46, 263-273.
- ABELLAN VAN KAN, G., CESARI, M., GILLETTE-GUYONNET, S., DUPUY, C., NOURHASHEMI, F., SCHOTT, A. M., BEAUCHET, O., ANNWEILER, C., VELLAS, B. & ROLLAND, Y. 2013. Sarcopenia and cognitive impairment in elderly women: results from the EPIDOS cohort. *Age & Ageing*, 42, 196-202.
- ABELLAN VAN KAN, G., ROLLAND, Y., ANDRIEU, S., BAUER, J., BEAUCHET, O., BONNEFOY, M., CESARI, M., DONINI, L. M., GILLETTE GUYONNET, S., INZITARI, M., NOURHASHEMI, F., ONDER, G., RITZ, P., SALVA, A., VISSER, M. & VELLAS, B. 2009. Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force. *J Nutr Health Aging*, 13, 881-9.
- ADAMS, G. R. & HADDAD, F. 1996. The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *Journal of Applied Physiology*, 81, 2509-2516.
- ADAMS, G. R. & MCCUE, S. A. 1998. Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats. *Journal of Applied Physiology*, 84, 1716-1722.
- AIELLO, A. E., HAAN, M., BLYTHE, L., MOORE, K., GONZALEZ, J. M. & JAGUST, W. 2006. The influence of latent viral infection on rate of cognitive decline over 4 years. *J Am Geriatr Soc*, 54, 1046-54.
- AIELLO, A. E., HAAN, M. N., PIERCE, C. M., SIMANEK, A. M. & LIANG, J. 2008. Persistent infection, inflammation, and functional impairment in older Latinos. *J Gerontol A Biol Sci Med Sci*, 63, 610-8.
- AIHIE-SAYER, A., SYDDALL, H., GILBODY, H., DENNISON, E. & COOPER, C. 2004. Does sarcopenia originate in early life? Findings from the Hertfordshire cohort study - ePrints Soton. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2004; 59(9): M930-M934.
- AKAMIZU, T. & KANGAWA, K. 2007. Emerging results of anticatabolic therapy with ghrelin. *Current Opinion in Clinical Nutrition and Metabolic Care*, 10, 278-283.
- AL SNIH, S., MARKIDES, K. S., OTTENBACHER, K. J. & RAJI, M. A. 2004. Hand grip strength and incident ADL disability in elderly Mexican Americans over a seven-year period. *Aging Clin Exp Res*, 16, 481-6.
- ALEXANDRE TDA, S., DUARTE, Y. A., SANTOS, J. L., WONG, R. & LEBRAO, M. L. 2014. Prevalence and associated factors of sarcopenia among elderly in Brazil: findings from the SABE study. *J Nutr Health Aging*, 18, 284-90.
- ALFARO-ACHA, A., AL SNIH, S., RAJI, M. A., KUO, Y.-F., MARKIDES, K. S. & OTTENBACHER, K. J. 2006. Handgrip strength and cognitive decline in older Mexican American's. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 61, 859-865.
- ALI, M. S., HARMER, M. & LATTO, I. P. 2001. Jugular bulb oximetry during cardiac surgery. *Anaesthesia*, 56, 24-37.
- ANDERSEN, J. L. 2003. Muscle fibre type adaptation in the elderly human muscle. *Scandinavian Journal of Medicine & Science in Sports*, 13, 40-47.



- ANDREW, R., SMITH, K., JONES, G. C. & WALKER, B. R. 2002. Distinguishing the activities of 11beta-hydroxysteroid dehydrogenases in vivo using isotopically labeled cortisol. *J Clin Endocrinol Metab*, 87, 277-85.
- ANDREW, R., WESTERBACKA, J., WAHREN, J., YKI-JARVINEN, H. & WALKER, B. R. 2005. The contribution of visceral adipose tissue to splanchnic cortisol production in healthy humans. *Diabetes*, 54, 1364-70.
- ANDREWS, P. J. & COLQUHOUN, A. D. 1994. Detection of cerebral hypoperfusion during cardiopulmonary bypass. Continuous measurement of cerebral venous oxyhaemoglobin saturation during myocardial revascularisation. *Anaesthesia*, 49, 949-53.
- ANDREWS, P. J., DEARDEN, N. M. & MILLER, J. D. 1991. Jugular bulb cannulation: description of a cannulation technique and validation of a new continuous monitor. *Br J Anaesth*, 67, 553-8.
- ANGEVAREN, M., AUFDEMKAMPE, G., VERHAAR, H. J., ALEMAN, A. & VANHEES, L. 2008. Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev*, CD005381.
- ANIANSSON, A., GRIMBY, G. & HEDBERG, M. 1992. Compensatory muscle-fiber hypertrophy in elderly men. *Journal of Applied Physiology*, 73, 812-816.
- ANIANSSON, A., HEDBERG, M., HENNING, G. B. & GRIMBY, G. 1986. Muscle morphology, enzymatic-activity, and muscle strength in elderly men - a follow-up-study. *Muscle & Nerve*, 9, 585-591.
- ANIANSSON, A., SPERLING, L., RUNDGREN, A. & LEHNBERG, E. 1983. Muscle function in 75-year-old men and women - a longitudinal-study. *Scandinavian Journal of Rehabilitation Medicine*, 92-102.
- ANKER, S. D., PONIKOWSKI, P., VARNEY, S., CHUA, T. P., CLARK, A. L., WEBBPEPLOE, K. M., HARRINGTON, D., KOX, W. J., POOLEWILSON, P. A. & COATS, A. J. S. 1997. Wasting as independent risk factor for mortality in chronic heart failure. *Lancet*, 349, 1050-1053.
- ANSTEY, K. J., LORD, S. R. & WILLIAMS, P. 1997. Strength in the lower limbs, visual contrast sensitivity, and simple reaction time predict cognition in older women. *Psychology and Aging*, 12, 137-144.
- ANSTEY, K. J., MACK, H. A., CHRISTENSEN, H., LI, S.-C., REGLADE-MESLIN, C., MALLER, J., KUMAR, R., DEAR, K., EASTEAL, S. & SACHDEV, P. 2007a. Corpus callosum size, reaction time speed and variability in mild cognitive disorders and in a normative sample. *Neuropsychologia*, 45, 1911-1920.
- ANSTEY, K. J. & SMITH, G. A. 1999. Interrelationships among biological markers of aging, health, activity, acculturation, and cognitive performance in late adulthood. *Psychology and Aging*, 14, 605-618.
- ANSTEY, K. J., VON SANDEN, C., SALIM, A. & O'KEARNEY, R. 2007b. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. *American Journal of Epidemiology*, 166, 367-378.
- ARIBISALA, B. S., GOW, A. J., BASTIN, M. E., VALDES HERNANDEZ, M. D. C., MURRAY, C., ROYLE, N. A., MANIEGA, S. M., STARR, J. M., DEARY, I. J. & WARDLAW, J. M. 2013. Associations between level and change in physical function and brain volumes. *PLoS ONE*, 8.
- ASHTON, W. S., DEGNAN, B. M., DANIEL, A. & FRANCIS, G. L. 1995. Testosterone increases insulin-like growth-factor-i and insulin-like growth factor-binding protein. *Annals of Clinical and Laboratory Science*, 25, 381-388.

- ATKINS, J. L., WHINCUP, P. H., MORRIS, R. W. & WANNAMETHEE, S. G. 2014. Low muscle mass in older men: the role of lifestyle, diet and cardiovascular risk factors. *J Nutr Health Aging*, 18, 26-33.
- ATKINSON, H. H., ROSANO, C., SIMONSICK, E. M., WILLIAMSON, J. D., DAVIS, C., AMBROSIUS, W. T., RAPP, S. R., CESARI, M., NEWMAN, A. B., HARRIS, T. B., RUBIN, S. M., YAFFE, K., SATTERFIELD, S. & KRITCHEVSKY, S. B. 2007. Cognitive function, gait speed decline, and comorbidities: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci*, 62, 844-50.
- AUYEUNG, T., LEE, J., KWOK, T. & WOO, J. 2011. Physical frailty predicts future cognitive decline - A four-year prospective study in 2737 cognitively normal older adults. *Journal of Nutrition, Health & Aging*, 15, 690-694.
- AUYEUNG, T. W., KWOK, T., LEE, J., LEUNG, P. C., LEUNG, J. & WOO, J. 2008. Functional decline in cognitive impairment--The relationship between physical and cognitive function. *Neuroepidemiology*, 31, 167-173.
- AUYEUNG, T. W., LEE, J. S. W., LEUNG, J., KWOK, T. & WOO, J. 2013. Adiposity to muscle ratio predicts incident physical limitation in a cohort of 3,153 older adults-an alternative measurement of sarcopenia and sarcopenic obesity. *Age*, 35, 1377-1385.
- BAGGER, Y. Z., TANKO, L. B., ALEXANDERSEN, P., QIN, G. & CHRISTIANSEN, C. 2004. The implications of body fat mass and fat distribution for cognitive function in elderly women. *Obesity Research*, 12, 1519-26.
- BAKER, L. D., BARSNESS, S. M., BORSON, S., MERRIAM, G. R., FRIEDMAN, S. D., CRAFT, S. & VITIELLO, M. V. 2012. Effects of growth hormone-releasing hormone on cognitive function in adults with mild cognitive impairment and healthy older adults: results of a controlled trial. *Archives of Neurology*, 69, 1420-9.
- BALAGOPAL, P., ROOYACKERS, O. E., ADEY, D. B., ADES, P. A. & NAIR, K. S. 1997. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *American Journal of Physiology-Endocrinology and Metabolism*, 273, E790-E800.
- BALTES, P. B. & LINDENBERGER, U. 1997. Emergence of a powerful connection between sensory and cognitive functions across the adult life span: A new window to the study of cognitive aging? *Psychology and Aging*, 12, 12-21.
- BANKS, W. A., MORLEY, J. E., FARR, S. A., PRICE, T. O., ERCAL, N., VIDAURRE, I. & SCHALLY, A. V. 2010. Effects of a growth hormone-releasing hormone antagonist on telomerase activity, oxidative stress, longevity, and aging in mice. *Proc Natl Acad Sci U S A*. United States.
- BANNERMAN, E., REILLY, J. J., MACLENNAN, W. J., KIRK, T. & PENDER, F. 1997. Evaluation of validity of British anthropometric reference data for assessing nutritional state of elderly people in Edinburgh: cross sectional study. *British Medical Journal*, 315, 338-341.
- BARNES, D. E., COVINSKY, K. E., WHITMER, R. A., KULLER, L. H., LOPEZ, O. L. & YAFFE, K. 2009. Predicting risk of dementia in older adults: The late-life dementia risk index. *Neurology*, 73, 173-9.
- BARTON, P. M. & HAYES, K. C. 1996. Neck flexor muscle strength, efficiency, and relaxation times in normal subjects and subjects with unilateral neck pain and headache. *Arch Phys Med Rehabil*, 77, 680-7.
- BARTON-DAVIS, E. R., SHOTURMA, D. I. & SWEENEY, H. L. 1999. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiologica Scandinavica*, 167, 301-305.
- BASU, R., SINGH, R. J., BASU, A., CHITILAPILLY, E. G., JOHNSON, C. M., TOFFOLO, G., COBELLI, C. & RIZZA, R. A. 2004. Splanchnic cortisol production

- occurs in humans: evidence for conversion of cortisone to cortisol via the 11-beta hydroxysteroid dehydrogenase (11beta-hsd) type 1 pathway. *Diabetes*, 53, 2051-9.
- BAUMGARTNER, R. N., KOEHLER, K. M., GALLAGHER, D., ROMERO, L., HEYMSFIELD, S. B., ROSS, R. R., GARRY, P. J. & LINDEMAN, R. D. 1998. Epidemiology of sarcopenia among the elderly in New Mexico. *American Journal of Epidemiology*, 147, 755-763.
- BAUMGARTNER, R. N., WATERS, D. L., GALLAGHER, D., MORLEY, J. E. & GARRY, P. J. 1999. Predictors of skeletal muscle mass in elderly men and women. *Mechanisms of Ageing and Development*, 107, 123-136.
- BEARDS, S. C., YULE, S., KASSNER, A. & JACKSON, A. 1998. Anatomical variation of cerebral venous drainage: the theoretical effect on jugular bulb blood samples. *Anaesthesia*, 53, 627-33.
- BENDLIN, B. B., FITZGERALD, M. E., RIES, M. L., XU, G., KASTMAN, E. K., THIEL, B. W., ROWLEY, H. A., LAZAR, M., ALEXANDER, A. L. & JOHNSON, S. C. 2010. White matter in aging and cognition: a cross-sectional study of microstructure in adults aged eighteen to eighty-three. *Dev Neuropsychol*, 35, 257-77.
- BERGSTRÖM, J. 1975. Percutaneous Needle Biopsy of Skeletal Muscle in Physiological and Clinical Research. *Scandinavian Journal of Clinical & Laboratory Investigation*, 35, 609-616.
- BERRYMAN, N., BHERER, L., NADEAU, S., LAUZIÈRE, S., LEHR, L., BOBEUF, F., KERGOAT, M. J., VU, T. T. M. & BOSQUET, L. 2013. Executive functions, physical fitness and mobility in well-functioning older adults. *Experimental Gerontology*, 48, 1402-1409.
- BEST, R. & WALKER, B. R. 1997. Additional value of measurement of urinary cortisone and unconjugated cortisol metabolites in assessing the activity of 11 beta-hydroxysteroid dehydrogenase in vivo. *Clinical Endocrinology*, 47, 231-236.
- BEYER, I., METS, T. & BAUTMANS, I. 2012. Chronic low-grade inflammation and age-related sarcopenia. *Curr Opin Clin Nutr Metab Care*, 15, 12-22.
- BINNERTS, A., DEURENBERG, P., SWART, G. R., WILSON, J. H. P. & LAMBERTS, S. W. J. 1992. Body-composition in growth-hormone deficient adults. *American Journal of Clinical Nutrition*, 55, 918-923.
- BITES, A. C., BUNOUT, D., BARRERA, G., HIRSCH, S., LEIVA, L. & DE LA MAZA, M. P. 2013. Association between functional measures and mortality in older persons. *International Journal of Gerontology*, 7, 17-21.
- BLAND, J. M. & ALTMAN, D. G. 2009. Analysis of continuous data from small samples. *BMJ*, 338, a3166.
- BLANKENBERG, S., RUPPRECHT, H. J., BICKEL, C., ESPINOLA-KLEIN, C., RIPPIN, G., HAFNER, G., OSSENDORF, M., STEINHAGEN, K., MEYER, J. & GROUP, F. T. A. 2001. Cytomegalovirus Infection With Interleukin-6 Response Predicts Cardiac Mortality in Patients With Coronary Artery Disease.
- BLASCO, M. A. 2005. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet*, 6, 611-22.
- BLUMENTHAL, H. T., SHOCK, N., GREULICH, R., ANDRES, R., ARRENBURG, D., COSTA, P., LAKATTA, E. & TOBIN, J. 1985. Normal human aging - the Baltimore Longitudinal Study of Aging. *Journals of Gerontology*, 40, 767-767.
- BOONEN, E., VERVENNE, H., MEERSSEMAN, P., ANDREW, R., MORTIER, L., DECLERCQ, P. E., VANWIJNGAERDEN, Y. M., SPRIET, I., WOUTERS, P. J., VANDER PERRE, S., LANGOUCHE, L., VANHOREBEEK, I., WALKER, B. R. & VAN DEN BERGHE, G. 2013. Reduced cortisol metabolism during critical illness. *N Engl J Med*, 368, 1477-88.

- BORSHEIM, E., BUI, Q. U., TISSIER, S., KOBAYASHI, H., FERRANDO, A. A. & WOLFE, R. R. 2008. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. *Clin Nutr*, 27, 189-95.
- BOVE, R. M., BRICK, D. J., HEALY, B. C., MANCUSO, S. M., GERWECK, A. V., BREDELLA, M. A., SHERMAN, J. C. & MILLER, K. K. 2013. Metabolic and endocrine correlates of cognitive function in healthy young women. *Obesity*, 21, 1343-1349.
- BOYLE, P. A., BUCHMAN, A. S., WILSON, R. S., LEURGANS, S. E. & BENNETT, D. A. 2009. Association of Muscle Strength With the Risk of Alzheimer Disease and the Rate of Cognitive Decline in Community-Dwelling Older Persons. *Archives of Neurology*, 66, 1339-1344.
- BROUSSARD, S. R., MCCUSKER, R. H., NOVAKOFSKI, J. E., STRLE, K., SHEN, W. H., JOHNSON, R. W., DANTZER, R. & KELLEY, K. W. 2004. IL-1 beta impairs insulin-like growth factor I-induced differentiation and downstream activation signals of the insulin-like growth factor I receptor in myoblasts. *Journal of Immunology*, 172, 7713-7720.
- BROWN, R. W., DIAZ, R., ROBSON, A. C., KOTELEVTSSEV, Y. V., MULLINS, J. J., KAUFMAN, M. H. & SECKL, J. R. 1996. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology*, 137, 794-7.
- BROWN, W. F., STRONG, M. J. & SNOW, R. 1988. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. *Muscle & Nerve*, 11, 423-432.
- BRUUNSGAARD, H., PEDERSEN, M. & PEDERSEN, B. K. 2001. Aging and proinflammatory cytokines. *Curr Opin Hematol*, 8, 131-6.
- BUNOUT, D., BARRERA, G., AVENDANO, M., GATTAS, V., LEIVA, L. & HIRSCH, S. 2005. Results of a community-based weight-bearing resistance training programme for healthy Chilean elderly subjects. *Age & Ageing*, 34, 80-83.
- BURNS, J. M., JOHNSON, D. K., WATTS, A., SWERDLOW, R. H. & BROOKS, W. M. 2010. Reduced lean mass in early Alzheimer disease and its association with brain atrophy. *Archives of Neurology*, 67, 428-33.
- CAGNIE, B., D'HOOGHE, R., ACHTEN, E., CAMBIER, D. & DANNEELS, L. 2010. A Magnetic resonance imaging investigation into the function of the deep cervical flexors during the performance of craniocervical flexion. *Journal of Manipulative and Physiological Therapeutics*, 33, 286-291.
- CALLISAYA, M. L., BEARE, R., PHAN, T. G., BLIZZARD, L., THRIFT, A. G., CHEN, J. & SRIKANTH, V. K. 2013. Brain structural change and gait decline: A longitudinal population-based study. *Journal of the American Geriatrics Society*, 61, 1074-1079.
- CAMPBELL, M. J., MCCOMAS, A. J. & PETITO, F. 1973. Physiological changes in aging muscles. *Journal of Neurology Neurosurgery and Psychiatry*, 36, 174-182.
- CAMPBELL, W. W. 1995. Effects of resistance training and dietary-protein intake on protein-metabolism in older adults. *American Journal of Physiology-Endocrinology and Metabolism*, 268, E1143-E1153.
- CANON, M. E. & CRIMMINS, E. M. 2011. Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline. *Journal of Nutrition, Health & Aging*, 15, 695-8.
- CARLSON, B. M. & FAULKNER, J. A. 1996. The regeneration of noninnervated muscle grafts and marcaine-treated muscles in young and old rats. *J Gerontol A Biol Sci Med Sci*, 51, B43-9.

- CARLSON, B. M. & FAULKNER, J. A. 1998. Muscle regeneration in young and old rats: Effects of motor nerve transection with and without Marcaine treatment. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 53, B52-B57.
- CARLSON, M. E., SUETTA, C., CONBOY, M. J., AAGAARD, P., MACKAY, A., KJAER, M. & CONBOY, I. 2009. Molecular aging and rejuvenation of human muscle stem cells. *Embo Molecular Medicine*, 1, 381-391.
- CASTELLI, W. P. 1984. Epidemiology of coronary heart disease: the Framingham study. *Am J Med*, 76, 4-12.
- CASTELLI, W. P., ABBOTT, R. D. & MCNAMARA, P. M. 1983. Summary estimates of cholesterol used to predict coronary heart disease. *Circulation*, 67, 730-4.
- CELLERINO, A. 2009. Life Extension in the Short-Lived Fish *Nothobranchius furzeri*. *Aging Medicine*, 5, 157-171.
- CESARI, M., KRITCHEVSKY, S. B., NEWMAN, A. B., SIMONSICK, E. M., HARRIS, T. B., PENNINX, B. W., BRACH, J. S., TYLAVSKY, F. A., SATTERFIELD, S., BAUER, D. C., RUBIN, S. M., VISSER, M. & PAHOR, M. 2009. Added value of physical performance measures in predicting adverse health-related events: results from the Health, Aging And Body Composition Study. *J Am Geriatr Soc*, 57, 251-9.
- CHAN, K. H., MILLER, J. D., DEARDEN, N. M., ANDREWS, P. J. & MIDGLEY, S. 1992. The effect of changes in cerebral perfusion pressure upon middle cerebral artery blood flow velocity and jugular bulb venous oxygen saturation after severe brain injury. *J Neurosurg*, 77, 55-61.
- CHAPMAN, K. E. & SECKL, J. R. 2008. 11 beta-HSD1, inflammation, metabolic disease and age-related cognitive (dys)Function. *Neurochemical Research*, 33, 624-636.
- CHIEN, M. Y., HUANG, T. Y. & WU, Y. T. 2008. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. *Journal of the American Geriatrics Society*, 56, 1710-1715.
- CHOUDHARI, R., ANAP, D., RAO, K. & IYER, C. 2012. Comparison of Upper, Middle, and Lower Trapezius Strength in Individuals with Unilateral Neck Pain. *Spine*, 1, 115.
- CHOWDHURY, B., SJOSTROM, L., ALPSTEN, M., KOSTANTY, J., KVIST, H. & LOFGREN, R. 1994. A multicompartiment body composition technique based on computerized tomography. *International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, 18, 219-34.
- CHRISTENSEN, H., KORTEN, A. E., MACKINNON, A. J., JORM, A. F., HENDERSON, A. S. & RODGERS, B. 2000. Are changes in sensory disability, reaction time, and grip strength associated with changes in memory and crystallized intelligence? A longitudinal analysis in an elderly community sample. *Gerontology*, 46, 276-292.
- CHRISTENSEN, H., MACKINNON, A. J., KORTEN, A. & JORM, A. F. 2001. The "common cause hypothesis" of cognitive aging: Evidence for not only a common factor but also specific associations of with vision and grip strength in a cross-sectional analysis. *Psychology and Aging*, 16, 588-599.
- CHUANG, Y. F., HAYDEN, K. M., NORTON, M. C., TSCHANZ, J., BREITNER, J. C., WELSH-BOHMER, K. A. & ZANDI, P. P. 2010. Association between APOE epsilon4 allele and vascular dementia: The Cache County study. *Dement Geriatr Cogn Disord*, 29, 248-53.
- CLEMENT, F. J. 1974. Longitudinal and cross-sectional assessments of age-changes in physical strength as related to sex, social-class, and mental-ability. *Journals of Gerontology*, 29, 423-429.
- COGGAN, A. R., ABDULJALIL, A. M., SWANSON, S. C., EARLE, M. S., FARRIS, J. W., MENDENHALL, L. A. & ROBITAILLE, P. M. 1993. Muscle metabolism during

- exercise in young and older untrained and endurance-trained men. *Journal of Applied Physiology*, 75, 2125-2133.
- COHEN, J. 1988. *Statistical power analysis for the behavioral sciences*, Hillsdale, New Jersey, Lawrence Erlbaum Associates.
- COLCOMBE, S. J., ERICKSON, K. I., SCALF, P. E., KIM, J. S., PRAKASH, R., MCAULEY, E., ELAVSKY, S., MARQUEZ, D. X., HU, L. & KRAMER, A. F. 2006. Aerobic exercise training increases brain volume in aging humans. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences*, 61, 1166-1170.
- COLEY, N., ANDRIEU, S., GARDETTE, V., GILLETTE-GUYONNET, S., SANZ, C., VELLAS, B. & GRAND, A. 2008. Dementia prevention: methodological explanations for inconsistent results. *Epidemiologic reviews*, 30, 35.
- CONLEY, K. E., JUBRIAS, S. A. & ESSELMAN, P. C. 2000. Oxidative capacity and ageing in human muscle. *Journal of Physiology-London*, 526, 203-210.
- COOPER, M. S., RABBITT, E. H., GODDARD, P. E., BARTLETT, W. A., HEWISON, M. & STEWART, P. M. 2002. Osteoblastic 11beta-hydroxysteroid dehydrogenase type 1 activity increases with age and glucocorticoid exposure. *J Bone Miner Res*, 17, 979-86.
- CORLEY, J., JIA, X., BRETT, C. E., GOW, A. J., STARR, J. M., KYLE, J. A., MCNEILL, G. & DEARY, I. J. 2011. Alcohol intake and cognitive abilities in old age: the Lothian Birth Cohort 1936 study. *Neuropsychology*, 25, 166-75.
- CORPAS, E., HARMAN, S. M., PINEYRO, M. A., ROBERSON, R. & BLACKMAN, M. R. 1992. Growth hormone (GH)-releasing hormone-(1-29) twice daily reverses the decreased GH and insulin-like growth factor-I levels in old men. *J Clin Endocrinol Metab*, 75, 530-5.
- COX, S. R., BASTIN, M. E., FERGUSON, K. J., MANIEGA, S. M., MACPHERSON, S. E., DEARY, I. J., WARDLAW, J. M. & MACLULLICH, A. M. 2014. Brain white matter integrity and cortisol in older men: the Lothian Birth Cohort 1936. *Neurobiol Aging*.
- CRAWFORD, J. R., DEARY, I. J., STARR, J. & WHALLEY, L. J. 2001. The NART as an index of prior intellectual functioning: a retrospective validity study covering a 66-year interval. *Psychol Med*, 31, 451-8.
- CRUZ-JENTOFT, A. J., BAEYENS, J. P., BAUER, J. M., BOIRIE, Y., CEDERHOLM, T., LANDI, F., MARTIN, F. C., MICHEL, J. P., ROLLAND, Y., SCHNEIDER, S. M., TOPINKOVA, E., VANDEWOUDE, M. & ZAMBONI, M. 2010. Sarcopenia: European consensus on definition and diagnosis. *Age and Ageing*, 39, 412-423.
- CUTHBERTSON, D. J., BABRAJ, J., SMITH, K., WILKES, E., FEDELE, M. J., ESSER, K. & RENNIE, M. 2006. Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 290, E731-E738.
- DAI, Q., BORENSTEIN, A. R., WU, Y., JACKSON, J. C. & LARSON, E. B. 2006. Fruit and vegetable juices and Alzheimer's disease: the Kame Project. *Am J Med*, 119, 751-9.
- DAI, T. H., LIU, J. Z., SAHGAL, V., BROWN, R. W. & YUE, G. H. 2001. Relationship between muscle output and functional MRI-measured brain activation. *Experimental Brain Research*, 140, 290-300.
- DANNESKIOLD-SAMSOE, B. & GRIMBY, G. 1986. The influence of prednisone on the muscle morphology and muscle enzymes in patients with rheumatoid arthritis. *Clin Sci (Lond)*, 71, 693-701.
- DAO, E., DAVIS, J. C., SHARMA, D., CHAN, A., NAGAMATSU, L. S. & LIU-AMBROSE, T. 2013. Change in body fat mass is independently associated with

- executive functions in older women: a secondary analysis of a 12-month randomized controlled trial. *PLoS ONE [Electronic Resource]*, 8, e52831.
- DAVIES, G., HARRIS, S. E., REYNOLDS, C. A., PAYTON, A., KNIGHT, H. M., LIEWALD, D. C., LOPEZ, L. M., LUCIANO, M., GOW, A. J., CORLEY, J., HENDERSON, R., MURRAY, C., PATTIE, A., FOX, H. C., REDMOND, P., LUTZ, M. W., CHIBA-FALEK, O., LINNERTZ, C., SAITH, S., HAGGARTY, P., MCNEILL, G., KE, X., OLLIER, W., HORAN, M., ROSES, A. D., PONTING, C. P., PORTEOUS, D. J., TENESA, A., PICKLES, A., STARR, J. M., WHALLEY, L. J., PEDERSEN, N. L., PENDLETON, N., VISSCHER, P. M. & DEARY, I. J. 2014. A genome-wide association study implicates the APOE locus in nonpathological cognitive ageing. *Mol Psychiatry*, 19, 76-87.
- DAVIS, C. L., TOMPOROWSKI, P. D., MCDOWELL, J. E., AUSTIN, B. P., MILLER, P. H., YANASAK, N. E., ALLISON, J. D. & NAGLIERI, J. A. 2011. Exercise improves executive function and achievement and alters brain activation in overweight children: a randomized, controlled trial. *Health Psychol*, 30, 91-8.
- DE REKENEIRE, N., RESNICK, H. E., SCHWARTZ, A. V., SHORR, R. I., KULLER, L. H., SIMONSICK, E. M., VELLAS, B. & HARRIS, T. B. 2003a. Diabetes is associated with subclinical functional limitation in nondisabled older individuals: the Health, Aging, and Body Composition study. *Diabetes Care*, 26, 3257-3263.
- DE REKENEIRE, N., VISSER, M., PEILA, R., NEVITT, M. C., CAULEY, J. A., TYLAVSKY, F. A., SIMONSICK, E. M. & HARRIS, T. B. 2003b. Is a fall just a fall: correlates of falling in healthy older persons. The Health, Aging and Body Composition Study. *Journal of the American Geriatrics Society*, 51, 841-846.
- DEARY, I., DER, G. & FORD, G. 2001. Reaction times and intelligence differences: A population-based cohort study. 29, 389-399.
- DEARY, I., STRAND, S., SMITH, P. & FERNANDES, C. 2007a. Intelligence and educational achievement. *Intelligence*, 35, 13-21.
- DEARY, I. J., CORLEY, J., GOW, A. J., HARRIS, S. E., HOULIHAN, L. M., MARIONI, R. E., PENKE, L., RAFNSSON, S. B. & STARR, J. M. 2009. Age-associated cognitive decline. *British Medical Bulletin*, 92, 135-152.
- DEARY, I. J., GOW, A. J., TAYLOR, M. D., CORLEY, J., BRETT, C., WILSON, V., CAMPBELL, H., WHALLEY, L. J., VISSCHER, P. M., PORTEOUS, D. J. & STARR, J. M. 2007b. The Lothian Birth Cohort 1936: a study to examine influences on cognitive ageing from age 11 to age 70 and beyond. *BMC Geriatr*, 7, 28.
- DEARY, I. J., HARRIS, S. E., FOX, H. C., HAYWARD, C., WRIGHT, A. F., STARR, J. M. & WHALLEY, L. J. 2005. KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. *Neuroscience Letters*, 378, 22-27.
- DEARY, I. J., HAYWARD, C., PERMANA, P. A., NAIR, S., WHALLEY, L. J., STARR, J. M., CHAPMAN, K. E., WALKER, B. R. & SECKL, J. R. 2006a. Polymorphisms in the gene encoding 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (HSD11B1) and lifetime cognitive change. *Neurosci Lett*, 393, 74-7.
- DEARY, I. J., JOHNSON, W., GOW, A. J., PATTIE, A., BRETT, C. E., BATES, T. C. & STARR, J. M. 2011. Losing One's Grip: A Bivariate Growth Curve Model of Grip Strength and Nonverbal Reasoning From Age 79 to 87 Years in the Lothian Birth Cohort 1921. *J Gerontol B Psychol Sci Soc Sci*, 66, 699-707.
- DEARY, I. J., SIMONOTTO, E., MEYER, M., MARSHALL, A., MARSHALL, I., GODDARD, N. & WARDLAW, J. M. 2004a. The functional anatomy of inspection time: an event-related fMRI study. *Neuroimage*, 22, 1466-79.
- DEARY, I. J., SPINATH, F. M. & BATES, T. C. 2006b. Genetics of intelligence. *Eur J Hum Genet*, 14, 690-700.

- DEARY, I. J., WHITEMAN, M. C., STARR, J. M., WHALLEY, L. J. & FOX, H. C. 2004b. The impact of childhood intelligence on later life: following up the Scottish mental surveys of 1932 and 1947. *J Pers Soc Psychol*. United States.
- DEBETTE, S. & MARKUS, H. S. 2010. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *Bmj*, 341, c3666.
- DELBONO, O., OROUKE, K. S. & ETTINGER, W. H. 1995. Excitation calcium release uncoupling in aged single human skeletal muscle fibers. *Journal of Membrane Biology*, 148, 211-222.
- DIK, M. G., JONKER, C., HACK, C. E., SMIT, J. H., COMIJS, H. C. & EIKELENBOOM, P. 2005. Serum inflammatory proteins and cognitive decline in older persons. *Neurology*, 64, 1371-1377.
- DOHERTY, T. J. 2003. Aging and sarcopenia. *Journal of Applied Physiology*, 95, 1717-1727.
- DOI, T., MAKIZAKO, H., SHIMADA, H., YOSHIDA, D., ITO, K., KATO, T., ANDO, H. & SUZUKI, T. 2012. Brain atrophy and trunk stability during dual-task walking among older adults. *Journals of Gerontology Series A-Biological Sciences & Medical Sciences*, 67, 790-5.
- DONALDSON, K. E., CARPENTER, W. H., TOTH, M. J., GORAN, M. I., NEWHOUSE, P. & POEHLMAN, E. T. 1996. No evidence for a higher resting metabolic rate in noninstitutionalized Alzheimer's disease patients. *Journal of the American Geriatrics Society*, 44, 1232-1234.
- DRAGO, V., BABILONI, C., BARTRES-FAZ, D., CAROLI, A., BOSCH, B., HENSCH, T., DIDIC, M., KLAFKI, H. W., PIEVANI, M., JOVICICH, J., VENTURI, L., SPITZER, P., VECCHIO, F., SCHOENKNECHT, P., WILTFANG, J., REDOLFI, A., FORLONI, G., BLIN, O., IRVING, E., DAVIS, C., HARDEMARK, H. G. & FRISONI, G. B. 2011. Disease tracking markers for Alzheimer's disease at the prodromal (MCI) stage. *J Alzheimers Dis*, 26 Suppl 3, 159-99.
- DUFF, K., MOLD, J. W. & ROBERTS, M. M. 2008. Walking speed and global cognition: results from the OKLAHOMA Study. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn*, 15, 31-9.
- DUMURGIER, J., CRIVELLO, F., MAZOYER, B., AHMED, I., TAVERNIER, B., GRABLI, D., FRANCOIS, C., TZOURIO-MAZOYER, N., TZOURIO, C. & ELBAZ, A. 2012. MRI atrophy of the caudate nucleus and slower walking speed in the elderly. *Neuroimage*, 60, 871-8.
- DUMURGIER, J., ELBAZ, A., DUFOUIL, C., TAVERNIER, B. & TZOURIO, C. 2010. Hypertension and lower walking speed in the elderly: the Three-City study. *Journal of Hypertension*, 28, 1506-14.
- DURGA, J., VAN BOXTEL, M. P., SCHOUTEN, E. G., KOK, F. J., JOLLES, J., KATAN, M. B. & VERHOEF, P. 2007. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet*, 369, 208-16.
- DVORAK, R. V. & POEHLMAN, E. T. 1998. Appendicular skeletal muscle mass, physical activity, and cognitive status in patients with Alzheimer's disease. *Neurology*, 51, 1386-90.
- EDUCATION, S. C. F. R. I. 1949. *The trend of Scottish intelligence: a comparison of the 1947 and 1932 surveys of the intelligence of eleven-year-old pupils*, London, UK, University of London Press.
- EDWARDS, R. H. & HYDE, S. 1977. Methods of measuring muscle strength and fatigue. *Physiotherapy*, 63, 51-5.



- EGAN, M. F., KOJIMA, M., CALLICOTT, J. H., GOLDBERG, T. E., KOLACHANA, B. S., BERTOLINO, A., ZAITSEV, E., GOLD, B., GOLDMAN, D., DEAN, M., LU, B. & WEINBERGER, D. R. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112, 257-269.
- ELBAZ, A., VICENTE-VYTOPILOVA, P., TAVERNIER, B., SABIA, S., DUMURGIER, J., MAZOYER, B., SINGH-MANOUX, A. & TZOURIO, C. 2013. Motor function in the elderly: evidence for the reserve hypothesis. *Neurology*, 81, 417-26.
- ELLIOTT, J. M., JULL, G. A., TNOTEBOOM, J., DURBRIDGE, G. L. & GIBBON, W. W. 2007. Magnetic resonance imaging study of cross-sectional area of the cervical extensor musculature in an asymptomatic cohort. *Clinical Anatomy*, 20, 35-40.
- ELLIS, K. A., BUSH, A. I., DARBY, D., DE FAZIO, D., FOSTER, J., HUDSON, P., LAUTENSCHLAGER, N. I., LENZO, N., MARTINS, R. N., MARUFF, P., MASTERS, C., MILNER, A., PIKE, K., ROWE, C., SAVAGE, G., SZOEKE, C., TADDEI, K., VILLEMAGNE, V., WOODWARD, M., AMES, D. & GRP, A. R. 2009. The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease. *International Psychogeriatrics*, 21, 672-687.
- ELSANKARI, S., BALEDENT, O., VAN PESCH, V., SINDIC, C., DE BROQUEVILLE, Q. & DUPREZ, T. 2013. Concomitant analysis of arterial, venous, and CSF flows using phase-contrast MRI: a quantitative comparison between MS patients and healthy controls. *J Cereb Blood Flow Metab*, 33, 1314-21.
- ENGELHART, M. J., GEERLINGS, M. I., RUITENBERG, A., VAN SWIETEN, J. C., HOLMAN, A., WITTEMAN, J. C. M. & BRETILER, M. M. B. 2002. Dietary intake of antioxidants and risk of Alzheimer disease. *Jama-Journal of the American Medical Association*, 287, 3223-3229.
- ERSHLER, W. B. & KELLER, E. T. 2000. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annual Review of Medicine*, 51, 245-270.
- FALDUTO, M. T., CZERWINSKI, S. M. & HICKSON, R. C. 1990. Glucocorticoid-induced muscle atrophy prevention by exercise in fast-twitch fibers. *J Appl Physiol (1985)*, 69, 1058-62.
- FARMER, A. 2012. Use of HbA1c in the diagnosis of diabetes. *Bmj*, 345, e7293.
- FAULKNER, J. A., LARKIN, L. M., CLAFLIN, D. R. & BROOKS, S. V. 2007. Age-related changes in the structure and function of skeletal muscles. *Clinical and Experimental Pharmacology and Physiology*, 34, 1091-1096.
- FAZEKAS, F., CHAWLUK, J. B., ALAVI, A., HURTIG, H. I. & ZIMMERMAN, R. A. 1987. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*, 149, 351-6.
- FEARON, K. C. H., HANSELL, D. T., PRESTON, T., PLUMB, J. A., DAVIES, J., SHAPIRO, D., SHENKIN, A., CALMAN, K. C. & BURNS, H. J. G. 1988. Influence of whole-body protein-turnover rate on resting energy-expenditure in patients with cancer. *Cancer Research*, 48, 2590-2595.
- FENG, W., UTRIAINEN, D., TRIFAN, G., ELIAS, S., SETHI, S., HEWETT, J. & HAACKE, E. M. 2012. Characteristics of flow through the internal jugular veins at cervical C2/C3 and C5/C6 levels for multiple sclerosis patients using MR phase contrast imaging. *Neurol Res*, 34, 802-9.
- FERGUSON, K. J., WARDLAW, J. M., EDMOND, C. L., DEARY, I. J. & MACLULLICH, A. M. 2005. Intracranial area: a validated method for estimating intracranial volume. *J Neuroimaging*, 15, 76-8.

- FIATARONE, M. A., MARKS, E. C., RYAN, N. D., MEREDITH, C. N., LIPSITZ, L. A. & EVANS, W. J. 1990. High-intensity strength training in nonagenarians - effects on skeletal-muscle. *Jama-Journal of the American Medical Association*, 263, 3029-3034.
- FIATARONE, M. A., ONEILL, E. F., RYAN, N. D., CLEMENTS, K. M., SOLARES, G. R., NELSON, M. E., ROBERTS, S. B., KEHAYIAS, J. J., LIPSITZ, L. A. & EVANS, W. J. 1994. Exercise training and nutritional supplementation for physical frailty in very elderly people. *New England Journal of Medicine*, 330, 1769-1775.
- FIELDING, R. A., VELLAS, B., EVANS, W. J., BHASIN, S., MORLEY, J. E., NEWMAN, A. B., ABELLAN VAN KAN, G., ANDRIEU, S., BAUER, J., BREUILLE, D., CEDERHOLM, T., CHANDLER, J., DE MEYNARD, C., DONINI, L., HARRIS, T., KANNT, A., KEIME GUIBERT, F., ONDER, G., PAPANICOLAOU, D., ROLLAND, Y., ROOKS, D., SIEBER, C., SOUHAMI, E., VERLAAN, S. & ZAMBONI, M. 2011. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc*, 12, 249-56.
- FISCHER, P., JUNGWIRTH, S., ZEHETMAYER, S., WEISSGRAM, S., HOENIGSCHNABL, S., GELPI, E., KRAMPLA, W. & TRAGL, K. H. 2007. Conversion from subtypes of mild cognitive impairment to Alzheimer dementia. *Neurology*, 68, 288-91.
- FJELL, A. M. & WALHOVD, K. B. 2010. Structural brain changes in aging: courses, causes and cognitive consequences. *Rev Neurosci*, 21, 187-221.
- FOLSTEIN, M. F., FOLSTEIN, S. E. & MCHUGH, P. R. 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, 12, 189-98.
- FORSBERG, A. M., NILSSON, E., WERNEMAN, J., BERGSTROM, J. & HULTMAN, E. 1991. Muscle composition in relation to age and sex. *Clinical Science*, 81, 249-56.
- FRANCO-MARINA, F., GARCIA-GONZALEZ, J. J., WAGNER-ECHEAGARAY, F., GALLO, J., UGALDE, O., SANCHEZ-GARCIA, S., ESPINEL-BERMUDEZ, C., JUAREZ-CEDILLO, T., RODRIGUEZ, M. A. & GARCIA-PENA, C. 2010. The Mini-mental State Examination revisited: ceiling and floor effects after score adjustment for educational level in an aging Mexican population. *Int Psychogeriatr*, 22, 72-81.
- FRIED, L. P., TANGEN, C. M., WALSTON, J., NEWMAN, A. B., HIRSCH, C., GOTTDIENER, J., SEEMAN, T., TRACY, R., KOP, W. J., BURKE, G., MCBURNIE, M. A. & CARDIOVASCULAR HLTH STUDY, C. 2001. Frailty in older adults: Evidence for a phenotype. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 56, M146-M156.
- FRIEDLANDER, A. L., BUTTERFIELD, E., MOYNIHAN, S., GRILLO, J., POLLACK, M., HOLLOWAY, L., FRIEDMAN, L., YESAVAGE, J., MATTHIAS, D. F., LEE, S., MARCUS, R. & HOFFMAN, A. R. 2001. One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women. *Journal of Clinical Endocrinology & Metabolism*, 86, 1496-1503.
- FRONTERA, W. R., HUGHES, V. A., FIELDING, R. A., FIATARONE, M. A., EVANS, W. J. & ROUBENOFF, R. 2000. Aging of skeletal muscle: a 12-yr longitudinal study. *Journal of Applied Physiology*, 88, 1321-1326.
- GALEA, V. 1996. Changes in motor unit estimates with aging. *Journal of Clinical Neurophysiology*, 13, 253-260.
- GALLAGHER, D. & HEYMSFIELD, S. B. 1998. Muscle distribution: Variations with body weight, gender, and age. *Applied Radiation and Isotopes*, 49, 733-734.

- GALLAGHER, D., VISSER, M., DEMEERSMAN, R. E., SEPULVEDA, D., BAUMGARTNER, R. N., PIERSON, R. N., HARRIS, T. & HEYMSFIELD, S. B. 1997. Appendicular skeletal muscle mass: Effects of age, gender, and ethnicity. *Journal of Applied Physiology*, 83, 229-239.
- GARDNER, M. P., LIGHTMAN, S. L., GALLACHER, J., HARDY, R., KUH, D., EBRAHIM, S., BAYER, A. & BEN-SHLOMO, Y. 2011. Diurnal cortisol patterns are associated with physical performance in the Caerphilly Prospective Study. *Int J Epidemiol*, 40, 1693-702.
- GARIBALLA, S. & ALESSA, A. 2013. Sarcopenia: prevalence and prognostic significance in hospitalized patients. *Clin Nutr*, 32, 772-6.
- GARRY, P. J., WAYNE, S. J. & VELLAS, B. 2007. The New Mexico aging process study (1979-2003) a longitudinal study of nutrition, health and aging. *Journal of Nutrition, Health and Aging*, 11 (2), 125-130.
- GEIST, L. J. & DAI, L. Y. 1996. Cytomegalovirus modulates interleukin-6 gene expression. *Transplantation*, 62, 653-8.
- GENTON, L., KARSEGARD, V. L., CHEVALLEY, T., KOSSOVSKY, M. P., DARMON, P. & PICHARD, C. 2011. Body composition changes over 9 years in healthy elderly subjects and impact of physical activity. *Clinical Nutrition*, 30 (4), 436-442.
- GEUSENS, P., VANDEVYVER, C., VANHOOF, J., CASSIMAN, J. J., BOONEN, S. & RAUS, J. 1997. Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. *Journal of Bone and Mineral Research*, 12, 2082-2088.
- GIACCAGLIA, V., NICKLAS, B., KRITCHEVSKY, S., MYCHALECKYZ, J., MESSIER, S., BLEECKER, E. & PAHOR, M. 2008. Interaction between angiotensin converting enzyme insertion/deletion genotype and exercise training on knee extensor strength in older individuals. *International Journal of Sports Medicine*, 29, 40-44.
- GIMENO, D., MARMOT, M. G. & SINGH-MANOUX, A. 2008. Inflammatory markers and cognitive function in middle-aged adults: the Whitehall II study. *Psychoneuroendocrinology*, 33, 1322-34.
- GODARD, M. P., WILLIAMSON, D. L. & TRAPPE, S. W. 2002. Oral amino-acid provision does not affect muscle strength or size gains in older men. *Medicine & Science in Sports & Exercise*, 34, 1126-31.
- GOODPASTER, B. H., KRISHNASWAMI, S., RESNICK, H., KELLEY, D. E., HAGGERTY, C., HARRIS, T. B., SCHWARTZ, A. V., KRITCHEVSKY, S. & NEWMAN, A. B. 2003. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care*, 26, 372-379.
- GOODPASTER, B. H., PARK, S. W., HARRIS, T. B., KRITCHEVSKY, S. B., NEVITT, M., SCHWARTZ, A. V., SIMONSICK, E. M., TYLAVSKY, F. A., VISSER, M., NEWMAN, A. B. & HLTH, A. B. C. S. 2006. The loss of skeletal muscle strength, mass, and quality in older adults: The health, aging and body composition study. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 61, 1059-1064.
- GOODPASTER, B. H., THAETE, F. L. & KELLEY, D. E. 2000. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *American Journal of Clinical Nutrition*, 71, 885-892.
- GOUNTOUNA, V. E., JOB, D. E., MCINTOSH, A. M., MOORHEAD, T. W. J., LYMER, G. K. L., WHALLEY, H. C., HALL, J., WAITER, G. D., BRENNAN, D., MCGONIGLE, D. J., AHEARN, T. S., CAVANAGH, J., CONDON, B., HADLEY, D. M., MARSHALL, I., MURRAY, A. D., STEELE, J. D., WARDLAW, J. M. &

- LAWRIE, S. M. 2010. Functional Magnetic Resonance Imaging (fMRI) reproducibility and variance components across visits and scanning sites with a finger tapping task. *Neuroimage*, 49, 552-560.
- GRAY, C., MACGILLIVRAY, T. J., EELEY, C., STEPHENS, N. A., BEGGS, I., FEARON, K. C. & GREIG, C. A. 2011. Magnetic resonance imaging with k-means clustering objectively measures whole muscle volume compartments in sarcopenia/cancer cachexia. *Clinical Nutrition*, 30, 106-111.
- GREENWOOD, C. E. & WINOCUR, G. 2005. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol Aging*, 26 Suppl 1, 42-5.
- GREIG, C. A., YOUNG, A., SKELTON, D. A., PIPPET, E., BUTLER, F. M. M. & MAHMUD, S. M. 1994. Exercise studies with elderly volunteers. *Age and Ageing*, 23, 185-189.
- GREIWE, J. S., CHENG, B., RUBIN, D. C., YARASHESKI, K. E. & SEMENKOVICH, C. F. 2001. Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. *Faseb Journal*, 15, 475-482.
- GRIFFITHS, P. D., HOGGARD, N., DANNELS, W. R. & WILKINSON, I. D. 2001. In vivo measurement of cerebral blood flow: a review of methods and applications. *Vasc Med*, 6, 51-60.
- GRODSTEIN, F., CHEN, J. & WILLETT, W. C. 2003. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. *American Journal of Clinical Nutrition*, 77, 975-984.
- GRUNE, F., KAZMAIER, S., SONNTAG, H., STOLKER, R. J. & WEYLAND, A. 2014. Moderate hyperventilation during intravenous anesthesia increases net cerebral lactate efflux. *Anesthesiology*, 120, 335-42.
- GUERRERO-BERROA, E., RAVONA-SPRINGER, R., HEYMAN, A., SCHMEIDLER, J., SILVERMAN, J. M., SANO, M., KOIFMANN, K., PREISS, R., HOFFMAN, H. & SCHNAIDER BEERI, M. 2014. Decreased motor function is associated with poorer cognitive function in elderly with type 2 diabetes. *Dement Geriatr Cogn Dis Extra*, 4, 103-12.
- GUPTA, A. & GUPTA, Y. 2013. Glucocorticoid-induced myopathy: Pathophysiology, diagnosis, and treatment. *Indian J Endocrinol Metab*, 17, 913-6.
- GUTHRIE, J. R., DENNERSTEIN, L., TAFFE, J. R., LEHERT, P. & BURGER, H. G. 2004. The menopausal transition: a 9-year prospective population-based study. The Melbourne Women's Midlife Health Project. *Climacteric*, 7, 375-89.
- GUTTMANN, C. R., BENSON, R., WARFIELD, S. K., WEI, X., ANDERSON, M. C., HALL, C. B., ABU-HASABALLAH, K., MUGLER, J. P., 3RD & WOLFSON, L. 2000. White matter abnormalities in mobility-impaired older persons. *Neurology*, 54, 1277-83.
- HAARBO, J., MARSLEW, U., GOTFREDSEN, A. & CHRISTIANSEN, C. 1991. Postmenopausal hormone replacement therapy prevents central distribution of body-fat after menopause. *Metabolism-Clinical and Experimental*, 40, 1323-1326.
- HAGER, K., MACHEIN, U., KRIEGER, S., PLATT, D., SEEFRIED, G. & BAUER, J. 1994. Interleukin-6 and selected plasma-proteins in healthy-persons of different ages. *Neurobiology of Aging*, 15, 771-772.
- HAJJAR, I., ZHAO, P., ALSOP, D., ABDULJALIL, A., SELIM, M., NOVAK, P. & NOVAK, V. 2010. Association of blood pressure elevation and nocturnal dipping with brain atrophy, perfusion and functional measures in stroke and nonstroke individuals. *American Journal of Hypertension*, 23 (1), 17-23.

- HARDAN, A. Y., KILPATRICK, M., KESHAVAN, M. S. & MINSHEW, N. J. 2003. Motor performance and anatomic magnetic resonance imaging (MRI) of the basal ganglia in autism. *Journal of Child Neurology*, 18 (5), 317-324.
- HAREN, M. T., BANKS, W. A., PERRY III, H. M., PATRICK, P., MALMSTROM, T. K., MILLER, D. K. & MORLEY, J. E. 2008. Predictors of serum testosterone and DHEAS in African-American men. *International Journal of Andrology*, 31, 50-9.
- HARRIS, S. E., WRIGHT, A. F., HAYWARD, C., STARR, J. M., WHALLEY, L. J. & DEARY, I. J. 2005. The functional COMT polymorphism, Val 158 Met, is associated with logical memory and the personality trait intellect/imagination in a cohort of healthy 79 year olds. *Neurosci Lett*, 385, 1-6.
- HART, C. L., TAYLOR, M. D., DAVEY SMITH, G., WHALLEY, L. J., STARR, J. M., HOLE, D. J., WILSON, V. & DEARY, I. J. 2003. Childhood IQ, social class, deprivation, and their relationships with mortality and morbidity risk in later life: prospective observational study linking the Scottish Mental Survey 1932 and the Midspan studies. *Psychosom Med*, 65, 877-83.
- HAUS, J. M., CARRITHERS, J. A., TRAPPE, S. W. & TRAPPE, T. A. 2007. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *Journal of Applied Physiology*, 103, 2068-2076.
- HAWORTH, C. M., WRIGHT, M. J., LUCIANO, M., MARTIN, N. G., DE GEUS, E. J., VAN BEIJSTERVELDT, C. E., BARTELS, M., POSTHUMA, D., BOOMSMA, D. I., DAVIS, O. S., KOVAS, Y., CORLEY, R. P., DEFRIES, J. C., HEWITT, J. K., OLSON, R. K., RHEA, S. A., WADSWORTH, S. J., IACONO, W. G., MCGUE, M., THOMPSON, L. A., HART, S. A., PETRILL, S. A., LUBINSKI, D. & PLOMIN, R. 2010. The heritability of general cognitive ability increases linearly from childhood to young adulthood. *Mol Psychiatry*, 15, 1112-20.
- HEDDEN, T. & GABRIELI, J. D. 2004. Insights into the ageing mind: a view from cognitive neuroscience. *Nat Rev Neurosci*. England.
- HEINRICH, P. C., CASTELL, J. V. & ANDUS, T. 1990. Interleukin-6 and the acute phase response. *Biochem J*, 265, 621-36.
- HERNANDEZ, M., FERGUSON, K., CHAPPELL, F. & WARDLAW, J. 2010. New multispectral MRI data fusion technique for white matter lesion segmentation: method and comparison with thresholding in FLAIR images. *Eur Radiol*, 20, 1684-91.
- HET, S., RAMLOW, G. & WOLF, O. 2005. A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology*, 30, 771-84.
- HEYMSFIELD, S. B., MULLER, M. J., BOSY-WESTPHAL, A., THOMAS, D. & SHEN, W. 2012. Human brain mass: similar body composition associations as observed across mammals. *American Journal of Human Biology*, 24, 479-85.
- HOLDNACK, J. 2001. *WTAR: Wechsler Test of Adult Reading manual*, San Antonio, TX, Psychological Corporation.
- HOLMES, M. C., CARTER, R. N., NOBLE, J., CHITNIS, S., DUTIA, A., PATERSON, J. M., MULLINS, J. J., SECKL, J. R. & YAU, J. L. 2010. 11beta-hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus and parietal cortex and causes memory impairments. *J Neurosci*, 30, 6916-20.
- HONEA, R. A., THOMAS, G. P., HARSHA, A., ANDERSON, H. S., DONNELLY, J. E., BROOKS, W. M. & BURNS, J. M. 2009. Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer disease. *Alzheimer Disease & Associated Disorders*, 23, 188-97.
- HOOKE, P., SRIRAMOJU, V. & LARSSON, L. 2001. Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. *American Journal of Physiology-Cell Physiology*, 280, C782-C788.

- HOUSTON, D. K., TOOZE, J. A., NEIBERG, R. H., HAUSMAN, D. B., JOHNSON, M. A., CAULEY, J. A., BAUER, D. C., CAWTHON, P. M., SHEA, M. K., SCHWARTZ, G. G., WILLIAMSON, J. D., TYLAVSKY, F. A., VISSER, M., SIMONSICK, E. M., HARRIS, T. B. & KRITCHEVSKY, S. B. 2012. 25-hydroxyvitamin D status and change in physical performance and strength in older adults. *American Journal of Epidemiology*, 176, 1025-1034.
- HUGHES, K. A., MANOLOPOULOS, K. N., IQBAL, J., CRUDEN, N. L., STIMSON, R. H., REYNOLDS, R. M., NEWBY, D. E., ANDREW, R., KARPE, F. & WALKER, B. R. 2012. Recycling between cortisol and cortisone in human splanchnic, subcutaneous adipose, and skeletal muscle tissues in vivo. *Diabetes*, 61, 1357-64.
- HUGHES, K. A., WEBSTER, S. P. & WALKER, B. R. 2008. 11-Beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) inhibitors in type 2 diabetes mellitus and obesity. *Expert Opin Investig Drugs*, 17, 481-96.
- HUNTER, S. K., THOMPSON, M. W., RUELL, P. A., HARMER, A. R., THOM, J. M., GWINN, T. H. & ADAMS, R. D. 1999. Human skeletal sarcoplasmic reticulum Ca<sup>2+</sup> uptake and muscle function with aging and strength training. *Journal of Applied Physiology*, 86, 1858-1865.
- HUYGENS, W., THOMIS, M. A., PEETERS, M. W., AERSSSENS, J., JANSSEN, R., VLIETINCK, R. F. & BEUNEN, G. 2004. Linkage of myostatin pathway genes with knee strength in humans. *Physiological Genomics*, 17, 264-270.
- HUYGENS, W., THOMIS, M. A. I., PEETERS, M. W., AERSSSENS, J., VLIETINCK, R. & BEUNEN, G. P. 2005. Quantitative trait loci for human muscle strength: linkage analysis of myostatin pathway genes. *Physiological Genomics*, 22, 390-397.
- IANNELLI, C. & PATERSON, L. 2005. *Moving Up and Down the Social Class Ladder in Scotland* [Online]. Centre for Educational Sociology, University of Edinburgh. Available: <http://www.ces.ed.ac.uk/PDF%20Files/Brief033.pdf>.
- IKEZOE, T., MORI, N., NAKAMURA, M. & ICHIHASHI, N. 2011. Age-related muscle atrophy in the lower extremities and daily physical activity in elderly women. *Arch Gerontol Geriatr*, 53, e153-7.
- IMAMURA, K., ASHIDA, H., ISHIKAWA, T. & FUJII, M. 1983. Human major psoas muscle and sacrospinalis muscle in relation to age - a study by computed-tomography. *Journals of Gerontology*, 38, 678-681.
- INACIO, M., RYAN, A. S., BAIR, W. N., PRETTYMAN, M., BEAMER, B. A. & ROGERS, M. W. 2014. Gluteal muscle composition differentiates fallers from non-fallers in community dwelling older adults. *BMC Geriatr*, 14, 37.
- IOVINO, M., MONTELEONE, P. & STEARDO, L. 1989. Repetitive growth hormone-releasing hormone administration restores the attenuated growth hormone (GH) response to GH-releasing hormone testing in normal aging. *J Clin Endocrinol Metab*, 69, 910-3.
- IQBAL, J., ANDREW, R., CRUDEN, N. L., KENYON, C. J., HUGHES, K. A., NEWBY, D. E., HADDOKE, P. W. & WALKER, B. R. 2014. Displacement of cortisol from human heart by acute administration of a mineralocorticoid receptor antagonist. *J Clin Endocrinol Metab*, 99, 915-22.
- IZQUIERDO, M., HAKKINEN, K., ANTON, A., GARRUES, M., IBANEZ, J., RUESTA, M. & GOROSTIAGA, E. M. 2001. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. *Med Sci Sports Exerc*, 33, 1577-87.
- JACOBSEN, D. E., MELIS, R. J. F., VERHAAR, H. J. J. & OLDE RIKKERT, M. G. M. 2012. Raloxifene and tibolone in elderly women: A randomized, double-blind,

- double-dummy, placebo-controlled trial. *Journal of the American Medical Directors Association*, 13, 189.e1-189.e7.
- JAKOBSSON, F., BORG, K. & EDSTROM, L. 1990. Fiber-type composition, structure and cytoskeletal protein location of fibers in anterior tibial muscle - comparison between young-adults and physically active aged humans. *Acta Neuropathologica*, 80, 459-468.
- JANG, C., OBEYESEKERE, V. R., DILLEY, R. J., ALFORD, F. P. & INDER, W. J. 2006. 11 beta hydroxysteroid dehydrogenase type 1 is expressed and is biologically active in human skeletal muscle. *Clinical Endocrinology*, 65, 800-805.
- JANG, Y. C., LUSTGARTEN, M. S., LIU, Y. H., MULLER, F. L., BHATTACHARYA, A., LIANG, H. Y., SALMON, A. B., BROOKS, S. V., LARKIN, L., HAYWORTH, C. R., RICHARDSON, A. & VAN REMMEN, H. 2010. Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. *Faseb Journal*, 24, 1376-1390.
- JANSSEN, I. 2006. Influence of sarcopenia on the development of physical disability: the cardiovascular health study. *Journal of the American Geriatrics Society*, 54, 56-62.
- JANSSEN, I., HEYMSFIELD, S. B. & ROSS, R. 2002. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society*, 50, 889-896.
- JANSSEN, I., HEYMSFIELD, S. B., WANG, Z. M. & ROSS, R. 2000. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *Journal of Applied Physiology*, 89, 81-88.
- JANSSEN, I. & ROSS, R. 2005. Linking age-related changes in skeletal muscle mass and composition with metabolism and disease. *Journal of Nutrition Health & Aging*, 9, 408-419.
- JOHANSSON, M. M., MARCUSSON, J. & WRESSLE, E. 2012. Cognition, daily living, and health-related quality of life in 85-year-olds in Sweden. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn*, 19, 421-32.
- KACHIWALA, S. J., HARRIS, S. E., WRIGHT, A. F., HAYWARD, C., STARR, J. M., WHALLEY, L. J. & DEARY, I. J. 2005. Genetic influences on oxidative stress and their association with normal cognitive ageing. *Neuroscience Letters*, 386, 116-120.
- KAMEL, H. K. 2003. Sarcopenia and aging. *Nutrition Reviews*, 61, 157-167.
- KAMEN, G., SISON, S. V., DU, C. C. D. & PATTEN, C. 1995. Motor unit discharge behavior in older adults during maximal-effort contractions. *Journal of Applied Physiology*, 79, 1908-1913.
- KAMIJO, K., KHAN, N. A., PONTIFEX, M. B., SCUDDER, M. R., DROLLETTE, E. S., RAINE, L. B., EVANS, E. M., CASTELLI, D. M. & HILLMAN, C. H. 2012. The relation of adiposity to cognitive control and scholastic achievement in preadolescent children. *Obesity*, 20, 2406-2411.
- KAMIJO, K., PONTIFEX, M. B., KHAN, N. A., RAINE, L. B., SCUDDER, M. R., DROLLETTE, E. S., EVANS, E. M., CASTELLI, D. M. & HILLMAN, C. H. 2014. The negative association of childhood obesity to cognitive control of action monitoring. *Cerebral Cortex*, 24, 654-662.
- KANDA, K. & HASHIZUME, K. 1991. Recovery of motor-unit function after peripheral-nerve injury in aged rats. *Neurobiology of Aging*, 12, 271-276.
- KATZ, D. A., LIU, W., LOCKE, C., JACOBSON, P., BARNES, D. M., BASU, R., AN, G., RIESER, M. J., DASZKOWSKI, D., GROVES, F., HENEGHAN, G., SHAH, A., GEVORKYAN, H., JHEE, S. S., ERESHEFSKY, L. & MAREK, G. J. 2013. Peripheral and central nervous system inhibition of 11beta-hydroxysteroid

- dehydrogenase type 1 in man by the novel inhibitor ABT-384. *Transl Psychiatry*, 3, e295.
- KELLER, J. N. 2006. Age-related neuropathology, cognitive decline, and Alzheimer's disease. *Ageing Res Rev*, 5, 1-13.
- KENNY, A. M., BELLANTONIO, S., GRUMAN, C. A., ACOSTA, R. D. & PRESTWOOD, K. M. 2002. Effects of transdermal testosterone on cognitive function and health perception in older men with low bioavailable testosterone levels. *J Gerontol A Biol Sci Med Sci*, 57, M321-5.
- KENT-BRAUN, J. A., NG, A. V. & YOUNG, K. 2000. Skeletal muscle contractile and noncontractile components in young and older women and men. *Journal of Applied Physiology*, 88, 662-668.
- KILGOUR, A., SUBEDI, D., GRAY, C., DEARY, I., LAWRIE, S., WARDLAW, J. & STARR, J. 2012. Design and Validation of a Novel Method to Measure Cross-Sectional Area of Neck Muscles Included during Routine MR Brain Volume Imaging. *PLoS ONE*, 7.
- KILGOUR, A. H., FERGUSON, K. J., GRAY, C. D., DEARY, I. J., WARDLAW, J. M., MACLULLICH, A. M. & STARR, J. M. 2013. Neck muscle cross-sectional area, brain volume and cognition in healthy older men: a cohort study. *BMC Geriatrics*, 13, 20.
- KILGOUR, A. H., TODD, O. M. & STARR, J. M. 2014. A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse. *BMC Geriatr*, 14, 85.
- KIM, J., BASAK, J. M. & HOLTZMAN, D. M. 2009. The role of apolipoprotein E in Alzheimer's disease. *Neuron*, 63, 287-303.
- KIM, Y. C. 1999. Testosterone supplementation in the aging male. *International Journal of Impotence Research*, 11, 343-352.
- KLITGAARD, H., BERGMAN, O., BETTO, R., SALVIATI, G., SCHIAFFINO, S., CLAUSEN, T. & B, S. 1990a. Co-existence of myosin heavy chain I and IIa isoforms in human skeletal muscle fibres with endurance training. *Pflugers Arch*.
- KLITGAARD, H., MANTONI, M., SCHIAFFINO, S., AUSONI, S., GORZA, L., LAURENTWINTER, C., SCHNOHR, P. & SALTIN, B. 1990b. Function, morphology and protein expression of aging skeletal-muscle - a cross-sectional study of elderly men with different training backgrounds. *Acta Physiologica Scandinavica*, 140, 41-54.
- KOMULAINEN, P., LAKKA, T. A., KIVIPELTO, M., HASSINEN, M., PENTTILA, I. M., HELKALA, E. L., GYLLING, H., NISSINEN, A. & RAURAMAA, R. 2007. Serum high sensitivity C-reactive protein and cognitive function in elderly women. *Age Ageing*, 36, 443-8.
- KONGSGAARD, M., BACKER, V., JORGENSEN, K., KJAER, M. & BEYER, N. 2004. Heavy resistance training increases muscle size, strength and physical function in elderly male COPD-patients--a pilot study. *Respiratory Medicine*, 98, 1000-7.
- KOPELMAN, M., GLIK, A., GREENBERG, S. & SHELEF, I. 2013. Intracranial nonjugular venous pathways: a possible compensatory drainage mechanism. *AJNR Am J Neuroradiol*, 34, 1348-52.
- KOSTER, A., DING, J., STENHOLM, S., CASEROTTI, P., HOUSTON, D. K., NICKLAS, B. J., YOU, T., LEE, J. S., VISSER, M., NEWMAN, A. B., SCHWARTZ, A. V., CAULEY, J. A., TYLAVSKY, F. A., GOODPASTER, B. H., KRITCHEVSKY, S. B. & HARRIS, T. B. 2011. Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? *J Gerontol A Biol Sci Med Sci*, 66, 888-95.



- KOSTER, A., VISSER, M., SIMONSICK, E. M., YU, B., ALLISON, D. B., NEWMAN, A. B., VAN EIJK, J. T. M., SCHWARTZ, A. V., SATTERFIELD, S. & HARRIS, T. B. 2010. Association between fitness and changes in body composition and muscle strength. *Journal of the American Geriatrics Society*, 58, 219-226.
- KRAL, V. A. 1962. Senescent forgetfulness: benign and malignant. *Can Med Assoc J*, 86, 257-60.
- KRAMER, A. F., ERICKSON, K. I. & COLCOMBE, S. J. 2006. Exercise, cognition, and the aging brain. *Journal of Applied Physiology*, 101, 1237-1242.
- KROTKIEWSKI, M. & BJORNTORP, P. 1986. Muscle tissue in obesity with different distribution of adipose tissue. Effects of physical training. *Int J Obes*, 10, 331-41.
- KUNINGAS, M., DE RIJK, R. H., WESTENDORP, R. G., JOLLES, J., SLAGBOOM, P. E. & VAN HEEMST, D. 2007. Mental performance in old age dependent on cortisol and genetic variance in the mineralocorticoid and glucocorticoid receptors. *Neuropsychopharmacology*, 32, 1295-301.
- LANG, C., KRAWIEC, B., HUBER, D., MCCOY, J. & FROST, R. 2006. Sepsis and inflammatory insults downregulate IGFBP-5, but not IGFBP-4, in skeletal muscle via a TNF-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol*.
- LARSSON, L. 1978. Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study. *Acta Physiol Scand Suppl*, 457, 1-36.
- LASAITIS, L. & KRASAUSKIENE, A. 2009. Psychological state, quality of life, and body composition in postmenopausal women with osteoporosis in Lithuania. *Archives of Osteoporosis*, 4 (1-2), 85-90.
- LAURETANI, F., RUSSO, C. R., BANDINELLI, S., BARTALI, B., CAVAZZINI, C., DI IORIO, A., CORSI, A. M., RANTANEN, T., GURALNIK, J. M. & FERRUCCI, L. 2003. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol*, 95, 1851-60.
- LAURIN, D., DAVID CURB, J., MASAKI, K. H., WHITE, L. R. & LAUNER, L. J. 2009. Midlife C-reactive protein and risk of cognitive decline: a 31-year follow-up. *Neurobiol Aging*, 30, 1724-7.
- LEE, J. S., AUYEUNG, T. W., LEUNG, J., KWOK, T., LEUNG, P. C. & WOO, J. 2011. Physical frailty in older adults is associated with metabolic and atherosclerotic risk factors and cognitive impairment independent of muscle mass. *Journal of Nutrition, Health & Aging*, 15, 857-62.
- LEGER, B., DERAIVE, W., DE BOCK, K., FLESPEL, P. & RUSSELL, A. P. 2008. Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of akt phosphorylation. *Rejuvenation Research*, 11, 163-175.
- LERNER, A., FELD, L. G., RIDDLESBERGER, M. M., ROSSI, T. M. & LEBENTHAL, E. 1986. Computed axial tomographic scanning of the thigh - an alternative method of nutritional assessment in pediatrics. *Pediatrics*, 77, 732-737.
- LEXELL, J., HENRIKSSONLARSEN, K., WINBLAD, B. & SJOSTROM, M. 1983. Distribution of different fiber types in human skeletal-muscles - effects of aging studied in whole muscle cross-sections. *Muscle & Nerve*, 6, 588-595.
- LEXELL, J., TAYLOR, C. C. & SJOSTROM, M. 1988. What is the cause of the aging atrophy - total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15-year-old to 83-year-old men. *Journal of the Neurological Sciences*, 84, 275-294.
- LEZAK, M. 2004. Neuropsychological testing. Oxford, UK: Oxford University Press.
- LICHTENSTEIN, D., SAIFI, R., AUGARDE, R., PRIN, S., SCHMITT, J. M., PAGE, B., PIPIEN, I. & JARDIN, F. 2001. The Internal jugular veins are asymmetric. Usefulness of ultrasound before catheterization. *Intensive Care Med*, 27, 301-5.

- LINDENBERGER, U. & BALTES, P. B. 1994. Sensory functioning and intelligence in old-age - a strong connection. *Psychology and Aging*, 9, 339-355.
- LINDLE, R. S., METTER, E. J., LYNCH, N. A., FLEG, J. L., FOZARD, J. L., TOBIN, J., ROY, T. A. & HURLEY, B. F. 1997. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. *Journal of Applied Physiology*, 83, 1581-1587.
- LIU, C. J. & LATHAM, N. K. 2009. Progressive resistance strength training for improving physical function in older adults. *Cochrane Database Syst Rev*, Cd002759.
- LIU, L. K., LEE, W. J., CHEN, L. Y., HWANG, A. C., LIN, M. H., PENG, L. N. & CHEN, L. K. 2014. Sarcopenia, and its association with cardiometabolic and functional characteristics in Taiwan: Results from I-Lan Longitudinal Aging Study. *Geriatrics and Gerontology International*, 14, 36-45.
- LIU-AMBROSE, T., NAGAMATSU, L. S., GRAF, P., BEATTIE, B. L., ASHE, M. C. & HANDY, T. C. 2010. Resistance training and executive functions: a 12-month randomized controlled trial. *Archives of Internal Medicine*, 170, 170-8.
- LONGSTRETH, W., MANOLIO, T., ARNOLD, A., BURKE, G., BRYAN, N., JUNGREIS, C., ENRIGHT, P., O'LEARY, D. & FRIED, L. 1996. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. *Stroke*, 27, 1274-82.
- LOPO, S., VINAGRE, E., PALMINHA, P., PAIXAO, M. T., NOGUEIRA, P. & FREITAS, M. G. 2011. Seroprevalence to cytomegalovirus in the Portuguese population, 2002-2003. *Euro Surveill*, 16.
- LUBECK, P. R., DOERR, H. W. & RABENAU, H. F. 2010. Epidemiology of human cytomegalovirus (HCMV) in an urban region of Germany: what has changed? *Med Microbiol Immunol*, 199, 53-60.
- LUCIANO, M., MARIONI, R. E., GOW, A. J., STARR, J. M. & DEARY, I. J. 2009. Reverse Causation in the Association Between C-Reactive Protein and Fibrinogen Levels and Cognitive Abilities in an Aging Sample. *Psychosomatic Medicine*, 71, 404-409.
- LUDATSCHER, R. M., SILBERMANN, M., GERSHON, D. & REZNICK, A. 1985. Evidence of schwann-cell degeneration in the aging mouse motor endplate region. *Experimental Gerontology*, 20, 81-91.
- LUFF, A. R. 1998. Age-associated changes in the innervation of muscle fibers and changes in the mechanical properties of motor units. *Towards Prolongation of the Healthy Life Span: Practical Approaches to Intervention*, 854, 92-101.
- LUNARDI, C. C., LIMA, R. M., PEREIRA, R. W., LEITE, T. K., SIQUEIRA, A. B. & OLIVEIRA, R. J. 2013. Association between polymorphisms in the TRHR gene, fat-free mass, and muscle strength in older women. *Age*, 35, 2477-83.
- LUPIEN, S. J., DE LEON, M., DE SANTI, S., CONVIT, A., TARSHISH, C., NAIR, N. P., THAKUR, M., MCEWEN, B. S., HAUGER, R. L. & MEANEY, M. J. 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci*, 1, 69-73.
- MA, K., MALLIDIS, C., BHASIN, S., MAHABADI, V., ARTAZA, J., GONZALEZ-CADAVID, N., ARIAS, J. & SALEHIAN, B. 2003. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *American Journal of Physiology-Endocrinology and Metabolism*, 285, E363-E371.
- MACKINTOSH, N. J. 1998. *IQ and human intelligence.*, Oxford University Press.
- MACLULLICH, A. M., DEARY, I. J., STARR, J. M., FERGUSON, K. J., WARDLAW, J. M. & SECKL, J. R. 2005. Plasma cortisol levels, brain volumes and cognition in healthy elderly men. *Psychoneuroendocrinology*, 30, 505-15.

- MACLULLICH, A. M., FERGUSON, K. J., REID, L. M., DEARY, I. J., STARR, J. M., WARDLAW, J. M., WALKER, B. R., ANDREW, R. & SECKL, J. R. 2012. 11beta-hydroxysteroid dehydrogenase type 1, brain atrophy and cognitive decline. *Neurobiol Aging*, 33, 207 e1-8.
- MAGEE, P., PEARSON, S. & ALLEN, J. 2008. The omega-3 fatty acid, eicosapentaenoic acid (EPA), prevents the damaging effects of tumour necrosis factor (TNF)-alpha during murine skeletal muscle cell differentiation. *Lipids in Health and Disease*, 7.
- MAGGIO, M., LAURETANI, F. & CEDA, G. P. 2013. Sex hormones and sarcopenia in older persons. *Curr Opin Clin Nutr Metab Care*, 16, 3-13.
- MAGRI, F., GABELLIERI, E., BUSCONI, L., GUAZZONI, V., CRAVELLO, L., VALDES, V., SORRENTINO, A. R., CHYTIRIS, S. & FERRARI, E. 2006. Cardiovascular, anthropometric and neurocognitive features of healthy postmenopausal women: Effects of hormone replacement therapy. *Life Sciences*, 78 (22), 2625-2632.
- MALAGUARNERA, M., CAMMALLERI, L., GARGANTE, M. P., VACANTE, M., COLONNA, V. & MOTTA, M. 2007. L-Carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial. *American Journal of Clinical Nutrition*, 86, 1738-44.
- MANLY, J. J., TANG, M. X., SCHUPF, N., STERN, Y., VONSATTEL, J. P. & MAYEUX, R. 2008. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann Neurol*, 63, 494-506.
- MANOR, B., ABDULJALIL, A., NEWTON, E. & NOVAK, V. 2012. The relationship between brain volume and walking outcomes in older adults with and without diabetic peripheral neuropathy. *Diabetes Care*, 35, 1907-1912.
- MAREK, G. J., KATZ, D. A., MEIER, A., GRECO, N. T., ZHANG, W., LIU, W. & LENZ, R. A. 2014. Efficacy and safety evaluation of HSD-1 inhibitor ABT-384 in Alzheimer's disease. *Alzheimers Dement*.
- MARIONI, R. E., STEWART, M. C., MURRAY, G. D., DEARY, I. J., FOWKES, F. G., LOWE, G. D., RUMLEY, A. & PRICE, J. F. 2009. Peripheral levels of fibrinogen, C-reactive protein, and plasma viscosity predict future cognitive decline in individuals without dementia. *Psychosom Med*, 71, 901-6.
- MARQUIS, S., MOORE, M. M., HOWIESON, D. B., SEXTON, G., PAYAMI, H., KAYE, J. A. & CAMICIOLI, R. 2002. Independent predictors of cognitive decline in healthy elderly persons. *Archives of Neurology*, 59, 601-6.
- MARSLAND, A. L., PETERSEN, K. L., SATHANOORI, R., MULDOON, M. F., NEUMANN, S. A., RYAN, C., FLORY, J. D. & MANUCK, S. B. 2006. Interleukin-6 covaries inversely with cognitive performance among middle-aged community volunteers. *Psychosom Med*, 68, 895-903.
- MARTIN, J. C., FARRAR, R. P., WAGNER, B. M. & SPIRDUSO, W. W. 2000. Maximal power across the lifespan. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 55, M311-M316.
- MARZETTI, E., CALVANI, R., CESARI, M., BUFORD, T. W., LORENZI, M., BEHNKE, B. J. & LEEUWENBURGH, C. 2013. Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials. *Int J Biochem Cell Biol*, 45, 2288-301.
- MARZETTI, E., CARTER, C. S., WOHLGEMUTH, S. E., LEES, H. A., GIOVANNINI, S., ANDERSON, B., QUINN, L. S. & LEEUWENBURGH, C. 2009. Changes in IL-15 expression and death-receptor apoptotic signaling in rat gastrocnemius muscle with

- aging and life-long calorie restriction. *Mechanisms of Ageing and Development*, 130, 272-280.
- MASLEY, S. C., WEAVER, W., PERI, G. & PHILLIPS, S. E. 2008. Efficacy of lifestyle changes in modifying practical markers of wellness and aging. *Alternative Therapies in Health & Medicine*, 14, 24-29.
- MATSUSHITA, K., VAN DER VELDE, M., ASTOR, B. C., WOODWARD, M., LEVEY, A. S., DE JONG, P. E., CORESH, J. & GANSEVOORT, R. T. 2010. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*, 375, 2073-81.
- MATSUSHITA, T., KANDA, F., SUGIO, T., OKUDA, S. & CHIHARA, K. 1996. [Growth hormone prevents the steroid myopathy in rats]. *Rinsho Shinkeigaku*, 36, 752-6.
- MCCANN, J. C. & AMES, B. N. 2005. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am J Clin Nutr*, 82, 281-95.
- MCDANIEL, M. 2005. Big-brained people are smarter: A meta-analysis of the relationship between in vivo brain volume and intelligence. *Intelligence*, 33, 337-346.
- MCINTYRE, C. W., SELBY, N. M., SIGRIST, M., PEARCE, L. E., MERCER, T. H. & NAISH, P. F. 2006. Patients receiving maintenance dialysis have more severe functionally significant skeletal muscle wasting than patients with dialysis-independent chronic kidney disease. *Nephrology Dialysis Transplantation*, 21, 2210-6.
- MCKEATING, E. G., ANDREWS, P. J. & MASCIA, L. 1998. The relationship of soluble adhesion molecule concentrations in systemic and jugular venous serum to injury severity and outcome after traumatic brain injury. *Anesth Analg*, 86, 759-65.
- MEANEY, M. J., O'DONNELL, D., ROWE, W., TANNENBAUM, B., STEVERMAN, A., WALKER, M., NAIR, N. P. V. & LUPIEN, S. 1995. Individual-differences in hypothalamic-pituitary-adrenal activity in later life and hippocampal aging. *Experimental Gerontology*, 30, 229-251.
- MEDICAL RESEARCH COUNCIL 2009. Research changes lives. MRC strategic plan 2009–2014.
- MEDVEDEV, Z. A. 1990. An attempt at a rational classification of theories of ageing. *Biol Rev Camb Philos Soc*, 65, 375-98.
- MIDDLETON, L. E., MANINI, T. M., SIMONSICK, E. M., HARRIS, T. B., BARNES, D. E., TYLAVSKY, F., BRACH, J. S., EVERHART, J. E. & YAFFE, K. 2011. Activity energy expenditure and incident cognitive impairment in older adults. *Archives of Internal Medicine*, 171, 1251-7.
- MITCHELL, W. K., WILLIAMS, J., ATHERTON, P., LARVIN, M., LUND, J. & NARICI, M. 2012. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol*, 3, 260.
- MITNITSKI, A., SONG, X. & ROCKWOOD, K. 2013. Assessing biological aging: the origin of deficit accumulation. *Biogerontology*, 14, 709-17.
- MITSUI, T., AZUMA, H., NAGASAWA, M., IUCHI, T., AKAIKE, M., ODOMI, M. & MATSUMOTO, T. 2002. Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle. *Journal of Neurology*, 249, 1004-9.
- MIURA, Y., KATO, H. & NOGUCHI, T. 1992. Effect of dietary proteins on insulin-like growth factor-i (IGF-1) messenger-ribonucleic-acid content in rat-liver. *British Journal of Nutrition*, 67, 257-265.

- MOORE, A. Z., CATUREGLI, G., METTER, E. J., MAKROGIANNIS, S., RESNICK, S. M., HARRIS, T. B. & FERRUCCI, L. 2014. Difference in muscle quality over the adult life span and biological correlates in the baltimore longitudinal study of aging. *Journal of the American Geriatrics Society*, 62, 230-236.
- MOORHEAD, T. W. J., GOUNTOUNA, V.-E., JOB, D. E., MCINTOSH, A. M., ROMANIUK, L., LYMER, G. K. S., WHALLEY, H. C., WAITER, G. D., BRENNAN, D., AHEARN, T. S., CAVANAGH, J., CONDON, B., STEELE, J. D., WARDLAW, J. M. & LAWRIE, S. M. 2009. Prospective multi-centre Voxel Based Morphometry study employing scanner specific segmentations: procedure development using CaliBrain structural MRI data. *BMC Med Imaging*, 9, 8.
- MORALES, A. J., HAUBRICH, R. H., HWANG, J. Y., ASAKURA, H. & YEN, S. S. C. 1998. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. *Clinical Endocrinology*, 49, 421-432.
- MORAN, C., PHAN, T. G., CHEN, J., BLIZZARD, L., BEARE, R., VENN, A., MUNCH, G., WOOD, A. G., FORBES, J., GREENAWAY, T. M., PEARSON, S. & SRIKANTH, V. 2013. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care*, 36, 4036-42.
- MORI, E., HIRONO, N., YAMASHITA, H., IMAMURA, T., IKEJIRI, Y., IKEDA, M., KITAGAKI, H., SHIMOMURA, T. & YONEDA, Y. 1997. Premorbid brain size as a determinant of reserve capacity against intellectual decline in Alzheimer's disease. *Am J Psychiatry*, 154, 18-24.
- MORLEY, J. E. 2012. Sarcopenia in the elderly. *Fam Pract*, 29 Suppl 1, i44-i48.
- MORLEY, J. E., ABBATECOLA, A. M., ARGILES, J. M., BARACOS, V., BAUER, J., BHASIN, S., CEDERHOLM, T., COATS, A. J., CUMMINGS, S. R., EVANS, W. J., FEARON, K., FERRUCCI, L., FIELDING, R. A., GURALNIK, J. M., HARRIS, T. B., INUI, A., KALANTAR-ZADEH, K., KIRWAN, B. A., MANTOVANI, G., MUSCARITOLI, M., NEWMAN, A. B., ROSSI-FANELLI, F., ROSANO, G. M., ROUBENOFF, R., SCHAMBELAN, M., SOKOL, G. H., STORER, T. W., VELLAS, B., VON HAEHLING, S., YEH, S. S. & ANKER, S. D. 2011. Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc*, 12, 403-9.
- MORLEY, J. E., BAUMGARTNER, R. N., ROUBENOFF, R., MAYER, J. & NAIR, K. S. 2001. Sarcopenia. *Journal of Laboratory and Clinical Medicine*, 137, 231-243.
- MORRIS, M. C., EVANS, D. A., BIENIAS, J. L., TANGNEY, C. C., BENNETT, D. A., AGGARWAL, N., WILSON, R. S. & SCHERR, P. A. 2002. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease on a biracial community study. *Jama-Journal of the American Medical Association*, 287, 3230-3237.
- MORRIS, M. C., EVANS, D. A., TANGNEY, C. C., BIENIAS, J. L., WILSON, R. S., AGGARWAL, N. T. & SCHERR, P. A. 2005. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. *American Journal of Clinical Nutrition*, 81, 508-514.
- MORSE, C. I., THOM, J. M., DAVIS, M. G., FOX, K. R., BIRCH, K. M. & NARICI, M. V. 2004. Reduced plantarflexor specific torque in the elderly is associated with a lower activation capacity. *European Journal of Applied Physiology*, 92, 219-226.
- MOSCUFO, N., GUTTMANN, C. R. G., MEIER, D., CSAPO, I., HILDENBRAND, P. G., HEALY, B. C., SCHMIDT, J. A. & WOLFSON, L. 2011. Brain regional lesion burden and impaired mobility in the elderly. *Neurobiology of Aging*, 32, 646-54.

- MOSCUFO, N., WOLFSON, L., MEIER, D., LIGUORI, M., HILDENBRAND, P. G., WAKEFIELD, D., SCHMIDT, J. A., PEARLSON, G. D. & GUTTMANN, C. R. 2012. Mobility decline in the elderly relates to lesion accrual in the splenium of the corpus callosum. *Age*, 34, 405-14.
- MOSLEY, T. H., JR., KNOPMAN, D. S., CATELLIER, D. J., BRYAN, N., HUTCHINSON, R. G., GROTHUES, C. A., FOLSOM, A. R., COOPER, L. S., BURKE, G. L., LIAO, D. & SZKLO, M. 2005. Cerebral MRI findings and cognitive functioning: the Atherosclerosis Risk in Communities study. *Neurology*, 64, 2056-62.
- MUHLESTEIN, J. B., HORNE, B. D., CARLQUIST, J. F., MADSEN, T. E., BAIR, T. L., PEARSON, R. R. & ANDERSON, J. L. 2000. Cytomegalovirus Seropositivity and C-Reactive Protein Have Independent and Combined Predictive Value for Mortality in Patients With Angiographically Demonstrated Coronary Artery Disease.
- MURTON, A. J., CONSTANTIN, D. & GREENHAFF, P. L. 2008. The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy. *Biochimica Et Biophysica Acta-Molecular Basis of Disease*, 1782, 730-743.
- MUSCARITOLI, M., ANKER, S. D., ARGILES, J., AVERSA, Z., BAUER, J. M., BIOLO, G., BOIRIE, Y., BOSAEUS, I., CEDERHOLM, T., COSTELLI, P., FEARON, K. C., LAVIANO, A., MAGGIO, M., FANELLI, F. R., SCHNEIDER, S. M., SCHOLS, A. & SIEBER, C. C. 2010. Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". *Clinical Nutrition*, 29, 154-159.
- NADKARNI, N. K., LEVINE, B., MCILROY, W. E. & BLACK, S. E. 2012. Impact of subcortical hyperintensities on dual-tasking in Alzheimer disease and aging. *Alzheimer Disease & Associated Disorders*, 26, 28-35.
- NARICI, M. V. & MAFFULLI, N. 2010. Sarcopenia: characteristics, mechanisms and functional significance. *British Medical Bulletin*, 95, 139-159.
- NEISSER, U., BOODOO, G., BOUCHARD, T. J., BOYKIN, A. W., BRODY, N., CECI, S. J., HALPERN, D. F., LOEHLIN, J. C., PERLOFF, R., STERNBERG, R. J. & URBINA, S. 1996. Intelligence: Knowns and unknowns. *American Psychologist*, 51, 77-101.
- NELSON, H. & WILLISON, J. 1991. *National Adult Reading Test (NART) Test Manual (Part II)* Windsor, UK, NFER-Nelson.
- NEWMAN, A. B., KUPELIAN, V., VISSER, M., SIMONSICK, E., GOODPASTER, B., NEVITT, M., KRITCHEVSKY, S. B., TYLAVSKY, F. A., RUBIN, S. M., HARRIS, T. B. & HLTH, A. B. C. S. I. 2003. Sarcopenia: Alternative definitions and associations with lower extremity function. *Journal of the American Geriatrics Society*, 51, 1602-1609.
- NIH. 2014. *NIH Toolbox 4-Meter Walk Gait Speed Test* [Online]. Available: <http://www.nihtoolbox.org/WhatAndWhy/Motor/Locomotion/Pages/NIH-Toolbox-4-Meter-Walk-Gait-Speed-Test.aspx>.
- NILWIK, R., SNIJDERS, T., LEENDERS, M., GROEN, B. B., VAN KRANENBURG, J., VERDIJK, L. B. & VAN LOON, L. J. 2013. The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. *Exp Gerontol*, 48, 492-8.
- NOURHASHEMI, F., ANDRIEU, S., GILLETTE-GUYONNET, S., REYNISH, E., ALBAREDE, J. L., GRANDJEAN, H. & VELLAS, B. 2002. Is there a relationship between fat-free soft tissue mass and low cognitive function? Results from a study of 7,105 women. *J Am Geriatr Soc*, 50, 1796-801.

- NOURHASHEMI, F., ANDRIEU, S., GILLETTE-GUYONNET, S., VELLAS, B., ALBAREDE, J. L. & GRANDJEAN, H. 2001. Instrumental activities of daily living as a potential marker of frailty: a study of 7364 community-dwelling elderly women (the EPIDOS study). *Journals of Gerontology Series A-Biological Sciences & Medical Sciences*, 56, M448-53.
- NOVAK, V., HAERTLE, M., ZHAO, P., HU, K., MUNSHI, M., NOVAK, P., ABDULJALIL, A. & ALSOP, D. 2009. White matter hyperintensities and dynamics of postural control. *Magnetic Resonance Imaging*, 27 (6), 752-759.
- ODA, K. 1984. Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. *J Neurol Sci*, 66, 327-38.
- OGATA, S., KATO, K., HONDA, C. & HAYAKAWA, K. 2014. Common Genetic Factors Influence Hand Strength, Processing Speed, and Working Memory. *J Epidemiol*, 24, 31-8.
- OHKAWA, S., ODAMAKI, M., YONEYAMA, T., HIBI, I., MIYAJI, K. & KUMAGAI, H. 2000. Standardized thigh muscle area measured by computed axial tomography as an alternate muscle mass index for nutritional assessment of hemodialysis patients. *American Journal of Clinical Nutrition*, 71, 485-490.
- OVEREND, T. J., CUNNINGHAM, D. A., KRAMER, J. F., LEFCOE, M. S. & PATERSON, D. H. 1992a. Knee extensor and knee flexor strength - cross-sectional area ratios in young and elderly men. *Journals of Gerontology*, 47, M204-M210.
- OVEREND, T. J., CUNNINGHAM, D. A., PATERSON, D. H. & LEFCOE, M. S. 1992b. Thigh composition in young and elderly men determined by computed-tomography. *Clinical Physiology*, 12, 629-640.
- PAOLISSO, G., AMMENDOLA, S., DEL BUONO, A., GAMBARDELLA, A., RIONDINO, M., TAGLIAMONTE, M. R., RIZZO, M. R., CARELLA, C. & VARRICCHIO, M. 1997. Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function. *Journal of Clinical Endocrinology & Metabolism*, 82, 2204-9.
- PAPADAKIS, M. A., GRADY, D., BLACK, D., TIERNEY, M. J., GOODING, G. A., SCHAMBELAN, M. & GRUNFELD, C. 1996. Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann Intern Med*, 124, 708-16.
- PAPADAKIS, M. A., GRADY, D., TIERNEY, M. J., BLACK, D., WELLS, L. & GRUNFELD, C. 1995. Insulin-like growth factor 1 and functional status in healthy older men. *Journal of the American Geriatrics Society*, 43, 1350-5.
- PARK, D. C. & REUTER-LORENZ, P. 2009. The adaptive brain: aging and neurocognitive scaffolding. *Annu Rev Psychol*, 60, 173-96.
- PARK, S. K. & PROLLA, T. A. 2005. Lessons learned from gene expression profile studies of aging and caloric restriction. *Ageing Research Reviews*, 4 (1), 55-65.
- PAWELEC, G., AKBAR, A., BEVERLEY, P., CARUSO, C., DERHOVANESSIAN, E., FÜLÖP, T., GRIFFITHS, P., GRUBECK-LOEBENSTEIN, B., HAMPRECHT, K., JAHN, G., KERN, F., KOCH, S. D., LARBI, A., MAIER, A. B., MACALLAN, D., MOSS, P., SAMSON, S., STRINDHALL, J., TRANNOY, E. & WILLS, M. 2010. Immunosenescence and Cytomegalovirus: where do we stand after a decade? *Immunity & Ageing*, 7, 13.
- PAWELEC, G., KOCH, S., FRANCESCHI, C. & WIKBY, A. 2006. Human immunosenescence: does it have an infectious component? *Ann N Y Acad Sci*, 1067, 56-65.

- PAYTON, A. 2009. The impact of genetic research on our understanding of normal cognitive ageing: 1995 to 2009. *Neuropsychol Rev*, 19, 451-77.
- PAYTON, A., GIBBONS, L., DAVIDSON, Y., OLLIER, W., RABBITT, P., WORTHINGTON, J., PICKLES, A., PENDLETON, N. & HORAN, M. 2005. Influence of serotonin transporter gene polymorphisms on cognitive decline and cognitive abilities in a nondemented elderly population. *Mol Psychiatry*, 10, 1133-9.
- PEDERSEN, M., PEDERSEN, K. K., BRUUNSGAARD, H., KRABBE, K. S., THOMSEN, C., FAERCH, K., PEDERSEN, B. K. & MORTENSEN, E. L. 2012. Cognitive functions in middle aged individuals are related to metabolic disturbances and aerobic capacity: a cross-sectional study. *PLoS ONE [Electronic Resource]*, 7, e51132.
- PEETERS, G. M. E. E., VAN SCHOOR, N. M., VAN ROSSUM, E. F. C., VISSER, M. & LIPS, P. 2008. The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. *Clinical Endocrinology*, 69, 673-682.
- PETERSEN, R. C. 2004. Mild cognitive impairment as a diagnostic entity. *Journal of Internal Medicine*, 256, 183-194.
- PETERSEN, R. C., ROBERTS, R. O., KNOPMAN, D. S., BOEVE, B. F., GEDA, Y. E., IVNIK, R. J., SMITH, G. E. & JACK, C. R., JR. 2009. Mild cognitive impairment: ten years later. *Arch Neurol*. United States.
- PETERSEN, R. C., SMITH, G. E., WARING, S. C., IVNIK, R. J., TANGALOS, E. G. & KOKMEN, E. 1999. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol*, 56, 303-8.
- PFLEGER, C. M., WANG, J., FRIEDMAN, L., VITTORINO, R., CONLEY, L. M., HO, L., FIVECOAT, H. C. & PASINETTI, G. M. 2010. Grape-seed polyphenolic extract improves the eye phenotype in a Drosophila model of tauopathy. *Int J Alzheimers Dis*, 2010.
- PICKETT, K. E. & PEARL, M. 2001. Multilevel analyses of neighbourhood socioeconomic context and health outcomes: a critical review. *J Epidemiol Community Health*, 55, 111-22.
- PIGUET, O., CRAMSIE, J., BENNETT, H. P., KRIL, J. J., LYE, T. C., CORBETT, A. J., HAYES, M., CREASEY, H. & BROE, G. 2006. Contributions of age and alcohol consumption to cerebellar integrity, gait and cognition in non-demented very old individuals. *European Archives of Psychiatry and Clinical Neuroscience*, 256, 504-511.
- RANTANEN, T. 2003. Muscle strength, disability and mortality. *Scand J Med Sci Sports*, 13, 3-8.
- RANTANEN, T., GURALNIK, J. M., FOLEY, D., MASAKI, K., LEVEILLE, S., CURB, J. D. & WHITE, L. 1999. Midlife hand grip strength as a predictor of old age disability. *Jama*, 281, 558-60.
- RAPP, P. R. & AMARAL, D. G. 1992. Individual-differences in the cognitive and neurobiological consequences of normal aging. *Trends in Neurosciences*, 15, 340-345.
- RAZ, N. & RODRIGUE, K. M. 2006. Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neurosci Biobehav Rev*, 30, 730-48.
- REBUFFE-SCRIVE, M., KROTKIEWSKI, M., ELFVERSON, J. & BJORNTORP, P. 1988. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab*, 67, 1122-8.
- REZASOLTANI, A., YLINEN, J. & VIHKO, V. 2002. Isometric cervical extension force and dimensions of semispinalis capitis muscle. *J Rehabil Res Dev*, 39, 423-8.



- RICE, C. L., CUNNINGHAM, D. A., PATERSON, D. H. & LEFCOE, M. S. 1989. Arm and leg composition determined by computed-tomography in young and elderly men. *Clinical Physiology*, 9, 207-220.
- RICHARDS, M., HARDY, R., KUH, D. & WADSWORTH, M. E. 2002. Birthweight, postnatal growth and cognitive function in a national UK birth cohort. *Int J Epidemiol*, 31, 342-8.
- RICHETTI, S. K., BLANK, M., CAPIOTTI, K. M., PIATO, A. L., BOGO, M. R., VIANNA, M. R. & BONAN, C. D. 2011. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behav Brain Res*, 217, 10-5.
- RIDKER, P. M., BURING, J. E., COOK, N. R. & RIFAI, N. 2003. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation*, 107, 391-7.
- RIFAI, Z., WELLE, S., KAMP, C. & THORNTON, C. A. 1995. Ragged-red fibers in normal aging and inflammatory myopathy. *Annals of Neurology*, 37, 24-29.
- ROBBINS, N. & FAHIM, M. A. 1985. Progression of age-changes in mature mouse motor-nerve terminals and its relation to locomotor-activity. *Journal of Neurocytology*, 14, 1019-1036.
- ROBERTS, E. T., HAAN, M. N., DOWD, J. B. & AIELLO, A. E. 2010. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol*, 172, 363-71.
- ROCKWOOD, K. & MITNITSKI, A. 2007. Frailty in Relation to the Accumulation of Deficits. *J Gerontol A Biol Sci Med Sci*, 62, 722-727.
- ROLLAND, Y., CZERWINSKI, S., VAN KAN, G. A., MORLEY, J. E., CESARI, M., ONDER, G., WOO, J., BAUMGARTNER, R., PILLARD, F., BOIRIE, Y., CHUMLEA, W. M. C. & VELLAS, B. 2008. Sarcopenia: Its assessment, etiology, pathogenesis, consequences and future perspectives. *Journal of Nutrition Health & Aging*, 12, 433-450.
- ROSANO, C., BRACH, J., LONGSTRETH JR, W. T. & NEWMAN, A. B. 2006. Quantitative measures of gait characteristics indicate prevalence of underlying subclinical structural brain abnormalities in high-functioning older adults. *Neuroepidemiology*, 26 (1), 52-60.
- ROSANO, C., KULLER, L. H., CHUNG, H., ARNOLD, A. M., LONGSTRETH JR, W. T. & NEWMAN, A. B. 2005. Subclinical brain magnetic resonance imaging abnormalities predict physical functional decline in high-functioning older adults. *Journal of the American Geriatrics Society*, 53 (4), 649-654.
- ROSANO, C., LONGSTRETH JR, W. T., BOUDREAU, R., TAYLOR, C. A., DU, Y., KULLER, L. H. & NEWMAN, A. B. 2011. High blood pressure accelerates gait slowing in well-functioning older adults over 18-years of follow-up. *Journal of the American Geriatrics Society*, 59 (3), 390-397.
- ROSANO, C., NEWMAN, A. B., KATZ, R., HIRSCH, C. H. & KULLER, L. H. 2008. Association between lower digit symbol substitution test score and slower gait and greater risk of mortality and of developing incident disability in well-functioning older adults. *Journal of the American Geriatrics Society*, 56 (9), 1618-1625.
- ROSANO, C., SIGURDSSON, S., SIGGEIRSDOTTIR, K., PHILLIPS, C. L., GARCIA, M., JONSSON, P. V., EIRIKSDOTTIR, G., NEWMAN, A. B., HARRIS, T. B., VAN BUCHEM, M. A., GUDNASON, V. & LAUNER, L. J. 2010. Magnetization transfer imaging, white matter hyperintensities, brain atrophy and slower gait in older men and women. *Neurobiology of Aging*, 31 (7), 1197-1204.
- ROSANO, C., STUDENSKI, S. A., AIZENSTEIN, H. J., BOUDREAU, R. M., LONGSTRETH JR, W. T. & NEWMAN, A. B. 2012. Slower gait, slower

- information processing and smaller prefrontal area in older adults. *Age and Ageing*, 41, 58-64.
- ROTH, S. M., FERRELL, R. E., PETERS, D. G., METTER, E. J., HURLEY, B. F. & ROGERS, M. A. 2002. Influence of age, sex, and strength training on human muscle gene expression determined by microarray. *Physiol Genomics*, 10, 181-90.
- ROTH, S. M., SCHRAGER, M. A., FERRELL, R. E., RIECHMAN, S. E., METTER, E. J., LYNCH, N. A., LINDLE, R. S. & HURLEY, B. F. 2001. CNTF genotype is associated with muscular strength and quality in humans across the adult age span. *Journal of Applied Physiology*, 90, 1205-1210.
- ROTH, S. M., ZMUDA, J. M., CAULEY, J. A., SHEA, P. R. & FERRELL, R. E. 2004. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 59, 10-15.
- ROUBENOFF, R. 2000. Sarcopenia and its implications for the elderly. *European Journal of Clinical Nutrition*, 54, S40-S47.
- ROUBENOFF, R. 2001. Origins and clinical relevance of sarcopenia. *Can J Appl Physiol*, 26, 78-89.
- ROUBENOFF, R. & HUGHES, V. A. 2000. Sarcopenia: Current concepts. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 55, M716-M724.
- ROUBENOFF, R., PARISE, H., PAYETTE, H. A., ABAD, L. W., D'AGOSTINO, R., JACQUES, P. F., WILSON, P. W. F., DINARELLO, C. A. & HARRIS, T. B. 2003. Cytokines, insulin-like growth factor 1, sarcopenia, and mortality in very old community-dwelling men and women: The Framingham Heart Study. *American Journal of Medicine*, 115, 429-435.
- ROVIO, S., KAREHOLT, I., HELKALA, E. L., VIITANEN, M., WINBLAD, B., TUOMILEHTO, J., SOININEN, H., NISSINEN, A. & KIVIPELTO, M. 2005. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurology*, 4, 705-711.
- SACHDEV, P. S., ANSTEY, K. J., PARSLOW, R. A., WEN, W., MALLER, J., KUMAR, R., CHRISTENSEN, H. & JORM, A. F. 2006. Pulmonary function, cognitive impairment and brain atrophy in a middle-aged community sample. *Dementia and Geriatric Cognitive Disorders*, 21 (5-6), 300-308.
- SACHDEV, P. S., PARSLOW, R., WEN, W., ANSTEY, K. J. & EASTEAL, S. 2009. Sex differences in the causes and consequences of white matter hyperintensities. *Neurobiology of Aging*, 30 (6), 946-956.
- SACHDEV, P. S., WEN, W., CHRISTENSEN, H. & JORM, A. F. 2005. White matter hyperintensities are related to physical disability and poor motor function. *Journal of Neurology, Neurosurgery and Psychiatry*, 76 (3), 362-367.
- SACHECK, J. M., OHTSUKA, A., MCLARY, S. C. & GOLDBERG, A. L. 2004. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *American Journal of Physiology-Endocrinology and Metabolism*, 287, E591-E601.
- SAKUMA, K., AOI, W. & YAMAGUCHI, A. 2014. Current understanding of sarcopenia: possible candidates modulating muscle mass. *Pflugers Arch*.
- SALLIS, J. F. 2000. Age-related decline in physical activity: a synthesis of human and animal studies. *Med Sci Sports Exerc*, 32, 1598-600.
- SALTHOUSE, T. 2012. Consequences of age-related cognitive declines. *Annu Rev Psychol*, 63, 201-26.
- SALTHOUSE, T. A. 2006. Mental Exercise and Mental Aging Evaluating the Validity of the "Use It or Lose It" Hypothesis. *Perspectives on Psychological Science*, 1, 68-87.

- SALTHOUSE, T. A. 2009. When does age-related cognitive decline begin? *Neurobiol Aging*, United States.
- SAMY MODELAR, S., SEVESTRE, M. A., DE CAGNY, B. & SLAMA, M. 2008. Ultrasound evaluation of central veins in the intensive care unit: effects of dynamic manoeuvres. *Intensive Care Med*, 34, 333-8.
- SANDEEP, T. C., YAU, J. L. W., MACLULLICH, A. M. J., NOBLE, J., DEARY, I. J., WALKER, B. R. & SECKL, J. R. 2004. 11 beta-Hydroxysteroid dehydrogenase inhibition improves cognitive function in healthy elderly men and type 2 diabetics. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 6734-6739.
- SAPOLSKY, R. M. & PULSINELLI, W. A. 1985. Glucocorticoids potentiate ischemic-injury to neurons - therapeutic implications. *Science*, 229, 1397-1400.
- SATO, K., OGOH, S., HIRASAWA, A., OUE, A. & SADAMOTO, T. 2011. The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J Physiol*, 589, 2847-56.
- SATO, T., AKATSUKA, H., KITO, K., TOKORO, Y., TAUCHI, H. & KATO, K. 1984. Age-changes in size and number of muscle-fibers in human minor pectoral muscle. *Mechanisms of Ageing and Development*, 28, 99-109.
- SAYER, A. A., SYDDALL, H. E., MARTIN, H. J., DENNISON, E. M., ROBERTS, H. C. & COOPER, C. 2006. Is grip strength associated with health-related quality of life? Findings from the Hertfordshire Cohort Study. *Age Ageing*, 35, 409-15.
- SCHAAP, L. A., PLUIJM, S. M. F., DEEG, D. J. H. & VISSER, M. 2006. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *American Journal of Medicine*, 119.
- SCHAKMAN, O., GILSON, H. & THISSEN, J. 2008. Mechanisms of glucocorticoid induced myopathy. *Journal of Endocrinology*, 197, 1-10.
- SCHAKMAN, O., KALISTA, S., BARBE, C., LOUMAYE, A. & THISSEN, J. P. 2013. Glucocorticoid-induced skeletal muscle atrophy. *Int J Biochem Cell Biol*, 45, 2163-72.
- SCHERDER, E., EGGERMONT, L., SWAAB, D., VAN HEUVELEN, M., KAMSMA, Y., DE GREEF, M., VAN WIJCK, R. & MULDER, T. 2007. Gait in ageing and associated dementias; its relationship with cognition. *Neurosci Biobehav Rev*, 31, 485-97.
- SCHMALTZ, H. N., FRIED, L. P., XUE, Q. L., WALSTON, J., LENG, S. X. & SEMBA, R. D. 2005. Chronic cytomegalovirus infection and inflammation are associated with prevalent frailty in community-dwelling older women. *J Am Geriatr Soc*, 53, 747-54.
- SCHMIDT, R., ROPELE, S., ENZINGER, C., PETROVIC, K., SMITH, S., SCHMIDT, H., MATTHEWS, P. M. & FAZEKAS, F. 2005. White matter lesion progression, brain atrophy, and cognitive decline: the Austrian stroke prevention study. *Ann Neurol*, 58, 610-6.
- SCHRAGER, M. A., ROTH, S. M., FERRELL, R. E., METTER, E. J., RUSSEK-COHEN, E., LYNCH, N. A., LINDLE, R. S. & HURLEY, B. F. 2004. Insulin-like growth factor-2 genotype, fat-free mass, and muscle performance across the adult life span. *Journal of Applied Physiology*, 97, 2176-2183.
- SCHRAM, M. T., EUSER, S. M., DE CRAEN, A. J., WITTEMAN, J. C., FROLICH, M., HOFMAN, A., JOLLES, J., BRETILER, M. M. & WESTENDORP, R. G. 2007. Systemic markers of inflammation and cognitive decline in old age. *J Am Geriatr Soc*, 55, 708-16.

- SCHWARTZ, D. H., LEONARD, G., PERRON, M., RICHER, L., SYME, C., VEILLETTE, S., PAUSOVA, Z. & PAUS, T. 2013. Visceral fat is associated with lower executive functioning in adolescents. *International Journal of Obesity*, 37, 1336-1343.
- SECKL, J. R. & WALKER, B. R. 2001. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology*, 142, 1371-6.
- SEN, J. & BELLI, A. 2007. S100B in neuropathologic states: the CRP of the brain? *J Neurosci Res*, 85, 1373-80.
- SHAH, O., KIMBALL, S. & JEFFERSON, L. 2000. Acute attenuation of translation initiation and protein synthesis by glucocorticoids in skeletal muscle. *Am J Physiol Endocrinol Metab*, 278, E76-82.
- SHENKIN, S. D., BASTIN, M. E., MACGILLIVRAY, T. J., DEARY, I. J., STARR, J. M., RIVERS, C. S. & WARDLAW, J. M. 2005. Cognitive correlates of cerebral white matter lesions and water diffusion tensor parameters in community-dwelling older people. *Cerebrovasc Dis*, 20, 310-8.
- SHENKIN, S. D., BASTIN, M. E., MACGILLIVRAY, T. J., DEARY, I. J., STARR, J. M. & WARDLAW, J. M. 2009. Birth parameters are associated with late-life white matter integrity in community-dwelling older people. *Stroke*, 40, 1225-8.
- SHERWIN, B. B. 2006. Estrogen and cognitive aging in women. *Neuroscience*, 138, 1021-1026.
- SIGDEL, M., KUMAR, A., GYAWALI, P., SHRESTHA, R., TULADHAR, E. T. & JHA, B. 2014. Association of high sensitivity C-reactive protein with the components of metabolic syndrome in diabetic and non-diabetic individuals. *J Clin Diagn Res*, 8, Cc11-3.
- SIGURJONSDOTTIR, H. A., ANDREW, R., STIMSON, R. H., JOHANNSSON, G. & WALKER, B. R. 2009. Lack of regulation of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 during short-term manipulation of GH in patients with hypopituitarism. *Eur J Endocrinol*, 161, 375-80.
- SIH, R., MORLEY, J. E., KAISER, F. E., PERRY, H. M., 3RD, PATRICK, P. & ROSS, C. 1997. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J Clin Endocrinol Metab*, 82, 1661-7.
- SILBERT, L. C., NELSON, C., HOWIESON, D. B., MOORE, M. M. & KAYE, J. A. 2008. Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline. *Neurology*, 71 (2), 108-113.
- SINCLAIR, J. 2008. Human cytomegalovirus: Latency and reactivation in the myeloid lineage. *J Clin Virol*, 41, 180-5.
- SINGH, M. A. F., DING, W. J., MANFREDI, T. J., SOLARES, G. S., O'NEILL, E. F., CLEMENTS, K. M., RYAN, N. D., KEHAYIAS, J. J., FIELDING, R. A. & EVANS, W. J. 1999. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *American Journal of Physiology-Endocrinology and Metabolism*, 277, E135-E143.
- SKELTON, D. A., GREIG, C. A., DAVIES, J. M. & YOUNG, A. 1994. Strength, power and related functional ability of healthy people aged 65-89 years. *Age and Ageing*, 23, 371-377.
- SKIPWORTH, R. J. E., STEWART, G. D., ROSS, J. A., GUTTRIDGE, D. C. & FEARON, K. C. H. 2006. The molecular mechanisms of skeletal muscle wasting: Implications for therapy. *Surgeon-Journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*, 4, 273-283.

- SLOBEDMAN, B., CAO, J. Z., AVDIC, S., WEBSTER, B., MCALLERY, S., CHEUNG, A. K., TAN, J. C. & ABENDROTH, A. 2010. Human cytomegalovirus latent infection and associated viral gene expression. *Future Microbiol*, 5, 883-900.
- SMALL, S. A. 2001. Age-related memory decline - Current concepts and future directions. *Archives of Neurology*, 58, 360-364.
- SMITH, G. D., HART, C., WATT, G., HOLE, D. & HAWTHORNE, V. 1998. Individual social class, area-based deprivation, cardiovascular disease risk factors, and mortality: the Renfrew and Paisley Study. *J Epidemiol Community Health*, 52, 399-405.
- SODERBERG-NAUCLER, C. & NELSON, J. Y. 1999. Human cytomegalovirus latency and reactivation - a delicate balance between the virus and its host's immune system. *Intervirology*, 42, 314-21.
- SONNTAG, W. E., RAMSEY, M. & CARTER, C. S. 2005. Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. *Ageing Res Rev*, 4, 195-212.
- SOOY, K., WEBSTER, S. P., NOBLE, J., BINNIE, M., WALKER, B. R., SECKL, J. R. & YAU, J. L. 2010. Partial deficiency or short-term inhibition of 11beta-hydroxysteroid dehydrogenase type 1 improves cognitive function in ageing mice. *J Neurosci*, 30, 13867-72.
- SORENSEN, M. B., ROSENFALCK, A. M., HOJGAARD, L. & OTTESEN, B. 2001. Obesity and sarcopenia after menopause are reversed by sex hormone replacement therapy. *Obesity Research*, 9, 622-626.
- SORWELL, K. G. & URBANSKI, H. F. 2010. Dehydroepiandrosterone and age-related cognitive decline. *Age (Dordr)*, 32, 61-7.
- SOUMARE, A., ELBAZ, A., ZHU, Y., MAILLARD, P., CRIVELLO, F., TAVERNIER, B., DUFOUIL, C., MAZOYER, B. & TZOURIO, C. 2009. White matter lesions volume and motor performances in the elderly. *Annals of Neurology*, 65, 706-15.
- SPEAKMAN, J. & MITCHELL, S. 2011. Caloric restriction. *Molecular Aspects of Medicine*, 32, 159-221.
- SRIKANTH, V., BEARE, R., BLIZZARD, L., PHAN, T., STAPLETON, J., CHEN, J., CALLISAYA, M., MARTIN, K. & REUTENS, D. 2009. Cerebral white matter lesions, gait, and the risk of incident falls: a prospective population-based study. *Stroke*, 40, 175-80.
- SRIKANTH, V., PHAN, T. G., CHEN, J., BEARE, R., STAPLETON, J. M. & REUTENS, D. C. 2010. The location of white matter lesions and gait--A voxel-based study. *Annals of Neurology*, 67, 265-269.
- STAMPFER, M. J., KANG, J. H., CHEN, J., CHERRY, R. & GRODSTEIN, F. 2005. Effects of moderate alcohol consumption on cognitive function in women. *New England Journal of Medicine*, 352, 245-253.
- STARR, J., LEAPER, S., MURRAY, A., LEMMON, H., STAFF, R., DEARY, I. & WHALLEY, L. 2003. Brain white matter lesions detected by magnetic resonance imaging are associated with balance and gait speed. *Journal of Neurology, Neurosurgery & Psychiatry*, 74, 94-98.
- STARR, J. M., DEARY, I. J., INCH, S., CROSS, S. & MACLENNAN, I. J. 1997. Age-associated cognitive decline in healthy old people. *Age and Ageing*, 26, 295-300.
- STARR, J. M., SHIELS, P. G., HARRIS, S. E., PATTIE, A., PEARCE, M. S., RELTON, C. L. & DEARY, I. J. 2008. Oxidative stress, telomere length and biomarkers of physical aging in a cohort aged 79 years from the 1932 Scottish Mental Survey. *Mech Ageing Dev*, 129, 745-51.
- STARR, J. M., TAYLOR, M. D., HART, C. L., DAVEY SMITH, G., WHALLEY, L. J., HOLE, D. J., WILSON, V. & DEARY, I. J. 2004. Childhood mental ability and blood

- pressure at midlife: linking the Scottish Mental Survey 1932 and the Midspan studies. *J Hypertens*. England.
- STEPHENS, N. A., GALLAGHER, I. J., ROOYACKERS, O., SKIPWORTH, R. J., TAN, B. H., MARSTRAND, T., ROSS, J. A., GUTTRIDGE, D. C., LUNDELL, L., FEARON, K. C. & TIMMONS, J. A. 2010. Using transcriptomics to identify and validate novel biomarkers of human skeletal muscle cancer cachexia. *Genome medicine*, 2, 1.
- STEWART, P. M. & KROZOWSKI, Z. S. 1999. 11 beta-Hydroxysteroid dehydrogenase. *Vitam Horm*, 57, 249-324.
- STIMSON, R. H., ANDERSSON, J., ANDREW, R., REDHEAD, D. N., KARPE, F., HAYES, P. C., OLSSON, T. & WALKER, B. R. 2009. Cortisol release from adipose tissue by 11beta-hydroxysteroid dehydrogenase type 1 in humans. *Diabetes*, 58, 46-53.
- STITT, T. N., DRUJAN, D., CLARKE, B. A., PANARO, F., TIMOFEYVA, Y., KLINE, W. O., GONZALEZ, M., YANCOPOULOS, G. D. & GLASS, D. J. 2004. The IGF-1/PI3K/Akt pathway prevents short article expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Molecular Cell*, 14, 395-403.
- STOQUART-ELSANKARI, S., BALEDENT, O., GONDRY-JOUET, C., MAKKI, M., GODEFROY, O. & MEYER, M. E. 2007. Aging effects on cerebral blood and cerebrospinal fluid flows. *J Cereb Blood Flow Metab*, 27, 1563-72.
- STRANDBERG, T. E., PITKALA, K. H. & TILVIS, R. S. 2009. Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease. *Jama*, 301, 380-2.
- STRAUB, R. H., KONECNA, L., HRACH, S., ROTHE, G., KREUTZ, M., SCHOLMERICH, J., FALK, W. & LANG, B. 1998. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: Possible link between endocrinosenescence and immunosenescence. *Journal of Clinical Endocrinology & Metabolism*, 83, 2012-2017.
- SULLIVAN, E. V., DESHMUKH, A., DE ROSA, E., ROSENBLOOM, M. J. & PFEFFERBAUM, A. 2005. Striatal and forebrain nuclei volumes: Contribution to motor function and working memory deficits in alcoholism. *Biological Psychiatry*, 57 (7), 768-776.
- SUMUKADAS, D., BAND, M., MILLER, S., CVORO, V., WITHAM, M., STRUTHERS, A., MCCONNACHIE, A., LLOYD, S. M. & MCMURDO, M. 2014. Do ACE inhibitors improve the response to exercise training in functionally impaired older adults? A randomized controlled trial. *J Gerontol A Biol Sci Med Sci*, 69, 736-43.
- TAAFFE, D. R., HENWOOD, T. R., NALLS, M. A., WALKER, D. G., LANG, T. F. & HARRIS, T. B. 2009. Alterations in muscle attenuation following detraining and retraining in resistance-trained older adults. *Gerontology*, 55, 217-23.
- TAN, L. J., LIU, S. L., LEI, S. F., PAPASIAN, C. J. & DENG, H. W. 2012. Molecular genetic studies of gene identification for sarcopenia. *Hum Genet*, 131, 1-31.
- THE ACADEMY OF MEDICAL SCIENTISTS 2009. Rejuvenating Ageing Research.
- THOMSON, P. A., HARRIS, S. E., STARR, J. M., WHALLEY, L. J., PORTEOUS, D. J. & DEARY, I. J. 2005. Association between genotype at an exonic SNP in DISC1 and normal cognitive aging. *Neuroscience Letters*, 389, 41-45.
- THORNELL, L. E. 2011. Sarcopenic obesity: satellite cells in the aging muscle. *Curr Opin Clin Nutr Metab Care*, 14, 22-7.
- TIGANESCU, A., TAHRANI, A., MORGAN, S., OTRANTO, M., DESMOULIÈRE, A., ABRAHAMS, L., HASSAN-SMITH, Z., WALKER, E., RABBITT, E., COOPER,

- M., AMREIN, K., LAVERY, G. & STEWART, P. 2013. 11 $\beta$ -Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest.*, 123, 3051-60.
- TISDALE, M. J. 2002. Cachexia in cancer patients. *Nature Reviews Cancer*, 2, 862-871.
- TOMLINSON, B. E. & IRVING, D. 1977. The numbers of limb motor neurons in the human lumbosacral cord throughout life. *J Neurol Sci.* Netherlands.
- TOMLINSON, J. W., MOORE, J., COOPER, M. S., BUJALSKA, I., SHAHMANESH, M., BURT, C., STRAIN, A., HEWISON, M. & STEWART, P. M. 2001. Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology*, 142, 1982-9.
- TROMPET, S., DE CRAEN, A. J., SLAGBOOM, P., SHEPHERD, J., BLAUW, G. J., MURPHY, M. B., BOLLEN, E. L., BUCKLEY, B. M., FORD, I., GAW, A., MACFARLANE, P. W., PACKARD, C. J., STOTT, D. J., JUKEMA, J. W. & WESTENDORP, R. G. 2008. Genetic variation in the interleukin-1 beta-converting enzyme associates with cognitive function. The PROSPER study. *Brain*, 131, 1069-77.
- TROUNCE, I., BYRNE, E. & MARZUKI, S. 1989. Decline in skeletal-muscle mitochondrial respiratory-chain function - possible factor in aging. *Lancet*, 1, 637-639.
- TRUELSEN, T., THUDIUM, D. & GRONBAEK, M. 2002. Amount and type of alcohol and risk of dementia: the Copenhagen City Heart Study. *Neurology*, 59, 1313-9.
- TSUJINAKA, T., FUJITA, J., EBISUI, C., YANO, M., KOMINAMI, E., SUZUKI, K., TANAKA, K., KATSUME, A., OHSUGI, Y., SHIOZAKI, H. & MONDEN, M. 1996. Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. *Journal of Clinical Investigation*, 97, 244-249.
- TSUYAMA, K., YAMAMOTO, Y., FUJIMOTO, H., ADACHI, T., NAKAZATO, K. & NAKAJIMA, H. 2001. Comparison of the isometric cervical extension strength and a cross-sectional area of neck extensor muscles in college wrestlers and judo athletes. *European Journal of Applied Physiology*, 84, 487-491.
- TUCKER, K. L., QIAO, N., SCOTT, T., ROSENBERG, I. & SPIRO, A. 2005. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. *American Journal of Clinical Nutrition*, 82, 627-635.
- TURRELL, G., LYNCH, J. W., KAPLAN, G. A., EVERSON, S. A., HELKALA, E. L., KAUKANEN, J. & SALONEN, J. T. 2002. Socioeconomic position across the lifecourse and cognitive function in late middle age. *J Gerontol B Psychol Sci Soc Sci*, 57, S43-51.
- ULBRICH, E. J., ANDERSON, S. E., BUSATO, A., ABDERHALDEN, S., BOESCH, C., ZIMMERMANN, H., HEINI, P., HODLER, J. & STURZENEGGER, M. 2011. Cervical Muscle Area Measurements in Acute Whiplash Patients and Controls. *Journal of Magnetic Resonance Imaging*, 33, 668-675.
- UNITED NATIONS PROGRAMME ON AGEING & THE INTERNATIONAL ASSOCIATION OF GERONTOLOGY AND GERIATRICS 2007. Research Agenda on Ageing for the 21st Century.
- VALDUEZA, J. M., VON MUNSTER, T., HOFFMAN, O., SCHREIBER, S. & EINHAUPL, K. M. 2000. Postural dependency of the cerebral venous outflow. *Lancet*, 355, 200-1.
- VAN BOXTEL, M. P. J., BUNTINX, F., HOUX, P. J., METSEMAKERS, J. F. M., KNOTTNERUS, A. & JOLLES, J. 1998. The relation between morbidity and

- cognitive performance in a normal aging population. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 53, M147-M154.
- VAN DEN KOMMER, T. N., DIK, M. G., COMIJS, H. C., JONKER, C. & DEEG, D. J. 2010. Homocysteine and inflammation: predictors of cognitive decline in older persons? *Neurobiol Aging*, 31, 1700-9.
- VAN ROSSUM, E. F. C., VOORHOEVE, P. G., TE VELDE, S. J., KOPER, J. W., DELEMARRE-VAN DE WAAL, H. A., KEMPER, H. C. G. & LAMBERTS, S. W. J. 2004. The ER22/23EK Polymorphism in the Glucocorticoid Receptor Gene Is Associated with a Beneficial Body Composition and Muscle Strength in Young Adults. *Journal of Clinical Endocrinology & Metabolism*, 89, 4004-4009.
- VANDERVOORT, A. A. 2002. Aging of the human neuromuscular system. *Muscle & Nerve*, 25, 17-25.
- VIITASALO, J. T., ERA, P., LESKINEN, A. L. & HEIKKINEN, E. 1985. Muscular strength profiles and anthropometry in random samples of men aged 31-35, 51-55 and 71-75 years. *Ergonomics*, 28, 1563-1574.
- VINA, J., BORRAS, C. & MIQUEL, J. 2007. Theories of ageing. *IUBMB Life*, 59, 249-54.
- VISSCHER, P. M., TYNAN, M., WHITEMAN, M. C., PATTIE, A., WHITE, I., HAYWARD, C., WRIGHT, A. F., STARR, J. M., WHALLEY, L. J. & DEARY, I. J. 2003. Lack of association between polymorphisms in angiotensin-converting-enzyme and methylenetetrahydrofolate reductase genes and normal cognitive ageing in humans. *Neurosci Lett*, 347, 175-8.
- VISSER, M., DEEG, D. J. H. & LIPS, P. 2003. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (Sarcopenia): The Longitudinal Aging Study Amsterdam. *Journal of Clinical Endocrinology & Metabolism*, 88, 5766-5772.
- VISSER, M., PAHOR, M., TAAFFE, D. R., GOODPASTER, B. H., SIMONSICK, E. M., NEWMAN, A. B., NEVITT, M. & HARRIS, T. B. 2002. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: The health ABC study. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 57, M326-M332.
- VOLKOW, N. D., WANG, G. J., FOWLER, J. S., LOGAN, J., GATLEY, S. J., MACGREGOR, R. R., SCHLYER, D. J., HITZEMANN, R. & WOLF, A. P. 1996. Measuring age-related changes in dopamine D2 receptors with 11C-raclopride and 18F-N-methylspiroperidol. *Psychiatry Res*, 67, 11-6.
- VOLPI, E., FERRANDO, A. A., YECKEL, C. W., TIPTON, K. D. & WOLFE, R. R. 1998. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *Journal of Clinical Investigation*, 101, 2000-2007.
- VOLPI, E. & RASMUSSEN, B. B. 2000. Nutrition and muscle protein metabolism in the elderly. *Diabetes Nutrition & Metabolism*, 13, 99-107.
- VOLPI, E., SHEFFIELD-MOORE, M., RASMUSSEN, B. B. & WOLFE, R. R. 2001. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *Jama-Journal of the American Medical Association*, 286, 1206-1212.
- WAKE, D. J., RASK, E., LIVINGSTONE, D. E. W., SODERBERG, S., OLSSON, T. & WALKER, B. R. 2003. Local and systemic impact of transcriptional up-regulation of 11 beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. *Journal of Clinical Endocrinology & Metabolism*, 88, 3983-3988.
- WANG, C., SWERDLOFF, R. S., IRANMANESH, A., DOBS, A., SNYDER, P. J., CUNNINGHAM, G., MATSUMOTO, A. M., WEBER, T. & BERMAN, N. 2000. Transdermal testosterone gel improves sexual function, mood, muscle strength, and



- body composition parameters in hypogonadal men. *J Clin Endocrinol Metab*, 85, 2839-53.
- WANG, G. C., KAO, W. H., MURAKAMI, P., XUE, Q. L., CHIOU, R. B., DETRICK, B., MCDYER, J. F., SEMBA, R. D., CASOLARO, V., WALSTON, J. D. & FRIED, L. P. 2010. Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. *Am J Epidemiol*, 171, 1144-52.
- WARDLAW, J., BASTIN, M., VALDÉS HERNÁNDEZ, M., MUÑOZ MANIEGA, S., ROYLE, N., MORRIS, Z., CLAYDEN, J., SANDEMAN, E., EADIE, E., MURRAY, C., STARR, J. & DEARY, I. 2011. Brain ageing, cognition in youth and old age, and vascular disease in the Lothian Birth Cohort 1936: rationale, design and methodology of the imaging protocol. *International Journal of Stroke*.
- WATERS, D. L., QUALLS, C. R., DORIN, R. I., VELDHUIS, J. D. & BAUMGARTNER, R. N. 2008. Altered growth hormone, cortisol, and leptin secretion in healthy elderly persons with sarcopenia and mixed body composition phenotypes. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences*, 63, 536-541.
- WATTS, A. S., VIDONI, E. D., LOSKUTOVA, N., JOHNSON, D. K. & BURNS, J. M. 2013. Measuring Physical Activity in Older Adults With and Without Early Stage Alzheimer's Disease. *Clinical Gerontologist*, 36, 356-374.
- WEAVER, J. D., HUANG, M. H., ALBERT, M., HARRIS, T., ROWE, J. W. & SEEMAN, T. E. 2002. Interleukin-6 and risk of cognitive decline - MacArthur studies of successful aging. *Neurology*, 59, 371-378.
- WEAVER, J. U., THAVENTHIRAN, L., NOONAN, K., BURRIN, J. M., TAYLOR, N. F., NORMAN, M. R. & MONSON, J. P. 1994. The effect of growth-hormone replacement on cortisol metabolism and glucocorticoid sensitivity in hypopituitary adults. *Clinical Endocrinology*, 41, 639-648.
- WEBER, M. M., MICHL, P., AUERNHAMMER, C. J. & ENGELHARDT, D. 1997. Interleukin-3 and interleukin-6 stimulate cortisol secretion from adult human adrenocortical cells. *Endocrinology*, 138, 2207-2210.
- WECHSLER, D. 1998a. *WAIS-IIIUK administration and scoring manual*, London, UK, Psychological Corporation.
- WECHSLER, D. 1998b. *WMS-IIIUK administration and scoring manual*, London, UK, Psychological Corporation.
- WEINDRUCH, R. 1995. Interventions based on the possibility that oxidative stress contributes to sarcopenia. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences*, 50, 157-161.
- WEISE, C. M., THIYYAGURA, P., REIMAN, E. M., CHEN, K. & KRAKOFF, J. 2013. Fat-free body mass but not fat mass is associated with reduced gray matter volume of cortical brain regions implicated in autonomic and homeostatic regulation. *NeuroImage*, 64, 712-721.
- WELLE, S. 1995. Myofibrillar protein-synthesis in young and old human-subjects after 3 months of resistance training. *American Journal of Physiology-Endocrinology and Metabolism*, 268, E422-E427.
- WELLE, S. 2002. Cellular and molecular basis of age-related sarcopenia. *Canadian Journal of Applied Physiology-Revue Canadienne De Physiologie Appliquee*, 27, 19-41.
- WELLE, S., BHATT, K. & THORNTON, C. A. 2000. High-abundance mRNAs in human muscle: comparison between young and old. *Journal of Applied Physiology*, 89, 297-304.
- WELLE, S., BROOKS, A. & THORNTON, C. A. 2001. Senescence-related changes in gene expression in muscle: similarities and differences between mice and men. *Physiological Genomics*, 5, 67-73.

- WELLE, S., THORNTON, C., STATT, M. & MCHENRY, B. 1996. Growth hormone increases muscle mass and strength but does not rejuvenate myofibrillar protein synthesis in healthy subjects over 60 years old. *The Journal of Clinical Endocrinology & Metabolism*.
- WELLE, S. & THORNTON, C. A. 1998. High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. *Am J Physiol*, 274, E677-83.
- WEN, W., SACHDEV, P. S., CHEN, X. & ANSTEY, K. 2006. Gray matter reduction is correlated with white matter hyperintensity volume: a voxel-based morphometric study in a large epidemiological sample. *Neuroimage*, 29, 1031-9.
- WETMORE, J. B., HONEA, R. A., VIDONI, E. D., ALMEHMI, A. & BURNS, J. M. 2011. Role of lean body mass in estimating glomerular filtration rate in Alzheimer disease. *Nephrology Dialysis Transplantation*, 26, 2222-31.
- WEUVE, J., KANG, J. H., MANSON, J. E., BRETELER, M. M., WARE, J. H. & GRODSTEIN, F. 2004. Physical activity, including walking, and cognitive function in older women. *JAMA*, 292, 1454-61.
- WEUVE, J., RIDKER, P. M., COOK, N. R., BURING, J. E. & GRODSTEIN, F. 2006. High-sensitivity C-reactive protein and cognitive function in older women. *Epidemiology*, 17, 183-189.
- WHALLEY, L. J., DEARY, I. J., STARR, J. M., WAHLE, K. W., RANCE, K. A., BOURNE, V. J. & FOX, H. C. 2008. n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. *Am J Clin Nutr*, 87, 449-54.
- WHORWOOD, C. B., DONOVAN, S. J., WOOD, P. J. & PHILLIPS, D. I. 2001. Regulation of glucocorticoid receptor alpha and beta isoforms and type I 11beta-hydroxysteroid dehydrogenase expression in human skeletal muscle cells: a key role in the pathogenesis of insulin resistance? *Journal of Clinical Endocrinology & Metabolism*, 86, 2296-308.
- WILSON, R., BIENIAS, J., EVANS, D. & BENNETT, D. 2004. Religious Orders Study: Overview and Change in Cognitive and Motor Speed. *Aging Neuropsychol Cogn*.
- WIRTH, R., SMOLINER, C., SIEBER, C. & VOLKERT, D. 2011. Cognitive function is associated with body composition and nutritional risk cognitive function is associated with body composition and nutritional risk of geriatric patients. *Journal of Nutrition, Health & Aging*, 15, 706-710.
- WISDOM, N. M., CALLAHAN, J. L. & HAWKINS, K. A. 2011. The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. *Neurobiol Aging*, 32, 63-74.
- WITHAM, M. D., SUMUKADAS, D. & MCMURDO, M. E. 2008. ACE inhibitors for sarcopenia--as good as exercise training? *Age Ageing*, 37, 363-5.
- WITHAM, M. D., SYDDALL, H. E., DENNISON, E., COOPER, C., MCMURDO, M. E. & SAYER, A. A. 2014. ACE inhibitors, statins and thiazides: no association with change in grip strength among community dwelling older men and women from the Hertfordshire Cohort Study. *Age Ageing*, 43, 661-6.
- WOLFSON, L., WEI, X., HALL, C. B., PANZER, V., WAKEFIELD, D., BENSON, R. R., SCHMIDT, J. A., WARFIELD, S. K. & GUTTMANN, C. R. G. 2005. Accrual of MRI white matter abnormalities in elderly with normal and impaired mobility. *Journal of the Neurological Sciences*, 232, 23-7.
- YAFFE, K., BARNES, D., NEVITT, M., LUI, L. Y. & COVINSKY, K. 2001. A prospective study of physical activity and cognitive decline in elderly women - Women who walk. *Archives of Internal Medicine*, 161, 1703-1708.

- YAFFE, K., KANAYA, A., LINDQUIST, K., SIMONSICK, E. M., HARRIS, T., SHORR, R. I., TYLAVSKY, F. A. & NEWMAN, A. B. 2004. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA*, 292, 2237-42.
- YAFFE, K., LINDQUIST, K., PENNINX, B. W., SIMONSICK, E. M., PAHOR, M., KRITCHEVSKY, S., LAUNER, L., KULLER, L., RUBIN, S. & HARRIS, T. 2003. Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology*, 61, 76-80.
- YARASHESKI, K. E., BHASIN, S., SINHA-HIKIM, I., PAK-LODUCA, J. & GONZALEZ-CADAVID, N. F. 2002. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *J Nutr Health Aging*, 6, 343-8.
- YARASHESKI, K. E., ZACHWIEJA, J. J. & BIER, D. M. 1993. Acute effects of resistance exercise on muscle protein-synthesis rate in young and elderly men and women. *American Journal of Physiology*, 265, E210-E214.
- YAU, J. L. & SECKL, J. R. 2012. Local amplification of glucocorticoids in the aging brain and impaired spatial memory. *Front Aging Neurosci*, 4, 24.
- YAU, J. L. W., NOBLE, J., KENYON, C. J., HIBBERD, C., KOTELEVTSSEV, Y., MULLINS, J. J. & SECKL, J. R. 2001. Lack of tissue glucocorticoid reactivation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. *Proc Natl Acad Sci*, 98, 4716-21.
- YLIHARSILA, H., KAJANTIE, E., OSMOND, C., FORSEN, T., BARKER, D. J. P. & ERIKSSON, J. G. 2007. Birth size, adult body composition and muscle strength in later life. *International Journal of Obesity*, 31, 1392-1399.
- YOUNG, A., STOKES, M. & CROWE, M. 1984. Size and strength of the quadriceps muscles of old and young women. *European Journal of Clinical Investigation*, 14, 282-287.
- YOUNG, A., STOKES, M. & CROWE, M. 1985. The size and strength of the quadriceps muscles of old and young men. *Clinical Physiology*, 5, 145-154.
- ZAMMIT, S., ALLEBECK, P., DAVID, A. S., DALMAN, C., HEMMINGSSON, T., LUNDBERG, I. & LEWIS, G. 2004. A longitudinal study of premorbid IQ score and risk of developing schizophrenia, bipolar disorder, severe depression, and other nonaffective psychoses. *Archives of General Psychiatry*, 61, 354-360.

# Appendices

## Appendix 1: Data extraction sheet used in the systematic review

|              |
|--------------|
| Study number |
|--------------|

### General information

|                                     |  |
|-------------------------------------|--|
| Date of extraction                  |  |
| Study title                         |  |
| Study authors                       |  |
| The journal and date of publication |  |
| Country of origin and language      |  |
| Source of funding                   |  |

### Study characteristics

|   |  |
|---|--|
| Aim/objectives of the study   |  |
| Study design  |  |
| Study inclusion and exclusion criteria                                |  |
| Recruitment procedures used (e.g. details of randomisation, blinding) |  |
| Part of larger study/trial?   |  |

### Participant characteristics

|  |  |
|--|--|
| n  |  |
| Age                                      |  |
| Gender                                   |  |
| Ethnicity                                |  |
| Socio-economic status                    |  |
| Disease characteristics if control group |  |
| Co-morbidities                           |  |

### Setting

|  |  |
|--|--|
| Where the patients were recruited from                                 |  |
| Where they underwent the testing (eg GP practice, research centre etc) |  |

**Outcome data/results**

|                         |  |  |
|-------------------------|--|--|
| <b>Brain structure</b>  | Whether reported   |  |
|                         | Definition used in study   |  |
|                         | Measurement tool or method used and unit of measurement            |  |
|                         | Length of follow-up, number and/or times of follow-up measurements |  |
| <b>Brain function</b>   | Whether reported   |  |
|                         | Definition used in study   |  |
|                         | Measurement tool or method used and unit of measurement            |  |
|                         | Length of follow-up, number and/or times of follow-up measurements |  |
| <b>Muscle structure</b> | Whether reported   |  |
|                         | Definition used in study   |  |
|                         | Measurement tool or method used and unit of measurement            |  |
|                         | Length of follow-up, number and/or times of follow-up measurements |  |
| <b>Muscle function</b>  | Whether reported   |  |
|                         | Definition used in study   |  |
|                         | Measurement tool or method used and unit of measurement            |  |
|                         | Length of follow-up, number and/or times of follow-up measurements |  |

**Outcomes in paper**

|   |  |
|---|--|
| Primary and secondary outcomes re: muscle/brain association |  |
| Correlations and associations of muscle and brain           |  |
| Additional outcomes   |  |
| Statistical techniques used                                 |  |

**Outcomes from enquiry**

|   |            |
|---|------------|
| Study written to                                  | Yes/No     |
| Replied   | Yes/No     |
| Gave associations/correlations                    | Yes/No/n/a |
| Gave data   | Yes/No/n/a |
| If no reason                                      |            |
| Correlations and associations of muscle and brain |            |
| Statistical techniques used                       |            |

## Appendix 2: Publications arising from the work contained in this PhD

1. Design and validation of a novel method to measure cross-sectional area of neck muscles included during routine MR brain volume imaging.  
*Kilgour AH, Subedi D, Gray CD, Deary IJ, Lawrie SM, Wardlaw JM, Starr JM.*  
PLoS One. 2012;7(4):e34444.
2. Neck muscle cross-sectional area, brain volume and cognition in healthy older men: a cohort study.  
*Kilgour AH, Ferguson KJ, Gray CD, Deary IJ, Wardlaw JM, MacLulich AM, Starr JM.*  
BMC Geriatrics. 2013 Feb 28;13:20.
3. Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly.  
*Kilgour AH, Firth C, Harrison R, Moss P, Bastin ME, Wardlaw JM, Deary IJ, Starr JM.*  
Immunity and Ageing. 2013 Aug 13;10(1):33.
4. Increased skeletal muscle 11 $\beta$ HSD1 mRNA is associated with lower muscle strength in ageing.  
*Kilgour AH, Gallagher IJ, MacLulich AM, Andrew R, Gray CD, Hyde P, Wackerhage H, Husi H, Ross JA, Starr JM, Chapman KE, Fearon KC, Walker BR, Greig CA.*  
PLoS One. 2013 Dec 31;8(12):e84057.
5. A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse.  
*Kilgour AH, Todd OM, Starr JM.*  
BMC Geriatr. 2014 Jul 10;14:85.
6. 11beta-Hydroxysteroid dehydrogenase activity in brain does not contribute to systemic interconversion of cortisol and cortisone in healthy men.  
*Kilgour AH, Semple S, Marshall I, Andrews P, Andrew R, Walker BR.*  
JCEM. 2015 Feb;100(2):483-489. Epub 2014 Nov 13.



# Design and Validation of a Novel Method to Measure Cross-Sectional Area of Neck Muscles Included during Routine MR Brain Volume Imaging

Alixé H. M. Kilgour<sup>1\*</sup>, Deepak Subedi<sup>2</sup>, Calum D. Gray<sup>3,4</sup>, Ian J. Deary<sup>1</sup>, Stephen M. Lawrie<sup>4,5</sup>, Joanna M. Wardlaw<sup>1,2,4</sup>, John M. Starr<sup>1</sup>

**1** Geriatric Medicine Unit, Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, United Kingdom, **2** Clinical Neurosciences, Brain Research Imaging Centre, University of Edinburgh, Edinburgh, United Kingdom, **3** Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom, **4** Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE), Edinburgh, United Kingdom, **5** Division of Psychiatry, University of Edinburgh, Edinburgh, United Kingdom

## Abstract

**Introduction:** Low muscle mass secondary to disease and ageing is an important cause of excess mortality and morbidity. Many studies include a MR brain scan but no peripheral measure of muscle mass. We developed a technique to measure posterior neck muscle cross-sectional area (CSA) on volumetric MR brain scans enabling brain and muscle size to be measured simultaneously.

**Methods:** We performed four studies to develop and test: feasibility, inter-rater reliability, repeatability and external validity. We used T1-weighted MR brain imaging from young and older subjects, obtained on different scanners, and collected mid-thigh MR data.

**Results:** After developing the technique and demonstrating feasibility, we tested it for inter-rater reliability in 40 subjects. Intraclass correlation coefficients (ICC) between raters were 0.99 (95% confidence intervals (CI) 0.98–1.00) for the combined group (trapezius, splenius and semispinalis), 0.92 (CI 0.85–0.96) for obliquus and 0.92 (CI 0.85–0.96) for sternocleidomastoid. The first unrotated principal component explained 72.2% of total neck muscle CSA variance and correlated positively with both right ( $r = 0.52$ ,  $p = .001$ ) and left ( $r = 0.50$ ,  $p = .002$ ) grip strength. The 14 subjects in the repeatability study had had two MR brain scans on three different scanners. The ICC for between scanner variation for total neck muscle CSA was high at 0.94 (CI 0.86–0.98). The ICCs for within scanner variations were also high, with values of 0.95 (CI 0.86–0.98), 0.97 (CI 0.92–0.99) and 0.96 (CI 0.86–0.99) for the three scanners. The external validity study found a correlation coefficient for total thigh CSA and total neck CSA of 0.88.

**Discussion:** We present a feasible, valid and reliable method for measuring neck muscle CSA on T1-weighted MR brain scans. Larger studies are needed to validate and apply our technique with subjects differing in age, ethnicity and geographical location.

**Citation:** Kilgour AHM, Subedi D, Gray CD, Deary IJ, Lawrie SM, et al. (2012) Design and Validation of a Novel Method to Measure Cross-Sectional Area of Neck Muscles Included during Routine MR Brain Volume Imaging. PLoS ONE 7(4): e34444. doi:10.1371/journal.pone.0034444

**Editor:** Yong Fan, Institution of Automation, CAS, China

**Received:** August 23, 2011; **Accepted:** March 5, 2012; **Published:** April 3, 2012

**Copyright:** © 2012 Kilgour et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The LBC1936 data were collected by a Research Into Ageing programme grant; this research continues as part of the Age UK-funded (formerly Help the Aged) Disconnected Mind project. The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC), Economic and Social Research Council (ESRC) and Medical Research Council (MRC) is gratefully acknowledged. This funding fully supported A. Kilgour during this research. The CaliBrain study was funded by a Chief Scientist Office (Scotland) Project Grant (CZB/4/427). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: ahmkilgour@doctors.org.uk

## Introduction

Low muscle mass secondary to disease and ageing is an important cause of excess mortality and morbidity [1–4]. Studies investigating correlates of muscle loss or potential interventions to slow or reverse muscle loss require accurate measurements of muscle size. Current imaging techniques used to measure muscle size include whole body or regional DEXA scans and volumetric or cross-sectional area measurements on MR or CT scans of the arm or leg [5]. Arm and thigh cross-sectional area (CSA) have

been used in previous studies as they are large and are viewed to be used in everyday tasks. However thigh muscle CSA has been shown to correlate well with total muscle mass and it may be that other muscle groups around the body are equally useful as a guide of general muscle bulk [6,7]. Whilst the above techniques remain the current gold standard, they are not commonly employed in clinical practice or in studies out with those directly investigating muscle mass (eg studies of sarcopenia or cachexia). Volumetric MR brain scans are commonly used in both research and clinical

practice. These scans often include much of the posterior neck muscles. A technique to measure posterior neck muscle CSA on volumetric MR brain scans would therefore enhance the value of volumetric MR brain scans: both brain and muscle size could be measured without additional scanning.

Recent studies have shown a correlation between grip strength and cognition, which has implications for studying rates of ageing [8,9]. However, few studies have investigated the relationship between muscle size and brain size. This is likely due in part to the fact that two different scans would be required in each subject to obtain these data. Both brain and muscle size are known to decrease with age, therefore studying the pattern of their inter-relationship would allow investigation of their shared risk factors which, in turn, may suggest underlying mechanisms. Many longitudinal aging studies include a volumetric MR brain scan [10–12]. If it were possible to measure muscle CSA reliably from volumetric MR brain scans and this measure was representative of general body muscle bulk, the relationship between muscle and brain size could be investigated using a single scan.

MR measurement of neck muscle cross sectional area (CSA) has been shown to be feasible in young healthy adults using scans dedicated to this purpose (ie MR Imaging of the neck), but older adults have not been studied [13–15]. These studies have demonstrated good inter-rater reliability [14,15]. Moreover, we found no previous studies documenting a technique to measure neck muscle size on MR brain scans. The limited data that are available suggest that neck muscle CSA and strength are correlated, indicating that neck muscle CSA has good construct validity [16]. We aimed to establish a novel method for measuring neck muscle CSAs from routine MR brain volume acquisitions. This paper details the technique we developed and further studies to test its reliability, validity and repeatability.

## Methods

### Study 1: Feasibility study

**Goal.** To investigate whether it is feasible to measure neck muscle CSA on MRI volumetric brain scans.

**Ethics & Sample.** The volumetric MR brain scans used in this study had already been performed as part of the Lothian Birth Cohort 1936 (LBC) study, as a primary outcome for that study was brain volume measurement. Ethics permission for the LBC1936 study protocol was obtained from the Multi-Centre Research Ethics Committee for Scotland (MREC/01/0/56) and from the Lothian Research Ethics Committee (LREC/2003/2/29) and covers this sub-study because the ethics approval included the use of the data for future research purposes. The research was carried out in compliance with the Helsinki Declaration. All participants gave written, informed consent. Twenty consecutive scans from a final total of 735 were selected between 02/02/09 and 30/03/09. Participants were community-resident, all born in 1936 and without any known major musculoskeletal disease. Height, weight and grip strength in both hands were measured by trained research nurses at a clinical research facility [11].

**Imaging Protocol.** The MR imaging was performed with participants in the supine position on a 1.5 tesla MR imaging unit (Signa HDxt, GE Healthcare, Milwaukee, USA) at the Brain Research Imaging Centre ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)). A phased array eight channel head coil was used and inversion recovery prepared volumetric T1 weighted images were acquired on a coronal plane for each patient. For this set of images, the alignment was perpendicular to the long axis of the hippocampus determined from a preliminary T2 weighted sagittal sequence. The flip angle was 8°, bandwidth 15.63 KHz, echo time (TE) 4 ms minimum to

13 ms maximum, repetition time (TR) 9.6 ms and inversion or preparation time (TI) 500 ms. The field of view (FOV), fixed superiorly at the cranial vertex, was 25.6 cm×25.6 cm, slice thickness 1.3 mm with no slice gap leading to 160 slices, displayed on a 192×192 matrix. These images took 8.13 minutes to acquire per patient. Full details of imaging protocol [17].

**Development of neck muscle cross sectional area measurement technique.** The image data were transferred to a Kodak Carestream picture archiving and communication systems (PACS) workstation where 3-D multiplanar reconstructions were performed. Freehand cursor was used to draw a region of interest (ROI) around the neck muscle of interest in the axial plane to obtain the cross sectional area on each side separately.

Two raters tested feasibility in ten of the participants. We sought to develop a technique that ensured raters found the same level from which to make their CSA measurements. Our first attempt involved finding the MR slice in which the CSA of the obliquus capitis inferior was at its maximum in the axial plane. We chose the obliquus capitis inferior because it is a short muscle and its width varies more along its length than the other neck muscles in the scan. We then measured the CSAs for the largest muscles in that slice of the scan; sternocleidomastoid (SCM), obliquus capitis inferior, semispinalis capitis, splenius capitis and trapezius for both right and left sides (Figure 1). Although it was possible to measure the CSA of neck muscles using this technique, there were occasional large discrepancies between raters indicating that this method lacked reliability, particularly with regard to finding the level to take the measurements.

Therefore, in the second attempt, we measured the neck muscles' CSAs of a further ten participants, but this time we started with the images in the sagittal view with the volume images loaded into the multiplanar reformat view. We chose the slice where the C2 vertebral body height was at its maximum. We then identified the midpoint of the C2 vertebral body height including the odontoid by measuring along its vertical length from the odontoid tip to lower end plate using the cursor and then marked the midpoint. We then switched to the axial view of the multiplanar reformat at the vertical midpoint of C2 and measured the CSAs of the neck muscles in that axial image.

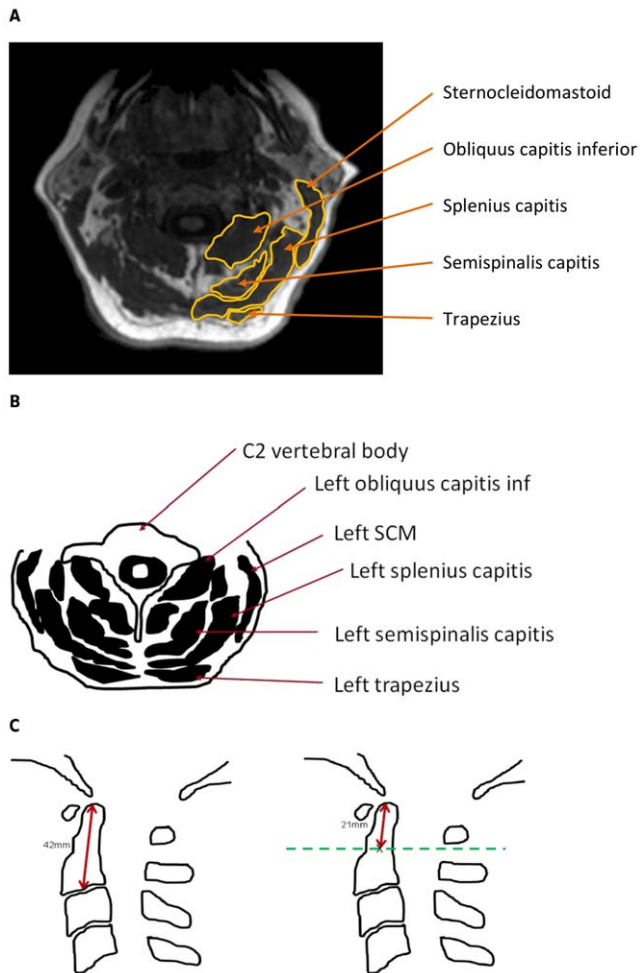
We initially attempted to standardise the plane of the axial image on a line parallel to C2 end plates, but the variability of tilt in endplates meant that occasionally that line could go as high as suboccipital level posteriorly and thereby miss the muscles of interest. Setting the axial slice perpendicular to the vertical line of measurement through C2 did not work either as it proved difficult to manipulate the axial slice by small angle changes precisely enough. Therefore, we used the midpoint of C2 in the sagittal plane while viewing the images in the multiplanar reformat and then clicked on the corresponding axial image. This resulted in the axial slice being parallel to the lower border of the volume scan, but not related to any particular line in the participant. This time we measured the three posterior neck muscles (trapezius, splenius capitis and semispinalis capitis) individually and in combination. See Figure 2 for the chosen method.

### Study 2: Study to measure inter-rater reliability

**Goal.** To investigate and quantify whether two raters using this technique would produce the same measurements.

**Sample.** A further 20 scans from the LBC 1936 study were studied in addition to the 20 from the feasibility study, to give us a total of 40 scans to measure with the newly-developed technique.

**Neck muscle cross sectional area measurements.** We performed the measurements with the chosen technique, as



**Figure 1. Figure of the posterior neck muscles and diagram demonstrating how the measurement plane was selected.** A. Non-contrast T1-weighted MR of transverse plane of the neck at mid-infero-superior-C2 level. B. Outline diagram showing the neck muscles whose cross-sectional areas were measured. C. Outline diagram demonstrating how measurement plane is selected with an example C2 height of 42 mm.

doi:10.1371/journal.pone.0034444.g001

described in the feasibility study, on all 40 MRI scans. (Figure 2) Each scan was measured three times by both raters and the median value for C2 body height and each of the three muscle measurements (SCM, obliquus capitis inferior and combined trapezius, splenius capitis and semispinalis capitis) were recorded separately for right and left sides for analysis. We noted that, unlike thigh muscles, there was only minimal intramuscular fat in the images of these neck muscles, so we did not seek to adjust for this or the small area of interfascial fat between trapezius, splenius capitis and semispinalis capitis in the combined group measure.

**Analysis.** To compare inter-rater reliability, we calculated the percentage difference in CSA as measured by the two raters and the two-way random effects absolute agreement intraclass correlation coefficients. Multiple linear regression analysis was used to estimate effects of sex and body mass index (BMI) on neck muscle CSA. Principal components analysis was used to extract a general trait for neck muscle CSA from the three individual muscle CSA measures: we accepted components with eigenvalues greater than unity and inspected the scree plot to identify the number of

components. All analyses were performed using the SPSS 16.0 statistical package.

### Study 3: Study to measure repeatability of technique

**Goal.** To assess whether the technique would provide the same results on scans measured:

- on the same subject and the same MRI scanner on different days
- on the same subject and different MRI scanners on different days

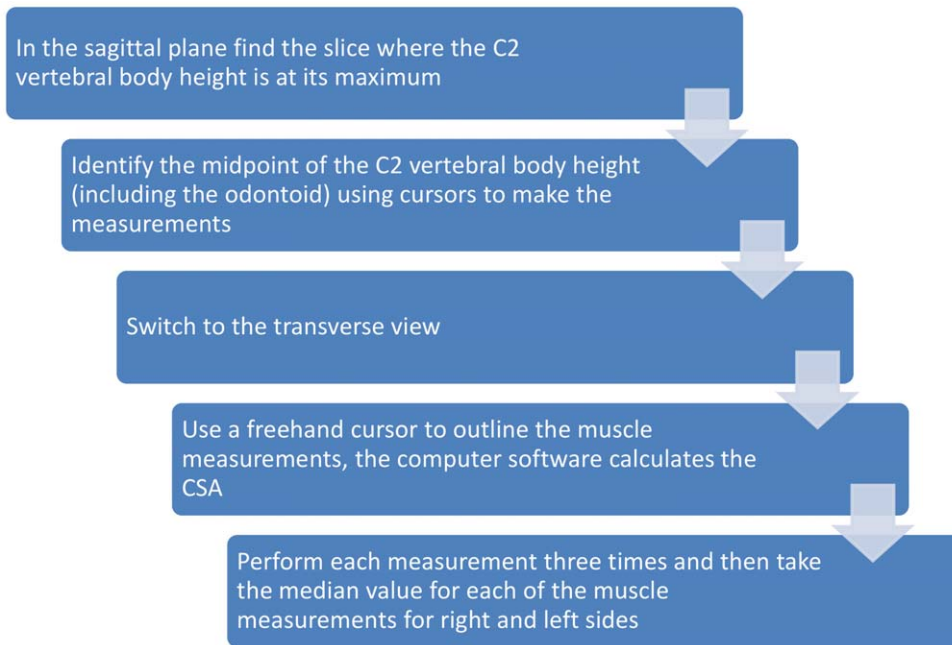
**Ethics.** All subjects provided written consent and ethics approval was gained by the local ethics research committee for the original CaliBrain study (REC 05/S0801/105) [18,19]. This included further use of the data for research purposes and therefore further ethics permission for this study was not required.

**Sample.** The CaliBrain study investigated the reliability of repeat volumetric brain MR measures with the same scanner and between different scanners [8]. We therefore used these data to test the reliability of repeat neck muscle CSA measurement from volumetric brain MR scans. The participants of the CaliBrain study were 14 normal volunteers from the three participating centres aged between 25 and 51 years, see below for details of the centres. As the data had been collected as part of the CaliBrain study no power calculations were carried out and we analysed all the available data. Exclusion criteria for the CaliBrain study were: previous history of a diagnosed neurological disorder or a major psychiatric disorder, treatment with psychotropic medication, including treatment for substance misuse and not meeting the MR safety criteria.

**Imaging protocol.** Each of the fourteen participants twice underwent a structural and functional MR brain scan at three imaging research centres around Scotland; The Department of Radiology, University of Aberdeen; The Brain Research Imaging Centre, Western General Hospital, University of Edinburgh; and The Neuroradiology Department, Southern General Hospital, NHS Greater Glasgow South University Hospitals Division. Therefore each participant underwent 6 separate scans. Each scan took place on a separate day and there were nominally 2 weeks between the scans at the same site. We only used the structural data for our study. The three scanners used were all manufactured by General Electric (GE Healthcare, Milwaukee, Wisconsin) and had primary field strengths of 1.5 T however the machines had differing software and hardware. Images were taken in the coronal plane at a slice thickness of 1.7 mm with no slice gap. 3D reconstruction was used to make the measurements with our technique. Further details of the imaging protocol can be found in the paper by Moorhead et al. [19]

**Cross-sectional area measurement technique.** The measurements were performed using Analyze, the biomedical image analysis software (Mayo Foundation, Rochester, Minnesota, USA).

The CaliBrain images were aligned with the ACPC line, an anatomical line which runs between the superior surface of the anterior commissure and the center of the posterior commissure. The feasibility and reliability studies had used images which were perpendicular to the MR table. Therefore the images underwent pre-processing prior to the measurements being made. This involved the images being tilted 15 degrees forward on the axial axis. This was actually preferable to the original study where the angle of the brain as viewed on PACS was not standardised, but just depended on how the patient placed their head in the scanner, as all the images in the CaliBrain study were standardised to an



**Figure 2. Flowchart summarizing method to measure craniad muscles cross-sectional areas.**  
doi:10.1371/journal.pone.0034444.g002

anatomical landmark, the ACPC line. Neck muscle CSA was measured as described in the feasibility study.

**Analysis.** ICCs were calculated for comparing within scanner and between scanner variations. When calculating the ICC for within scanner variation the measurements taken from the first and second scans were compared for each of the three individual measurements (ie combined group, SCM and obliquus) and the total neck muscle CSA (ie all three measurements for right and left sides added together). The ICC for between scanner variation were calculated using the mean total muscle CSA for each measurement on that site (eg (Total neck muscle CSA Edinburgh scan 1+Total neck muscle CSA Edinburgh scan 2)/2).

All data were analysed using the SPSS 17.0 statistics package. Three nonsynchronous sets of measurements were taken for each scan and the median values were used for the analysis.

#### Study 4: External validity study

**Goal.** To assess and quantify whether neck CSA is related to mid-thigh CSA, which has been previously shown to be related to general muscle mass [6,20].

**Ethics.** Ethics permission for the MR brain scans undertaken as part of the Lothian Birth Cohort (LBC) 1936 project had already been obtained as per study 1. A substantial amendment was submitted to allow us to recruit 25 subjects from the LBC 1936 pool and perform a MR scan of their mid-thigh. This was approved in April 2010.

**Sample.** 735 LBC1936 participants had brain MR data available. Power calculations indicated that for a minimum Pearson correlation coefficient of 0.6 at  $\alpha = 0.05$ ,  $n = 20$  provided 80% power and  $n = 26$  provided 90% power. We therefore chose to scan 25 subjects. We contacted the subjects who had most recently had their MR brain scan, within a few weeks, to ensure that the effect of any variable on the thigh muscle bulk in the time lapsing between the MR brain and thigh scans was as small as possible. Participants were excluded if they had severe osteoarthritis affecting the knee or hip or a previous stroke,

previous total hip replacement, or a history of any degenerative neurological disorder

**Imaging protocol.** The MR brain scans had been collected as part of the LBC 1936 study. See study 1 (above) for details of the protocol.

We used anatomical landmarks to identify the midpoint of the femur. We palpated for the protuberance of the greater trochanter and the upper border of the patella and then measured down the lateral aspect of the thigh using these landmarks and marked the midpoint. A cod liver oil capsule was then taped there to allow us to identify the corresponding MR slice. This was performed separately for each leg.

The scan was performed using a 3.0 tesla Siemens Verio research MR scanner (Siemens Medical, Germany) at the Clinical Research Imaging Centre within the Queen's Medical Research Institute. Images were acquired using a combination of body and spine matrix coil elements. The subjects lay supine for the scan. A coronal scouting scan was performed and then 5–10 axial images were taken with the cod liver oil capsules in the middle slices. Slice thickness was 3 mm with no slice gap.

**Cross-sectional Area Measurements.** Measurements for sternocleidomastoid, obliquus and the combined group (trapezius, splenius and semispinalis) were made using the above described technique on a PACS workstation.

The thigh muscle CSA measurements were also performed on a PACS workstation using the slice on each side where the cod liver oil capsule was at its widest which should indicate the anatomically chosen midpoint. Three measurements were taken on each leg: the anterior group, the medial group and the posterior group. The anterior group consisted of the quadriceps (vastus lateralis, intermedius and medialis and rectus femoris) and sartorius, the medial group of gracilis and the adductors (longus, brevis and magnus) and the posterior group of the hamstrings (biceps femoris, semitendinosus and semimembranosus).

Both the thigh and neck measurements were repeated 3 times for the left and right sides.



**Analysis.** All data were analysed using the SPSS 17.0 statistics package. Three nonsynchronous sets of measurements were taken for each subject and the median values were used for the analysis.

Please see figure 3 for a summary of the methods for the above four studies.

## Results

### Study 1: Feasibility study

The measurements made with the chosen technique were used to calculate intra-class correlation coefficients (ICC) to compare the median value of 3 measurements made by rater A against rater B. In this study we measured each of the three posterior muscles (trapezius, splenius and semispinalis) separately and together in a single measurement as a combined group. The boundaries between these three muscles are not clear and we thought that the differences in CSA measurements were a reflection of where the boundary was taken to be rather than true measurement differences in the size of the muscles themselves. The ICC and associated 95% confidence intervals support this view, as the values for the ICC for the three respective individual muscles were 0.78 (CI 0.16–0.94), 0.86 (CI 0.48–0.97) and 0.90 (CI 0.60–0.97) and the combined group ICC was much stronger at 0.99 (CI 0.95–1.00). Therefore we decided to use the combined measurement from thereon with individual measurements of the stand alone muscle, obliquus and sternocleidomastoid. For full results please see table 1.

### Study 2: Study to measure inter-rater reliability

Of the 40 scans from the LBC 1936 cohort, one proved to be a duplicate and was excluded. One rater considered one scan to be

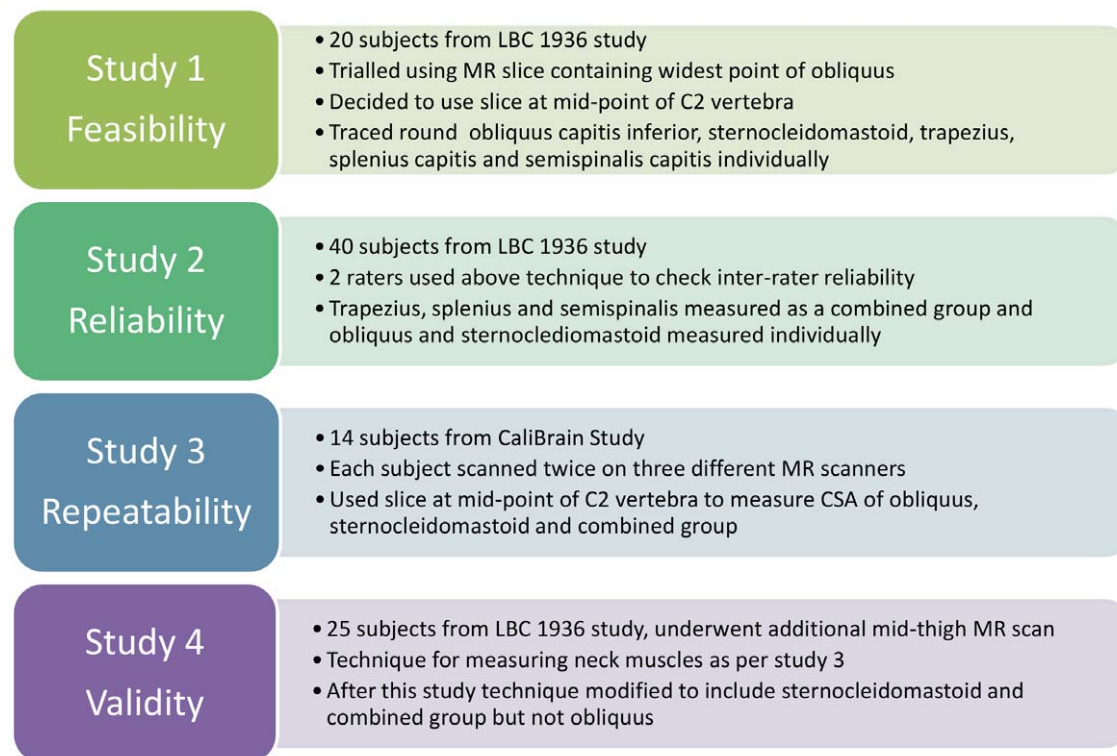
**Table 1.** Intra-class correlation coefficients for the second technique trialled in the feasibility study (Study 1).

| Measurement  | ICC   | 95% CI      |
|--|-------|-------------|
| <b>Trapezius</b>   | 0.778 | 0.160–0.944 |
| <b>Splenius</b>  | 0.861 | 0.475–0.965 |
| <b>Semispinalis</b>  | 0.895 | 0.603–0.974 |
| <b>Summation of Trapezius, Splenius &amp; Semispinalis</b> | 0.978 | 0.916–0.994 |
| <b>Single measurement of combined group</b>                | 0.986 | 0.946–0.996 |
| <b>Obliquus</b>  | 0.900 | 0.623–0.975 |
| <b>SCM</b>   | 0.894 | 0.598–0.973 |

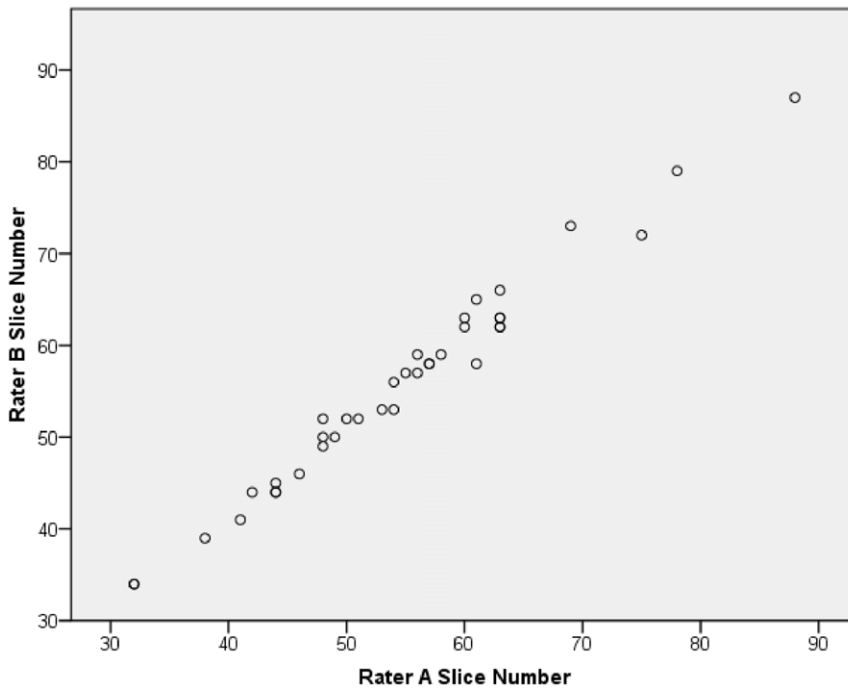
doi:10.1371/journal.pone.0034444.t001

unmeasurable whilst the other considered two scans unmeasurable. Scans were thus measured for 37 (18 male, 19 female) participants of mean age 72.0 (standard deviation 0.38) years when weighed and mean age 72.2 (sd 0.25) years when scanned. Men had a mean height of 1.73 m (sd 0.07), mean weight of 85.0 kg (sd 11.2) and mean BMI of 28.2 kg/m<sup>2</sup> (sd 3.2). Women had a mean height of 1.59 m (sd 0.04), mean weight of 71.0 kg (sd 14.0) and mean BMI of 27.9 kg/m<sup>2</sup> (sd 5.2).

Rater A measured mean C2 height as 3.7 cm (sd 0.5) in men and 3.6 cm (sd 0.2) in women. Rater B measured mean C2 height as 4.0 cm (sd 0.4) in men and 3.7 cm (sd 0.2) in women. These differences had no effect on slice chosen as midpoint of C2 for muscle CSA measurement (Figure 4).



**Figure 3.** Diagram summarizing the methods for the four studies. doi:10.1371/journal.pone.0034444.g003



**Figure 4.** Plot of MR slice chosen as representing the mid-point of C2 for both raters. doi:10.1371/journal.pone.0034444.g004

Table 2 shows the mean CSAs per rater, absolute mean difference and mean difference as percentage of CSA. Intraclass correlation coefficients between raters were 0.99 (95% confidence intervals 0.98–1.00) for the combined group CSA, 0.92 (95% C.I. 0.85–0.96) for obliquus CSA and 0.92 (95% C.I. 0.85–0.96) for sternocleidomastoid CSA (Table 3). Obliquus CSA was predicted by sex (beta = -0.54 for women,  $p < .001$ ) and BMI (beta = 0.36,  $p = .01$ ) adjusted  $R^2$  for model = 0.40, sternocleidomastoid CSA by sex (beta = -0.60 for women,  $p < .001$ ) and BMI (beta = 0.41,  $p = .001$ ) adjusted  $r^2$  for model = 0.52, and combined CSA by sex (beta = -0.74 for women,  $p < .001$  only) adjusted  $r^2$  for model = 0.55.

There were no significant associations between inter-rater CSA difference and mean CSA for the combined group ( $r = 0.08$ ,  $p = .66$ ), but larger inter-rater differences were significantly associated with smaller CSAs for both obliquus ( $r = -0.61$ ,  $p < .001$ ) and sternocleidomastoid ( $r = -0.39$ ,  $p = .018$ ). CSAs all correlated highly significantly with each other ( $p < .001$ ): combined-obliquus ( $r = 0.59$ ), combined-sternocleidomastoid ( $r = 0.66$ ), obliquus-sternocleidomastoid ( $r = 0.50$ ).

**Table 3.** Intra-class correlation coefficients with 95% confidence intervals for the reliability study (Study 2).

| Muscle Measurement        | ICC  | 95% Confidence Intervals |
|---------------------------|------|--------------------------|
| Combined group            | 0.99 | 0.98–0.995               |
| Obliquus capitis inferior | 0.92 | 0.85–0.96                |
| SCM                       | 0.92 | 0.85–0.96                |

doi:10.1371/journal.pone.0034444.t003

A Bland-Altman plot for total neck muscle CSA demonstrates a degree of linear bias with Rater 2 reporting bigger measurements for the small neck muscle CSAs and smaller measurements for the bigger neck muscle CSAs (Figure 5A). If obliquus is removed, leaving the combined group plus SCM, this linear bias appears to resolve (Figure 5B). However the bias of measurement is small for both graphs.

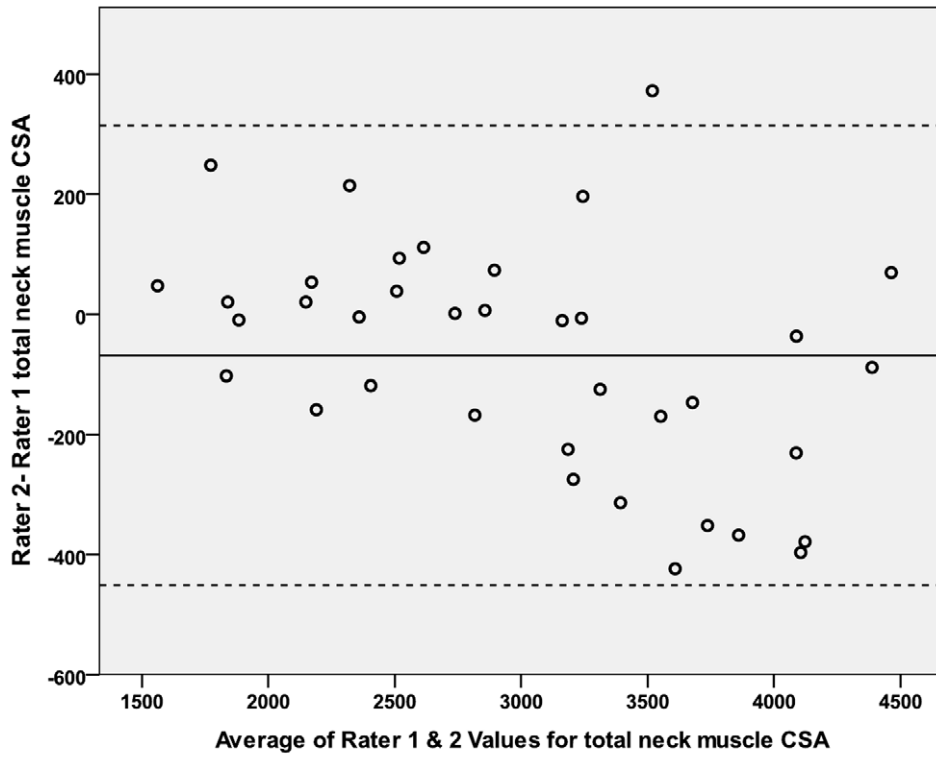
Table 4 shows coefficients of variation (CV) for both raters and a Levene’s test for homogeneity which found no significant

**Table 2.** Mean cross-sectional areas (CSAs) as measured by each rater summed for left and right, together with absolute mean difference and mean difference as percentage of CSA between raters (Study 2).

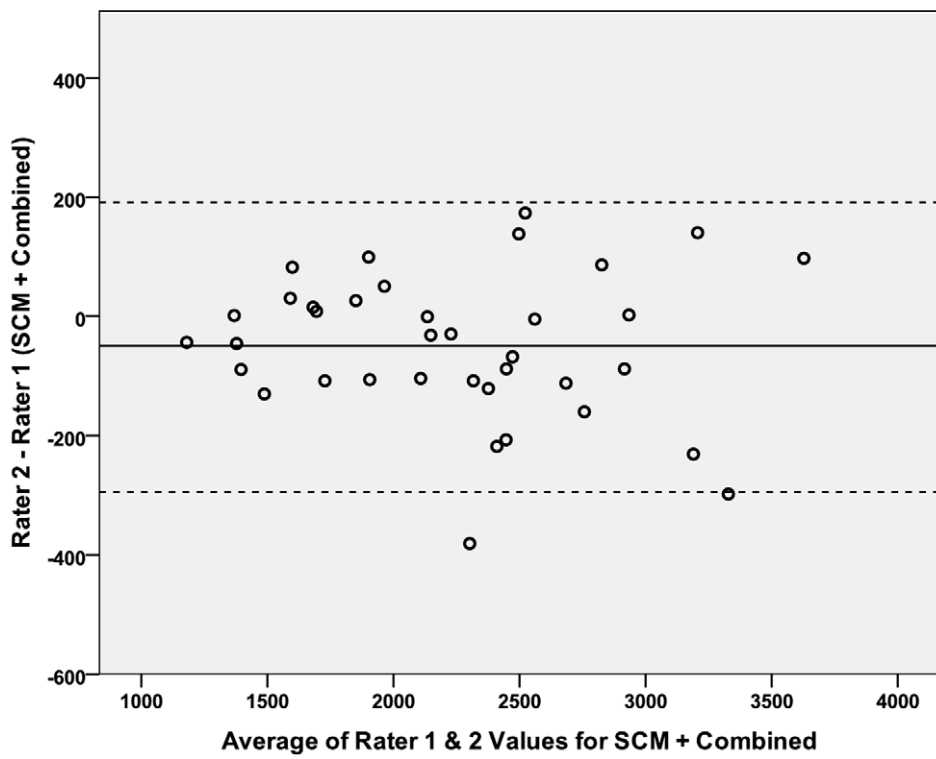
|   | Combined Group  | Obliquus Capitis Inferior | Sternocleidomastoid |
|---|-----------------|---------------------------|---------------------|
| Mean CSA rater A (mm <sup>2</sup> )                     | 1850            | 773                       | 422                 |
| Mean CSA rater B (mm <sup>2</sup> )                     | 1847            | 753                       | 376                 |
| Mean inter-rater difference (95% CI) (mm <sup>2</sup> ) | 3 (-30, 36)     | 20 (-33, 73)              | 46 (-29, 63)        |
| Mean difference as percentage of mean CSA (95% CI)      | 0.3 (-1.5, 2.0) | 4.1 (-6.3, 14.4)          | 11.3 (7.1, 15.5)    |

doi:10.1371/journal.pone.0034444.t002

**A**



**B**



**Figure 5. Bland-Altman plots for total neck muscle CSA and SCM+combined CSA measured by 2 raters.** Bias of measurement between 2 different raters (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively. A. Bland-Altman plot for measurements of total neck muscle CSA by 2 different raters. B. Bland-Altman plot for measurements of SCM+combined CSA by 2 different raters.

doi:10.1371/journal.pone.0034444.g005

difference between the CV for the two raters for any of the muscle measurements.

The first unrotated principal component explained 72.2% of total CSA variance for the three muscles (loadings were 0.89 for the combined group, 0.81 for obliquus and 0.85 for sternocleidomastoid) and correlated positively with grip strength of both right ( $r = 0.52$ ,  $p = .001$ ) and left ( $r = 0.50$ ,  $p = .002$ ) hands. The second principal component had an eigenvalue of 0.51.

### Study 3: Study to measure repeatability of technique

Data were analysed for all 14 participants. Thirteen of the participants had undergone all 6 scans and one had undergone 5 of the scans having not completed their second scan in one location. There were 10 men and 4 women. The mean age was 36.3 years (range 25–51).

Mean values (sd) for the measurements across all six scanners for right and left sides added together were: SCM 4.97 cm<sup>2</sup> (1.11), combined group 20.12 cm<sup>2</sup> (5.74), obliquus 9.88 cm<sup>2</sup> (3.23) and total neck muscle CSA 34.97 cm<sup>2</sup> (8.67). ICCs were calculated for within scanner and between scanner variability (Tables 5 & 6). Within scanner ICCs for the Edinburgh scanner used for studies 1, 2 and 4 ranged from 0.83 for SCM to 0.96 for the combined group.

Bland-Altman plots show no definite linear bias between the Edinburgh and Glasgow scanners and the Aberdeen and Edinburgh scanners (Figures 6A & 6B). The Aberdeen-Glasgow plot indicates that the Aberdeen scanner may overestimate larger neck muscle CSA and underestimate smaller neck muscle CSA (Figure 6C). However the numbers involved in this study were small ( $n = 14$ ).

Table 7 shows the coefficients of variance (CV) for the mean values for each of the three scanners and a Levene's test of homogeneity which found no significant difference in CV for any of the three scanners, for any of the muscle measurements.

### Study 4: External validity study

25 subjects underwent the additional thigh scan; however, only 24 could be used in the analyses as one patient had not tolerated the full MR brain scan so we were unable to make the neck muscle CSA measurements. There was no overlap between subjects in

study 2 and study 4. Of these 24 subjects, 11 were female and 13 male. Mean age (sd) was 73.8 years (0.27). Mean weight (sd) for the women was 63.2 kg (15.4) and for the men was 85.6 kg (10.9).

Mean total neck muscle CSA (sd) was 22.5 cm<sup>2</sup> (3.7) for the female subgroup and 38.1 cm<sup>2</sup> (6.3) for the male subgroup. Mean total thigh muscle CSA was 184.3 cm<sup>2</sup> (36.5) for the female subgroup and 277.0 cm<sup>2</sup> (31.3) for the male subgroup. An independent t test showed that both total neck muscle CSA ( $p < 0.0005$ ) and total thigh muscle CSA ( $p < 0.0005$ ) were significantly different between the female and male subgroups. The correlation coefficient for all subjects for total thigh CSA and total neck CSA was 0.88 indicating that each explained at least 77.4% of the variance of the other.

## Discussion

### Summary of findings

This study sought to develop a technique to measure neck muscle cross-sectional area on volumetric MR brain scans. An initial feasibility study led to the formation of the technique. (Figure 2) The reliability study then demonstrated that the technique had high inter-rater reliability for measurement of the CSA of the combined trapezius, splenius and semispinalis group in older adults. Obliquus capitis inferior and sternocleidomastoid CSAs are smaller muscles and measurements were less reliable between raters, though intraclass correlation coefficients remained high.

Study 3 demonstrated that the technique has good within scanner and between scanner repeatability. The confidence intervals for the measurements of the combined group and the total neck muscle area are quite narrow however the confidence intervals for the SCM and obliquus capitis inferior measurements are wider. This is because the cross-sectional areas of the SCM and obliquus muscles are smaller than either the combined or the total measurements. This means that any measurement errors will account for a greater proportion of the CSA than for muscles with a large area.

The obliquus is a short muscle whose cross-sectional area varies greatly over its length, unlike the other four muscles, as it has a wide belly and comparatively narrow tails. Our technique meant

**Table 4.** Coefficients of variation (%) with 95% CI and Levene's significance test between the two raters (Study 2).

| Measurement           | Rater | Coefficients of Variation (CV) |               |                              |
|-----------------------|-------|--------------------------------|---------------|------------------------------|
|                       |       | (%)                            | 95% CI for CV | Levene's test (Significance) |
| Combined group        | 1     | 28.1                           | 22.9–36.5     | 0.96                         |
|                       | 2     | 28.5                           | 23.2–37.0     |                              |
| Obliquus              | 1     | 43.8                           | 35.6–56.9     | 0.10                         |
|                       | 2     | 32.3                           | 26.3–42.0     |                              |
| SCM                   | 1     | 31.0                           | 25.2–40.3     | 0.82                         |
|                       | 2     | 29.8                           | 24.2–38.7     |                              |
| Total neck muscle CSA | 1     | 28.2                           | 22.9–36.6     | 0.47                         |
|                       | 2     | 25.9                           | 21.1–33.6     |                              |

doi:10.1371/journal.pone.0034444.t004



**Table 5.** Between scanner intra-class correlation coefficients for the repeatability study (Study 3).

| Groups                          | ICC  | 95% Confidence Intervals | Sig     |
|---------------------------------|------|--------------------------|---------|
| <b>E, A &amp; G Total means</b> | 0.94 | 0.86–0.98                | p<0.001 |
| <b>E, A &amp; G SCM means</b>   | 0.76 | 0.53–0.90                | p<0.001 |
| <b>E, A &amp; G Comb means</b>  | 0.95 | 0.89–0.98                | p<0.001 |
| <b>E, A &amp; G Obliq means</b> | 0.78 | 0.56–0.92                | p<0.001 |

doi:10.1371/journal.pone.0034444.t005

that in some subjects we were measuring across the belly of the obliquus and in some just the tail end. It also runs at an oblique angle and we identified that this angle varies between subjects. This meant that the area of obliquus we were measuring was an inexact proxy. For these reasons we conclude that the obliquus measurement should not be included in our technique. The ICCs are not as strong for the SCM as the combined group in the repeatability and reliability studies, which is likely a reflection of its small size. However as there are not the same intrinsic anatomical problems in measuring this muscle as there are with the obliquus and as total neck muscle CSA including the SCM appears to have stronger ICCs than the combined group alone, it is probably beneficial to include the SCM in addition to the combined group to provide a better estimate of general muscle bulk.

The final study was designed to measure the external validity of the technique. It shows that there is a strong correlation between neck muscle cross sectional area and thigh muscle cross sectional area, which is often used as a proxy for general muscle mass [21–23]. The percentage variance (ie r-squared) is 76.7% and the 1<sup>st</sup> unrotated principal component of neck muscle CSA was found in

**Table 6.** Within scanner intra-class correlation coefficients for the repeatability study (Study 3).

| Groups                   | ICC  | 95% Confidence Intervals | Sig     |
|--------------------------|------|--------------------------|---------|
| <b>E1 &amp; E2 Total</b> | 0.95 | 0.86–0.98                | p<0.001 |
| <b>A1 &amp; A2 Total</b> | 0.97 | 0.92–0.99                | p<0.001 |
| <b>G1 &amp; G2 Total</b> | 0.96 | 0.86–0.99                | p<0.001 |
| <b>E1 &amp; E2 SCM</b>   | 0.83 | 0.55–0.94                | p<0.001 |
| <b>A1 &amp; A2 SCM</b>   | 0.80 | 0.48–0.93                | p<0.001 |
| <b>G1 &amp; G2 SCM</b>   | 0.90 | 0.70–0.97                | p<0.001 |
| <b>E1 &amp; E2 Comb</b>  | 0.96 | 0.88–0.99                | p<0.001 |
| <b>A1 &amp; A2 Comb</b>  | 0.97 | 0.92–0.99                | p<0.001 |
| <b>G1 &amp; G2 Comb</b>  | 0.96 | 0.88–0.99                | p<0.001 |
| <b>E1 &amp; E2 Obliq</b> | 0.93 | 0.79–0.98                | p<0.001 |
| <b>A1 &amp; A2 Obliq</b> | 0.83 | 0.56–0.94                | p<0.001 |
| <b>G1 &amp; G2 Obliq</b> | 0.83 | 0.53–0.95                | p<0.001 |

Key for Table 5 &amp; 6:

- E = scan performed in Edinburgh.
- A = Scan performed in Aberdeen.
- G = Scan performed in Glasgow.
- 1 & 2 = 1<sup>st</sup> and 2<sup>nd</sup> scan on that site.
- SCM = Sternocleidomastoid.
- Comb = Combined group (Trapezius, Splenius capitis, Semispinalis capitis).
- Obliq = Obliquus Capitis Inferior.
- Total = Total neck muscle CSA.

doi:10.1371/journal.pone.0034444.t006

the reliability study to explain 72.2% of variance. Extracting principal components is useful to reduce random measurement errors that might be associated with individuals' neck positions for example. The principal component correlated positively with grip strength, providing further support for neck muscle CSAs' validity as an index of sarcopenia. This means that posterior neck muscles can be used equally as well as thigh muscles as an index of general muscle bulk [6,7].

### Previous research on quantifying muscle mass

Previous studies quantifying neck muscle CSA have only focused on young subjects and have used scans performed specifically for that purpose. They have however shown good reliability in the techniques used [14,15]. We found no previous studies which measured neck muscle CSA on MRI scans on elderly subjects and none which used MRI brain scans for this purpose.

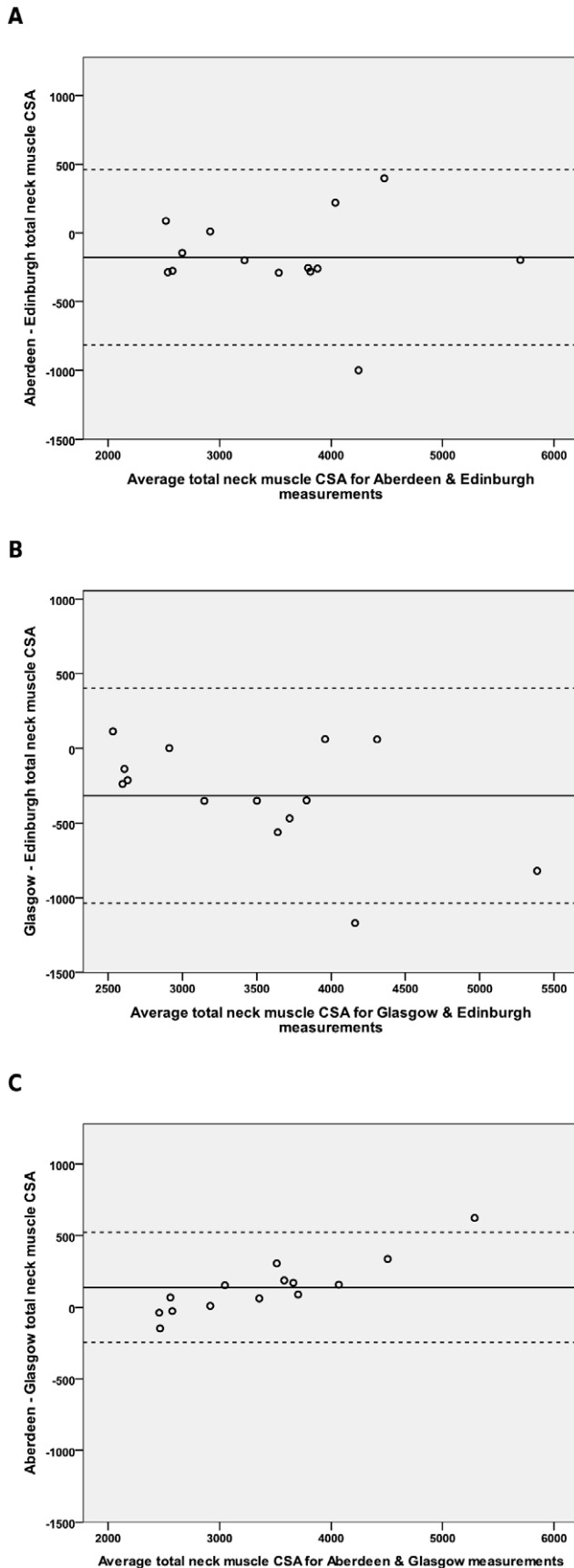
When considering the validity of using a cross-sectional measurement of muscle size to infer general muscle bulk we referred to previous studies on body composition. Studies investigating how differing muscle groups relate to each other have tended to compare upper and lower limb muscle mass alone [24–26]. We found no studies which compared muscle CSA, mass or volume between any other two or more areas of the body, including the neck. Three large studies on body composition suggest that the distribution of muscle between the upper and lower limbs varies with gender, age, height, weight and ethnicity [24–26].

### Strengths and limitations of the studies

Although there is no reason to suppose that this methodology is not applicable in younger adults and older adults (ie 75 y+), three of the studies were restricted to a narrow age cohort around 72 years old and the study of younger subjects only had a n = 14. The study participants were all community-resident volunteers and thus relatively healthy and were not diverse in terms of geography or ethnicity. The narrow geographical location of the subjects is important as it has been shown that anthropometric measurements vary across the UK. Bannerman et al collected data from residents of Edinburgh and compared their results with anthropometric reference data from South Wales and Nottingham. They found significant differences between the three groups confirming their hypothesis that anthropometric measurements vary across geographical area [27].

Skeletal muscle can be split into two groups; postural and phasic. Postural muscles have a larger percentage of type 1 fibres and show less fatigability. Phasic muscles are primarily involved in movement and have a higher proportion of type 2 muscle fibres. A feature of ageing muscle is that type 2 fibre width decreases more than type 1 fibre width, therefore the relation between neck CSA (mainly postural, ie more type 1 fibres) and thigh CSA (a mixture of postural and phasic) will change with age [28–31].

Despite not standardising the angle of the axial measurement slice relative to the patient, we still achieved very high inter-rater reliability. However, it is possible that the measurement variability would be larger in a longitudinal study if the patients were in different positions in the scanner on each occasion. Such differences are usually only slight because head, neck and back are passively supported during scanning leaving the neck muscles in a relaxed state. Lateral changes are unlikely to have a major effect because muscle CSAs are summed for both left and right so that reductions on one side could be compensated by the accompanying increase on the other. Such compensation does not apply to antero-posterior positioning; however, small differ-



**Figure 6. Bland-Altman plots for total neck muscle CSA measured on 3 different MRI scanners.** Bias of measurement between different MRI scanners (mean of the ordinate) and limits of agreement (2sd) are represented by a solid and two dashed lines respectively. A. Bland-Altman plot for total neck muscle CSA measured on the Aberdeen and Edinburgh MR images. B. Bland-Altman plot for total neck muscle CSA measured on the Edinburgh and Glasgow MR images. C. Bland-Altman plot for total neck muscle CSA measured on the Glasgow and Aberdeen MR images.  
doi:10.1371/journal.pone.0034444.g006

ences in this plane are also unlikely to be important. A difference in angle of C2 between two repeated scans in the sagittal plane of 5° would result in a CSA difference of 0.4%, 10° increases CSA by 1.5% and 15° by 3.5% all within the limits for inter-rater SCM CSA difference; even a 20° angle increases CSA by only 6.4%, probably at the limit of utility of the technique to detect medium effect sizes. If reliability in longitudinal studies was not acceptable (i.e. mean differences in plane angles measured at C2 > 20°), increased positional standardization would be necessary which might include using more than one anatomical marker from a T1-weighted volume scan for standardization.

Most of these limitations could be addressed by a larger study which included a wider spread of age, geographical area, ethnicity, health status and an equal gender balance. It would be interesting to look at muscles from elsewhere in the body also. For example including a measure of upper arm CSA and calf muscle CSA and to investigate how the comparative size of these muscles varies with age.

### Implications for future research

This new technique is particularly interesting because several of the longitudinal studies investigating ageing involve an MR brain scan, therefore the method could be used to measure changes in muscle size and consequently estimate sarcopenia in these studies without any further imaging. This will allow the wealth of variables already collected as part of these studies to be researched as possible correlates of sarcopenia. Longitudinal studies are important sources of information for researchers interested in age associated disease to allow identification of key risk factors. These in turn allow hypotheses to be generated which can lead to both an understanding of the mechanisms underlying these diseases, which may lead on to development of treatments, and the possibility of generating advice to prevent or slow down some of the disease processes. Although we developed the technique on MR volume brain images, it is now common to acquire volume data when performing a CT brain scan and, as there is often good differentiation between muscle and fat in the neck, the same approach could possibly work on CT scans as well. Further testing is required.

We now plan to use this technique on two longitudinal studies to allow us to investigate correlates of sarcopenia and identify possible causative factors from lifestyle and biomedical data which have been collected concurrently with the MRI scans.

### Conclusion

We have developed a feasible, valid and repeatable method for measuring neck muscle cross-sectional area on MR brain scans which has good inter-rater reliability. This technique can be used to measure neck muscle CSA which can serve as a proxy measure of muscle bulk as shown by the above factor analysis and shared variance measures. We have demonstrated that neck muscle CSA correlates strongly with grip strength, a commonly used functional measure. The development of a reliable method to measure neck muscle CSA from volumetric MR brain scans potentially opens up

**Table 7.** Coefficients of variation (%) with 95% CI and Levene's significance test between the three MRI scanners (Study 3).

| Measurement                  | Scanner   | Coefficients of Variation (CV) (%) | 95%CI for CV | Levene's test (Significance) |
|------------------------------|-----------|------------------------------------|--------------|------------------------------|
| <b>Combined</b>              | Aberdeen  | 32.2                               | 23.3–51.9    | 0.89                         |
|                              | Edinburgh | 27.1                               | 19.7–43.7    |                              |
|                              | Glasgow   | 26.0                               | 18.9–41.9    |                              |
| <b>Obliquus</b>              | Aberdeen  | 30.6                               | 22.2–49.3    | 0.53                         |
|                              | Edinburgh | 36.7                               | 26.6–59.1    |                              |
|                              | Glasgow   | 28.1                               | 20.4–45.3    |                              |
| <b>SCM</b>                   | Aberdeen  | 21.7                               | 15.7–35.0    | 0.95                         |
|                              | Edinburgh | 20.6                               | 14.9–33.2    |                              |
|                              | Glasgow   | 22.1                               | 16.0–35.6    |                              |
| <b>Total neck muscle CSA</b> | Aberdeen  | 26.4                               | 19.1–42.5    | 0.84                         |
|                              | Edinburgh | 25.5                               | 18.5–41.1    |                              |
|                              | Glasgow   | 22.3                               | 16.2–35.9    |                              |

doi:10.1371/journal.pone.0034444.t007

a new field of radiological aging research. This in turn will allow sarcopenia to be investigated in studies which have involved a brain scan but no measure of muscle bulk without involving any additional scanning. Additional studies are needed to investigate these important relationships further with particular reference to how the relationships change with age.

## Acknowledgments

A Gow, C Murray, J Corley, R Henderson and A Pattie at the Centre of Cognitive Aging and Cognitive Epidemiology, University of Edinburgh, UK, for data collection on the LBC1936.

The LBC1936 Study and Calibrain study participants.

S Semple, T MacGillivray and staff at the Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, UK.

## References

- Roubenoff R, Parise H, Payette HA, Abad LW, D'Agostino R, et al. (2003) Cytokines, insulin-like growth factor 1, sarcopenia, and mortality in very old community-dwelling men and women: The Framingham Heart Study. *American Journal of Medicine* 115: 429–435.
- Anker SD, Ponikowski P, Varney S, Chua TP, Clark AL, et al. (1997) Wasting as independent risk factor for mortality in chronic heart failure. *Lancet* 349: 1050–1053.
- Tisdale MJ (2002) Cachexia in cancer patients. *Nature Reviews Cancer* 2: 862–871.
- Janssen I, Heymsfield SB, Ross R (2002) Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society* 50: 889–896.
- Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, et al. (2010) Sarcopenia: European consensus on definition and diagnosis. *Age and Ageing* 39: 412–423.
- Ohkawa S, Odamaki M, Yoneyama T, Hibi I, Miyaji K, et al. (2000) Standardized thigh muscle area measured by computed axial tomography as an alternate muscle mass index for nutritional assessment of hemodialysis patients. *American Journal of Clinical Nutrition* 71: 485–490.
- Lerner A, Feld LG, Riddlesberger MM, Rossi TM, Lebenthal E (1986) Computed axial tomographic scanning of the thigh - an alternative method of nutritional assessment in pediatrics. *Pediatrics* 77: 732–737.
- Deary IJ, Johnson W, Gow AJ, Pattie A, Brett CE, et al. (2011) Losing One's Grip: A Bivariate Growth Curve Model of Grip Strength and Nonverbal Reasoning From Age 79 to 87 Years in the Lothian Birth Cohort 1921. *J Gerontol B Psychol Sci Soc Sci*.
- Boyle PA, Buchman AS, Wilson RS, Leurgans SE, Bennett DA (2009) Association of Muscle Strength With the Risk of Alzheimer Disease and the Rate of Cognitive Decline in Community-Dwelling Older Persons. *Archives of Neurology* 66: 1339–1344.
- Blumenthal HT, Shock N, Greulich R, Andres R, Arrenberg D, et al. (1985) Normal human aging - the Baltimore Longitudinal Study of Aging. *Journals of Gerontology* 40: 767–767.
- Deary IJ, Gow AJ, Taylor MD, Corley J, Brett C, et al. (2007) The Lothian Birth Cohort 1936: a study to examine influences on cognitive ageing from age 11 to age 70 and beyond. *BMC Geriatr* 7: 28.
- Ellis KA, Bush AI, Darby D, De Fazio D, Foster J, et al. (2009) The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease. *International Psychogeriatrics* 21: 672–687.
- Elliott JM, Jull GA, T Noteboom J, Durbridge GL, Gibbon WW (2007) Magnetic resonance imaging study of cross-sectional area of the cervical extensor musculature in an asymptomatic cohort. *Clinical Anatomy* 20: 35–40.
- Ulbrich EJ, Anderson SE, Busato A, Abderhalden S, Boesch C, et al. (2011) Cervical Muscle Area Measurements in Acute Whiplash Patients and Controls. *Journal of Magnetic Resonance Imaging* 33: 668–675.
- Cagnic B, D'Hooge R, Achten E, Cambier D, Danneels L (2010) A Magnetic resonance imaging investigation into the function of the deep cervical flexors during the performance of craniocervical flexion. *Journal of Manipulative and Physiological Therapeutics* 33: 286–291.
- Tsuyama K, Yamamoto Y, Fujimoto H, Adachi T, Nakazato K, et al. (2001) Comparison of the isometric cervical extension strength and a cross-sectional area of neck extensor muscles in college wrestlers and judo athletes. *European Journal of Applied Physiology* 84: 487–491.
- Wardlaw J, Bastin M, Valdés Hernández M, Muñoz Maniega S, Royle N, et al. (2011) Brain ageing, cognition in youth and old age, and vascular disease in the Lothian Birth Cohort 1936: rationale, design and methodology of the imaging protocol. *International Journal of Stroke*.
- Gountouna VE, Job DE, McIntosh AM, Moorhead TWJ, Lymer GKL, et al. (2010) Functional Magnetic Resonance Imaging (fMRI) reproducibility and variance components across visits and scanning sites with a finger tapping task. *Neuroimage* 49: 552–560.
- Moorhead TWJ, Gountouna V-E, Job DE, McIntosh AM, Romaniuk L, et al. (2009) Prospective multi-centre Voxel Based Morphometry study employing scanner specific segmentations: procedure development using CaliBrain structural MRI data. *BMC Med Imaging* 9: 8.

D Job, the radiographers and staff at The Brain Research Imaging Centre, Edinburgh, UK.

TWJ Moorhead, Division of Psychiatry, University of Edinburgh, UK.  
CA Greig, Department of Geriatric Medicine, University of Edinburgh, UK.

The SINAPSE Collaboration ([www.sinapse.ac.uk](http://www.sinapse.ac.uk)).

## Author Contributions

Conceived and designed the experiments: AK DS IJD SML JMW JMS. Performed the experiments: AK DS. Analyzed the data: AK DS JMW JMS. Contributed reagents/materials/analysis tools: CG IJD SML JMW JMS. Wrote the paper: AK DS CG IJD SML JMW JMS.

20. Dai TH, Liu JZ, Sahgal V, Brown RW, Yue GH (2001) Relationship between muscle output and functional MRI-measured brain activation. *Experimental Brain Research* 140: 290–300.
21. Kongsgaard M, Backer V, Jorgensen K, Kjaer M, Beyer N (2004) Heavy resistance training increases muscle size, strength and physical function in elderly male COPD-patients—a pilot study. *Respiratory Medicine* 98: 1000–1007.
22. Godard MP, Williamson DL, Trappe SW (2002) Oral amino-acid provision does not affect muscle strength or size gains in older men. *Medicine & Science in Sports & Exercise* 34: 1126–1131.
23. McIntyre CW, Selby NM, Sigrist M, Pearce LE, Mercer TH, et al. (2006) Patients receiving maintenance dialysis have more severe functionally significant skeletal muscle wasting than patients with dialysis-independent chronic kidney disease. *Nephrology Dialysis Transplantation* 21: 2210–2216.
24. Janssen I, Heymsfield SB, Wang ZM, Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology* 89: 81–88.
25. Gallagher D, Visser M, deMeersman RE, Sepulveda D, Baumgartner RN, et al. (1997) Appendicular skeletal muscle mass: Effects of age, gender, and ethnicity. *Journal of Applied Physiology* 83: 229–239.
26. Gallagher D, Heymsfield SB (1998) Muscle distribution: Variations with body weight, gender, and age. *Applied Radiation and Isotopes* 49: 733–734.
27. Bannerman E, Reilly JJ, MacLennan WJ, Kirk T, Pender F (1997) Evaluation of validity of British anthropometric reference data for assessing nutritional state of elderly people in Edinburgh: cross sectional study. *British Medical Journal* 315: 338–341.
28. Doherty TJ (2003) Aging and sarcopenia. *Journal of Applied Physiology* 95: 1717–1727.
29. Larsson L (1978) Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study. *Acta Physiol Scand Suppl* 457: 1–36.
30. Andersen JL (2003) Muscle fibre type adaptation in the elderly human muscle. *Scandinavian Journal of Medicine & Science in Sports* 13: 40–47.
31. Lexell J, Taylor CC, Sjoström M (1988) What is the cause of the aging atrophy - total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15-year-old to 83-year-old men. *Journal of the Neurological Sciences* 84: 275–294.

RESEARCH ARTICLE

Open Access

# Neck muscle cross-sectional area, brain volume and cognition in healthy older men; a cohort study

Alixé HM Kilgour<sup>1,2\*</sup>, Karen J Ferguson<sup>1,2</sup>, Calum D Gray<sup>3,6</sup>, Ian J Deary<sup>1,4</sup>, Joanna M Wardlaw<sup>1,3,5,6</sup>, Alasdair MJ MacLulich<sup>1,2</sup> and John M Starr<sup>1,2</sup>

## Abstract

**Background:** Two important consequences of the normal ageing process are sarcopenia (the age-related loss of muscle mass and function) and age-related cognitive decline. Existing data support positive relationships between muscle function, cognition and brain structure. However, studies investigating these relationships at older ages are lacking and rarely include a measure of muscle size. Here we test whether neck muscle size is positively associated with cognition and brain structure in older men.

**Methods:** We studied 51 healthy older men with mean age 73.8 (sd 1.5) years. Neck muscle cross-sectional area (CSA) was measured from T1-weighted MR-brain scans using a validated technique. We measured multiple cognitive domains including verbal and visuospatial memory, executive functioning and estimated prior cognitive ability. Whole brain, ventricular, hippocampal and cerebellar volumes were measured with MRI. General linear models (ANCOVA) were performed.

**Results:** Larger neck muscle CSA was associated with less whole brain atrophy ( $t = 2.86$ ,  $p = 0.01$ , partial eta squared 17%). Neck muscle CSA was not associated with other neuroimaging variables or current cognitive ability. Smaller neck muscle CSA was unexpectedly associated with higher prior cognition ( $t = -2.12$ ,  $p < 0.05$ , partial eta squared 10%).

**Conclusions:** In healthy older men, preservation of whole brain volume (i.e. less atrophy) is associated with larger muscle size. Longitudinal ageing studies are now required to investigate these relationships further.

**Keywords:** Sarcopenia, Cognition, Aging, Muscle cross-sectional area, Brain volume

## Background

As the population of the world ages, governments, research funding bodies and the general public are becoming increasingly interested in promoting healthy ageing. Healthy ageing is not just the avoidance of pathology but also the slowing down of the natural rate of ageing, mainly through lifestyle adaptations. Two important consequences of the normal ageing process are sarcopenia and age-related cognitive decline (ARCD).

Sarcopenia is the loss of muscle mass and function with advancing age [1-3]. It is a main component of the

frailty syndrome, and greater degrees of sarcopenia are associated with increased levels of falls, disability, morbidity and death [4-6]. Muscle mass is lost from the third decade at a rate of 1-2% per year, increasing with age [7-9]. Muscle function deteriorates more quickly, with studies showing strength to decline by 1-4% per year and power to decline by 3-4% per year [8,10]. ARCD is the normal and universal change in cognition seen with increasing age [11]. Such changes mainly affect the so-called 'fluid' abilities (eg working memory, speed of processing, reasoning). Crystallised abilities (eg. vocabulary, knowledge, autobiographical memory) remain largely intact [12,13]. Studies have shown that the decline seen in fluid cognitive abilities begins in early adulthood, with some deterioration seen by the early 20s [14].

\* Correspondence: a.kilgour@ed.ac.uk

<sup>1</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, 7 George Square, Edinburgh EH8 9JZ, UK

<sup>2</sup>Geriatric Medicine Unit, University of Edinburgh, Edinburgh, UK

Full list of author information is available at the end of the article

Until recently, muscle and brain ageing had rarely been studied in tandem; however, studies demonstrating improved cognition in later life with increased physical activity have highlighted this important area of research. For example a Cochrane review of randomised controlled trials found evidence that aerobic physical activities improved cognitive function in healthy older adults, with effects observed for motor function, cognitive speed, and auditory and visual attention [15]. Observational studies have also found positive relationships between physical activity and cognitive function [16,17]. There is some evidence that both grey and white matter brain volume can be significantly improved by aerobic exercise in older adults [18]. It was hypothesized that these relationships were due solely to cardiovascular fitness, and although this may play a role, animal studies have found other underlying mechanisms including: increased levels of brain-derived neurotrophic factor which may contribute to neurogenesis, effects on neurotransmitter systems and increased insulin-like growth factor 1 [19].

Several studies have also demonstrated a positive relationship between muscle function (eg handgrip strength, gait speed) and cognition [20-23]. Possible mechanisms which might account for the shared variance in muscle and brain size and function with healthy ageing include: the role of hormones and growth factors (eg glucocorticoids) [24,25]; immunosenescence and inflammation (eg IL6 and CRP) [26,27]; oxidative stress and mitochondrial ageing [28,29]; decreased stem cell activity [30,31]; and environmental and lifestyle factors (eg smoking) [32,33].

The above findings showing association between brain and muscle structure and function add support to the common cause hypothesis, that core underlying processes determine the rate of ageing in each organ throughout the body. If we are able to demonstrate an association between muscle size and brain size and function, this could add further weight to the common cause hypothesis [34-37]. This would have large implications for future treatments designed to modify the rate of ageing, which could possibly target several organs at once (eg muscle and brain) as opposed to individualised treatments being developed.

It is known that muscle size and function (ie strength or power) do not age in parallel [10,38], therefore the association between muscle size and either brain structure (eg whole brain volume) or cognitive function requires independent study. We found only one study which has investigated the relationship between muscle bulk and brain structure in older adults; however, this study included subjects with Alzheimer's disease along with normal controls [39]. The studies investigating muscle size and cognition have largely relied on simple cognitive screening tools (eg Mini Mental State Examination

(MMSE)) and do not contain a measure or estimate of prior cognitive ability [40-42].

Here we studied community-dwelling healthy older men, measuring: neck muscle cross-sectional area (CSA), multiple cognitive domains including estimated prior cognitive ability and neuroimaging volumes. We hypothesised that lower muscle bulk is associated with structural markers of brain ageing, and poorer cognitive ability in healthy older people.

## Methods

### Participants

Participants were 51 community-dwelling men involved in a longitudinal ageing study investigating healthy ageing, glucocorticoid status and brain structure [43,44]. The study was approved by the Lothian Health Ethics Committee. All subjects gave written informed consent and the research was carried out in compliance with the Helsinki Declaration. Data from the second wave of the study were used because the smaller MR head coil used in the first wave excluded the neck muscles. Exclusion criteria were previously provided [43,44]. Participants were healthy, lacking of significant illness, including dementia, stroke, ischaemic heart disease, depressive illness, excessive alcohol intake, and cancer. No participants were taking psychotropic medication.

### MR brain imaging

The full MR brain imaging protocol has been previously published [43]. In summary, imaging was performed on a GE Signa LX 1.5 T (General Electric) MR scanner. Participants received a sagittal T<sub>1</sub>-weighted spin echo sequence covering the whole head (TR 450 msec, TE 9 msec, FOV 24 cm, matrix 256 × 224, slice thickness 5 mm (no gap)) and a volume scan consisting of a T<sub>1</sub>-weighted 3D inversion recovery prepared sequence (3D IR\_PREP) acquired in the coronal plane with slices perpendicular to the long axis of the hippocampus and covering the whole head (TI 600 msec with TE set to minimum, FOV 22 cm, matrix 256 × 192, slice thickness 1.7 mm (no gap)).

### Brain structure measurements

Image analysis was performed using Analyze v7.0 for Windows (Mayo Clinic, Rochester, MA). Whole brain, hippocampal and ventricular volumes [43], and intracranial area (a validated estimate of intracranial volume [45]) were obtained by an experienced rater.

### Neck muscle cross-sectional area

We used neck muscle cross-sectional area (CSA) as a measure of muscle size. We have previously shown in a study of 24 subjects that neck muscle CSA is strongly correlated with thigh muscle CSA ( $R^2$  0.77), which is



often used as a proxy for general muscle bulk [46]. Neck muscle CSA is generally available in brain MRI studies, whereas thigh muscle CSA is not usually measured within longitudinal cognitive ageing studies. Neck muscle CSA was measured using a validated technique [46]. Full details can be obtained in the technique paper; however, in summary, the mid-point of the C2-vertebra was located in the sagittal slice of a 3D reconstructed image. The image was then converted to a transverse view and the posterior neck muscles were outlined using a cursor. The software then calculated the contained area. The muscles measured were the semispinalis capitis, splenius capitis and trapezius (measured as a combined group), and the sternocleidomastoid.

### Tests of cognitive function

The following tests of cognitive function were performed as part of the original study, as previously described [43]: the Mini-Mental State Examination (MMSE, a screening test for cognitive impairment); the Controlled Word Association Test (CWAT, tests verbal fluency, which is an aspect of executive function); the Digit-Symbol Substitution Test (DSST, tests attention and processing speed) from the Wechsler Adult Intelligence Scale; Raven's Standard Progressive Matrices (RSPM, tests non-verbal reasoning, an important aspect of fluid intelligence); Logical Memory (tests immediate and delayed verbal declarative memory); Visual Reproduction (tests immediate and delayed visual memory); Rey's Auditory-verbal Learning Test (tests verbal memory and learning); Benton's Visual Retention Test (tests visual memory); and the National Adult Reading Test (NART, provides an estimate of prior general cognitive ability). Due to the strong correlation between pre-morbid cognitive ability (of which NART provides an estimate) and educational achievement [47], it was decided to include only NART and not educational achievement as a predictor variable. One participant had a MMSE below 24 and was excluded from the analysis as this score may be reflective of an incipient diagnosis of dementia.

### Statistical analysis

Descriptive statistics, exploratory analyses, general linear modeling (Analysis of Covariance; ANCOVA) and principal components analysis were performed on SPSS version 18.0 for Windows. Missing values were excluded listwise. For the ANCOVA we constructed baseline models with the measures of brain structure and cognitive ability as dependent (i.e. outcome) variables and neck muscle CSA as an independent variable, adjusting for intracranial area (ICA) and age. ICA is thought not to change after the onset of age-related neuronal loss, particularly in men, therefore can be used as a marker of peak brain size, thus allowing the outcome variable to be

**Table 1 Neuroimaging and muscle cross-sectional area data**

|  | N  | Mean   | Std. Deviation |
|--|----|--------|----------------|
| Whole brain volume (cm <sup>3</sup> )        | 50 | 1149.8 | 90.4           |
| Intracranial area (cm <sup>2</sup> )         | 50 | 157.9  | 9.6            |
| Total ventricular volume (cm <sup>3</sup> )* | 50 | 31.2   | 24.0           |
| Total hippocampal volume (cm <sup>3</sup> )  | 48 | 7.0    | 0.8            |
| Cerebellar volume (cm <sup>3</sup> )         | 50 | 135.4  | 14.3           |
| Total SCM muscle area (cm <sup>2</sup> )     | 49 | 5.1    | 1.1            |
| Total comb muscle area (cm <sup>2</sup> )    | 49 | 22.8   | 3.1            |
| Total muscle area (cm <sup>2</sup> )         | 49 | 28.0   | 3.4            |
| Valid N (listwise)                           | 47 |        |                |

\*Non-parametric data, median and inter-quartile range presented.

more reflective of brain atrophy [48]. Also, without adjusting for ICA it could be argued that those with bigger skulls require larger neck muscles for support or that they are just larger in proportion; therefore, this adjustment also controls for this. We also adjusted for NART since this was found to correlate significantly with neck muscle CSA, brain volumes and current cognition. Furthermore, in the models with current cognitive ability as an outcome variable, adjusting for the NART score allows the model to reflect the degree of cognitive ageing that has taken place.

### Results

All participants (n = 51) were male with a mean age of 73.8 years (sd 1.5). Descriptive statistics for participant neuroimaging and neck muscle cross-sectional area data are in Table 1. Of the 51 MR brain scans reviewed, we were unable to measure neck muscle CSA on one scan as the scan did not extend far enough inferiorly to include the neck muscles at the required level for measurement

**Table 2 Prior and current cognition data**

|                                       | N  | Mean | Std. Deviation |
|---------------------------------------|----|------|----------------|
| National adult reading test *         | 47 | 42.0 | 13.0           |
| Mini mental state Examination *       | 50 | 28.0 | 4.0            |
| Controlled word association test      | 47 | 40.4 | 10.9           |
| Digit symbol substitution test        | 46 | 43.3 | 10.7           |
| Raven's standard progressive matrices | 46 | 40.0 | 9.1            |
| Logical memory                        | 47 | 51.6 | 15.0           |
| Visual reproduction                   | 47 | 46.4 | 14.8           |
| Auditory-verbal learning test         | 47 | 49.9 | 9.0            |
| Benton visual retention test *        | 47 | 17.0 | 4.0            |
| Factor 1 (Memory)                     | 45 | 0.0  | 1.0            |
| Factor 2 (Cognitive processing)       | 45 | 0.0  | 1.0            |
| Valid N (listwise)                    | 45 |      |                |

\*Non-parametric data, median and inter-quartile range presented.

**Table 3 Spearman's rho correlations between neuroimaging, muscle and cognitive variables (with p values)**

|                             | Total neck muscle CSA | NART            |
|-----------------------------|-----------------------|-----------------|
| Age                         | .21<br>(.15)          | -.03<br>(.82)   |
| Whole brain volume          | .07<br>(.62)          | .49<br>(<0.001) |
| Intracranial area           | -.11<br>(.44)         | .56<br>(<0.001) |
| Total ventricular volume    | .13<br>(.10)          | .10<br>(.50)    |
| Hippocampal volume          | -.07<br>(.67)         | .48<br>(<0.001) |
| Cerebellar volume           | .03<br>(.83)          | .46<br>(<0.001) |
| Memory factor               | -.28<br>(.07)         | .13<br>(.40)    |
| Cognitive processing factor | -.03<br>(.83)         | .52<br>(<0.001) |
| NART-                       | -.36<br>(.01)         | 1.00            |

(the mid-point of the C2-vertebra). In a further two cases, the hippocampal volume could not be measured.

Table 2 contains the descriptive statistics for the cognitive test data. To reduce the risk of type 1 statistical error by testing multiple associations, we performed principal components analysis. The Kaiser-Meyer-Olkin measure verified sampling adequacy. Two principal components were extracted employing varimax rotation; the first had an eigenvalue of 2.58, explaining 36.9% of the variance and the second had an eigenvalue of 2.4, explaining 34.3% of the variance. For comprehensibility, we refer to these components as 'factors' as is general usage. Together, the factors explain 71.2% of the variance in the cognitive test scores. The cognitive tests which focused on memory (ie Logical Memory, Visual

Reproduction, AVLT and BVRT) had high factor loadings for Factor 1; therefore, we hereafter call this factor the Memory Factor. Factor 2 had high factor loadings for the three other tests: CWAT, DSST and RSPM and hereafter is referred to as the Cognitive Processing Factor.

Initially bivariate statistics were performed (Spearman's rho) (Table 3). The only variable to significantly correlate with total neck muscle CSA was NART (rho  $-.36$ ,  $p = .01$ ). NART was also found to correlate strongly with the following variables: intracranial area; unadjusted whole brain, hippocampal, and cerebellar volumes; and the cognitive processing factor.

ANCOVA was performed to check for shared variance among neck muscle CSA and the neuroimaging measures, the cognitive factors, and NART. Models were corrected for age, intracranial area (ICA, to correct for head size) and NART, except in the model for NART where only age and ICA were adjusted for. Total neck muscle CSA was found to predict 17% of the variance in whole brain volume ( $t = 2.86$ ,  $p = 0.01$ ) (Table 4). However, total neck muscle CSA did not significantly predict the variance in ventricular, hippocampal or cerebellar volumes ( $p > 0.05$ ).

Neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor ( $p > 0.05$ ) (Tables 5 & 6). Using the NART score as an outcome variable, we found that total neck muscle CSA predicts 10% of the variance in the NART score ( $t = -2.12$ ,  $p < 0.05$ ) after adjusting for ICA and age.

## Discussion

We found that in healthy elderly men, preservation of whole brain volume was associated with larger total neck muscle cross-sectional area. Therefore in an elderly cohort, those that have a smaller muscle bulk have undergone more brain atrophy. This finding supports the common cause hypothesis, by demonstrating that the rate of sarcopenia and ARCD may occur in parallel within individuals, driven by core underlying biological processes. However, we found no significant association between total neck muscle CSA and ventricular volume

**Table 4 ANCOVA for whole brain volume with total neck muscle area, intracranial area, age and prior cognition as predictor variables**

| Source                                   | Degrees of freedom | F     | Unstandardised B* | t    | Sig. | Partial Eta squared |
|--|--------------------|-------|-------------------|------|------|---------------------|
| Corrected model                          | 4                  | 12.85 |                   |      | <.01 | .56                 |
| Intercept                                | 1                  | 1.06  | 491.40            | 1.03 | .31  | .03                 |
| Age (years)                              | 1                  | 0.79  | -5.64             | -.89 | .38  | .02                 |
| Intracranial area (cm <sup>2</sup> )     | 1                  | 13.13 | 0.04              | 3.62 | <.01 | .24                 |
| NART (Score out of 50)                   | 1                  | 8.43  | 3.85              | 2.90 | .01  | .17                 |
| Total neck muscle CSA (cm <sup>2</sup> ) | 1                  | 8.16  | 0.09              | 2.86 | .01  | .17                 |
| Corrected total                          | 45                 |       |                   |      |      |                     |

\*Unstandardised B coefficients reflect change in whole brain volume in cm<sup>3</sup>.



**Table 5 ANCOVA for Memory factor with total neck muscle area, intracranial area, age and prior cognition as predictor variables**

| Source                                   | Degrees of freedom | F    | Unstandardised B* | t     | Sig. | Partial Eta squared |
|--|--------------------|------|-------------------|-------|------|---------------------|
| Corrected model                          | 4                  | 1.23 |                   |       | .31  | .11                 |
| Intercept                                | 1                  | 1.39 | 8.91              | 1.18  | .25  | .03                 |
| Age (years)                              | 1                  | 1.30 | -.12              | -1.14 | .26  | .03                 |
| Intracranial area (cm <sup>2</sup> )     | 1                  | 0.02 | <-.01             | -.15  | .88  | .00                 |
| NART (Score out of 50)                   | 1                  | 1.56 | .03               | 1.25  | .22  | .04                 |
| Total neck muscle CSA (cm <sup>2</sup> ) | 1                  | 0.28 | <.01              | -.53  | .60  | .01                 |
| Corrected total                          | 43                 |      |                   |       |      |                     |

\*Unstandardised B coefficients reflect change in factor score for the Memory Factor.

(a different measure of brain atrophy), or hippocampal or cerebellar volumes.

We unexpectedly found that total neck muscle CSA was significantly negatively associated with estimated prior cognitive ability (NART) after adjustment for ICA and age, but we found no significant association between total neck muscle CSA and current cognitive abilities. This suggests that those with lower prior cognitive ability may have larger muscles in old age. Muscle mass in old age is determined by 2 factors. Firstly, peak muscle bulk obtained in young adulthood, and secondly, rate of muscle atrophy with ageing. Therefore we hypothesise that those with lower cognitive abilities may have undertaken more manual work [49,50] and therefore achieved a greater peak muscle bulk and a larger muscle mass in old age. We can find no plausible explanation as to why a lower prior cognitive ability would favour a slower rate of muscle atrophy. We unfortunately do not have sufficiently detailed previous occupational history or socio-economic class data for the participants to be able to test this theory further at this point.

We found only one previous study which investigated the relationship between muscle size and brain size, and this study also found a positive relationship between muscle bulk and whole brain volume. Burns et al. studied elderly people with early Alzheimer's disease (AD) (n = 70) or normal cognition (n = 70) and found that whole brain volume, normalized for head size, was

predictive of lean mass as measured by DEXA (Beta .20, p < .001) in both groups [39]. White matter volume was the primary driving factor for the relationship (Beta .19, p < .001) while grey matter volume showed no association with lean mass. This indicates that the cause of loss of lean muscle mass in AD may be different to normal ageing as it is primarily grey matter that is lost in AD.

In the above study Burns et al. also investigated the relationship between MMSE and a measure of global cognitive performance (a composite score made up of the results of a battery of tests, including the DSST and verbal fluency) with muscle mass [15]. They found a significant positive association between both the global cognitive performance score (Beta .12, p = .007) and MMSE (Beta .11, p = .009) and muscle mass, controlling for age and sex but not for prior cognition which we have shown to correlate with both brain and muscle size (Table 3). Our study was able to investigate the relationship between cognitive decline, by adjusting for prior cognition using the NART score, and current cognition, whereas this study only looked at cross-sectional data from current cognition. This may explain why they found an association between current cognition and muscle mass and we did not.

Several large studies have also investigated the links between muscle size and cognition. In a large cross-sectional study of community dwelling women aged 75 or over (n = 7105), Nourhashemi et al. found that low

**Table 6 ANCOVA for General Processing Factor with total neck muscle area, intracranial area, age and prior cognition as predictor variables**

| Source                                   | Degrees of freedom | F     | Unstandardised B* | t     | Sig. | Partial Eta squared |
|--|--------------------|-------|-------------------|-------|------|---------------------|
| Corrected model                          | 4                  | 5.19  |                   |       | <.01 | .35                 |
| Intercept                                | 1                  | 0.23  | 3.06              | .48   | .63  | .01                 |
| Age (years)                              | 1                  | 1.60  | -.11              | -1.26 | .21  | .04                 |
| Intracranial area (cm <sup>2</sup> )     | 1                  | 0.15  | <.01              | .39   | .70  | .00                 |
| NART (Score out of 50)                   | 1                  | 11.86 | .06               | 3.44  | <.01 | .23                 |
| Total neck muscle CSA (cm <sup>2</sup> ) | 1                  | 2.86  | <.01              | 1.69  | .10  | .07                 |
| Corrected total                          | 43                 |       |                   |       |      |                     |

\*Unstandardised B coefficients reflect change in factor score for the General Processing Factor.

cognitive function was associated with low fat free mass [41]. However the cognitive test used was the Short Portable Mental Status Questionnaire (SPMSQ), which consists of only 10 questions and is mainly used as a screening test for cognitive impairment.

Conversely, Wirth et al. studied 4095 consecutive geriatric hospital patients and found that fat-free mass was not associated with cognitive dysfunction, measured using MMSE, after adjusting for age, sex and Barthel index [42]. Also, Auyeung et al. studied 2737 cognitively normal older people and found that appendicular skeletal muscle mass (ASM) was significantly predictive of MMSE 4 years later in men but not women [40]. However, after adjustment for age, years of education and baseline MMSE score, the relationship in men was not significant either.

Our study has the benefit of including tests of both prior and current cognitive function. This allows us to look at cognitive decline rather than purely at current cognitive ability, and is the only study we could find that specifically tested the relationship between prior cognition and muscle size. Also, the three large studies mentioned above used cognitive tests which are primarily designed to screen for cognitive impairment (ie SPMSQ and MMSE) rather than to detect the subtleties of change in cognition with age [40-42], for which our cognitive tests were specifically chosen. Burns et al. used more detailed cognitive tests; however the numbers involved in their study are much smaller compared to the other three studies. Our study is the first to measure muscle cross-sectional area and cognition or brain size; the above mentioned studies used either bioimpedance analysis or DEXA as the measure of muscle bulk.

The main limitations of our study are the lack of longitudinal data and the small sample size. In ageing studies longitudinal data are crucial as it is the rate of loss of muscle size or brain size that is of interest rather than measurements at a cross-sectional time point. With brain size we can partially correct for this using intracranial area, but with muscle size we are unsure if someone has lost 10% of their lean body mass in the previous decade or 50%, as clearly the peak muscle bulk obtained will affect the final outcome greatly. The study also contained mainly white males and this will affect the generalisability of our results.

## Conclusion

In healthy older men preservation of whole brain volume is associated with larger muscle size and larger muscle size was associated with lower prior cognition, but not current cognition. These results support previous work in this area which has also found associations between brain and muscle variables in older adults.

Longitudinal ageing studies are now required to investigate these relationships further.

## Competing interests

The authors have declared that no competing interests exist.

## Authors' contributions

Conceived and designed the project: AK IJD JMW AMJM JMS. Performed the measurements: AK, KJF, AMJM. Analyzed the data: AK IJD JMW AMJM JMS. Contributed analysis tools: CDG. Wrote the paper: AK KJF CDG IJD JMW AMJM JMS. All authors read and approved the final manuscript.

## Acknowledgements

Dr AHM Kilgour was fully funded during this research by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC), Economic and Social Research Council (ESRC) and Medical Research Council (MRC) is gratefully acknowledged. The Chief Scientist's Office of the Scottish Government funded the original study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. S Semple, T MacGillivray and staff at the Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, UK. D Job, the radiographers and staff at The Brain Research Imaging Centre, Edinburgh, UK. The SINAPSE Collaboration ([www.sinapse.ac.uk](http://www.sinapse.ac.uk)).

## Author details

<sup>1</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, 7 George Square, Edinburgh EH8 9JZ, UK. <sup>2</sup>Geriatric Medicine Unit, University of Edinburgh, Edinburgh, UK. <sup>3</sup>Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK. <sup>4</sup>Department of Psychology, University of Edinburgh, Edinburgh, UK. <sup>5</sup>Brain Research Imaging Centre, Clinical Neurosciences, University of Edinburgh, Edinburgh, UK. <sup>6</sup>Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE), Edinburgh, UK.

Received: 30 October 2012 Accepted: 22 February 2013

Published: 28 February 2013

## References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinkova E, Vandewoude M, Zamboni M: **Sarcopenia: European consensus on definition and diagnosis.** *Age Ageing* 2010, **39**:412-423.
2. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, Abellan van Kan G, Andrieu S, Bauer J, Breuille D, Cederholm T, Chandler J, De Meynard C, Donini L, Harris T, Kann T, Keime Guibert F, Onder G, Papanicolaou D, Rolland Y, Rooks D, Sieber C, Souhami E, Verlaan S, Zamboni M: **Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia.** *J Am Med Dir Assoc* 2011, **12**:249-256.
3. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, Fearon KC, Laviano A, Maggio M, Fanelli FR, Schneider SM, Schols A, Sieber CC: **Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by special interest groups (sig) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics".** *Clin Nutr* 2010, **29**:154-159.
4. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, Corsi AM, Rantanen T, Guralnik JM, Ferrucci L: **Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia.** *J Appl Physiol* 2003, **95**:1851-1860.
5. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD: **Epidemiology of sarcopenia among the elderly in new mexico.** *Am J Epidemiol* 1998, **147**:755-763.
6. Rantanen T: **Muscle strength, disability and mortality.** *Scand J Med Sci Sports* 2003, **13**:3-8.

7. Janssen I, Heymsfield SB, Wang ZM, Ross R: **Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr.** *J Appl Physiol* 2000, **89**:81–88.
8. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, Newman AB, Hlth ABCS: **The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study.** *J Gerontol A Biol Sci Med Sci* 2006, **61**:1059–1064.
9. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R: **Aging of skeletal muscle: A 12-yr longitudinal study.** *J Appl Physiol* 2000, **88**:1321–1326.
10. Skelton DA, Greig CA, Davies JM, Young A: **Strength, power and related functional ability of healthy people aged 65–89 years.** *Age Ageing* 1994, **23**:371–377.
11. Deary IJ, Corley J, Gow AJ, Harris SE, Houlihan LM, Marioni RE, Penke L, Rafnsson SB, Starr JM: **Age-associated cognitive decline.** *Br Med Bull* 2009, **92**:135–152.
12. Hedden T, Gabrieli JD: **Insights into the ageing mind: a view from cognitive neuroscience;** *Nat rev neurosci. England* 2004, **5**:87–96.
13. Park DC, Reuter-Lorenz P: **The adaptive brain: aging and neurocognitive scaffolding.** *Annu Rev Psychol* 2009, **60**:173–196.
14. Salthouse TA: **When does age-related cognitive decline begin?** *Neurobiol aging United States* 2009, **30**:507–514.
15. Angevaren M, Aufdemkampe G, Verhaar HJ, Aleman A, Vanhees L: **Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment.** *Cochrane Database Syst Rev* 2008:CD005381.
16. Coley N, Andrieu S, Gardette V, Gillette-Guyonnet S, Sanz C, Vellas B, Grand A: **Dementia prevention: methodological explanations for inconsistent results.** *Epidemiol Rev* 2008, **30**:35.
17. Weuve J, Kang JH, Manson JE, Breteler MM, Ware JH, Grodstein F: **Physical activity, including walking, and cognitive function in older women.** *JAMA* 2004, **292**:1454–1461.
18. Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, Elavsky S, Marquez DX, Hu L, Kramer AF: **Aerobic exercise training increases brain volume in aging humans.** *J Gerontol A Biol Sci Med Sci* 2006, **61**:1166–1170.
19. Kramer AF, Erickson KI, Colcombe SJ: **Exercise, cognition, and the aging brain.** *J Appl Physiol* 2006, **101**:1237–1242.
20. Rosano C, Simonsick EM, Harris TB, Kritchevsky SB, Brach J, Visser M, Yaffe K, Newman AB: **Association between physical and cognitive function in healthy elderly: the health, aging and body composition study.** *Neuroepidemiology* 2005, **24**:8–14.
21. Fitzpatrick AL, Buchanan CK, Nahin RL, Dekosky ST, Atkinson HH, Carlson MC, Williamson JD: **Ginkgo evaluation of memory study I: associations of gait speed and other measures of physical function with cognition in a healthy cohort of elderly persons.** *J Gerontol A Biol Sci Med Sci* 2007, **62**:1244–1251.
22. Raji MA, Kuo YF, Snih SA, Markides KS, Peek MK, Ottenbacher KJ: **Cognitive status, muscle strength, and subsequent disability in older Mexican Americans.** *J Am Geriatr Soc* 2005, **53**:1462–1468.
23. Deary IJ, Johnson W, Gow AJ, Pattie A, Brett CE, Bates TC, Starr JM: **Losing one's grip: a bivariate growth curve model of grip strength and nonverbal reasoning from age 79 to 87 years in the lothian birth cohort 1921.** *J Gerontol B Psychol Sci Soc Sci* 2011, **66**:609–707.
24. Meaney MJ, Odonnell D, Rowe W, Tannenbaum B, Steverman A, Walker M, Nair NPV, Lupien S: **Individual-differences in hypothalamic-pituitary-adrenal activity in later life and hippocampal aging.** *Exp Gerontol* 1995, **30**:229–251.
25. Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, Arias J, Salehian B: **Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression.** *Am J Physiol Endocrinol Metab* 2003, **285**:E363–E371.
26. Weaver JD, Huang MH, Albert M, Harris T, Rowe JW, Seeman TE: **Interleukin-6 and risk of cognitive decline - macarthur studies of successful aging.** *Neurology* 2002, **59**:371–378.
27. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, Harris TB: **Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the health abc study.** *J Gerontol A Biol Sci Med Sci* 2002, **57**:M326–M332.
28. Kachiwala SJ, Harris SE, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ: **Genetic influences on oxidative stress and their association with normal cognitive ageing.** *Neurosci Lett* 2005, **386**:116–120.
29. Trounce I, Byrne E, Marzuki S: **Decline in skeletal-muscle mitochondrial respiratory-chain function - possible factor in aging.** *Lancet* 1989, **1**:637–639.
30. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn A-M, Nordborg C, Peterson DA, Gage FH: **Neurogenesis in the adult human hippocampus.** *Nat Med* 1998, **4**:1313–1317.
31. Welle S: **Cellular and molecular basis of age-related sarcopenia.** *Can J Appl Physiol* 2002, **27**:19–41.
32. Castillo EM, Goodman-Gruen D, Kritz-Silverstein D, Morton DJ, Wingard DL, Barrett-Connor E: **Sarcopenia in elderly men and women: the rancho bernardo study.** *Am J Prev Med* 2003, **25**:226–231.
33. Anstey KJ, von Sanden C, Salim A, O'Kearney R: **Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies.** *Am J Epidemiol* 2007, **166**:367–378.
34. Baltes PB, Lindenberger U: **Emergence of a powerful connection between sensory and cognitive functions across the adult life span: a new window to the study of cognitive aging?** *Psychol Aging* 1997, **12**:12–21.
35. Lindenberger U, Baltes PB: **Sensory functioning and intelligence in old-age - a strong connection.** *Psychol Aging* 1994, **9**:339–355.
36. Anstey KJ, Smith GA: **Interrelationships among biological markers of aging, health, activity, acculturation, and cognitive performance in late adulthood.** *Psychol Aging* 1999, **14**:605–618.
37. Christensen H, Mackinnon AJ, Korten A, Jorm AF: **The "common cause hypothesis" of cognitive aging: evidence for not only a common factor but also specific associations of with vision and grip strength in a cross-sectional analysis.** *Psychol Aging* 2001, **16**:588–599.
38. Young A, Stokes M, Crowe M: **The size and strength of the quadriceps muscles of old and young men.** *Clin Physiol* 1985, **5**:145–154.
39. Burns JM, Johnson DK, Watts A, Swerdlow RH, Brooks WM: **Reduced lean mass in early alzheimer disease and its association with brain atrophy.** *Arch Neurol* 2010, **67**:428–433.
40. Auyeung T, Lee J, Kwok T, Woo J: **Physical frailty predicts future cognitive decline - a four-year prospective study in 2737 cognitively normal older adults.** *J Nutr Health Aging* 2011, **15**:690–694.
41. Nourhashemi F, Andrieu S, Gillette-Guyonnet S, Reynish E, Albarede J-L, Grandjean H, Vellas B: **Is there a relationship between fat-free soft tissue mass and low cognitive function? Results from a study of 7,105 women.** *J Am Geriatr Soc* 2002, **50**:1796–1801.
42. Wirth R, Smoliner C, Sieber C, Volkert D: **Cognitive function is associated with body composition and nutritional risk cognitive function is associated with body composition and nutritional risk of geriatric patients.** *J Nutr Health Aging* 2011, **15**:706–710.
43. MacLulich AM, Ferguson KJ, Reid LM, Deary IJ, Starr JM, Wardlaw JM, Walker BR, Andrew R, Seckl JR: **11beta-hydroxysteroid dehydrogenase type 1, brain atrophy and cognitive decline.** *Neurobiol Aging* 2012, **33**:207. e201-208.
44. MacLulich AM, Deary IJ, Starr JM, Ferguson KJ, Wardlaw JM, Seckl JR: **Plasma cortisol levels, brain volumes and cognition in healthy elderly men.** *Psychoneuroendocrinology* 2005, **30**:505–515.
45. Ferguson KJ, Wardlaw JM, Deary IJ, MacLulich AMJ: **Intracranial area: a validated method for estimating intracranial volume.** *J Neuroimaging* 2005, **15**:76–78.
46. Kilgour A, Subedi D, Gray C, Deary I, Lawrie S, Wardlaw J, Starr J: **Design and validation of a novel method to measure cross-sectional area of neck muscles included during routine MR brain volume imaging.** *PLoS One* 2012, **7**.
47. Deary I, Strand S, Smith P, Fernandes C: **Intelligence and educational achievement.** *Intelligence* 2007, **35**:13–21.
48. Mori E, Hirono N, Yamashita H, Imamura T, Ikejiri Y, Ikeda M, Kitagaki H, Shimomura T, Yoneda Y: **Premorbid brain size as a determinant of reserve capacity against intellectual decline in alzheimer's disease.** *Am J Psychiatry* 1997, **154**:18–24.
49. Neisser U, Boodoo G, Bouchard TJ, Boykin AW, Brody N, Ceci SJ, Halpern DF, Loehlin JC, Perloff R, Sternberg RJ, Urbina S: **Intelligence: knowns and unknowns.** *Am Psychol* 1996, **51**:77–101.
50. Mackintosh NJ: **IQ and human intelligence.** New York, NY, US: Oxford University Press; 1998.

doi:10.1186/1471-2318-13-20

**Cite this article as:** Kilgour et al.: Neck muscle cross-sectional area, brain volume and cognition in healthy older men; a cohort study. *BMC Geriatrics* 2013 **13**:20.

RESEARCH

Open Access

# Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly

Alixé HM Kilgour<sup>1,2,5\*</sup>, Charlotte Firth<sup>6</sup>, Rowan Harrison<sup>2</sup>, Paul Moss<sup>6</sup>, Mark E Bastin<sup>1,3</sup>, Joanna M Wardlaw<sup>1,3</sup>, Ian J Deary<sup>1,4</sup> and John M Starr<sup>1,2</sup>

## Abstract

**Background:** Sarcopenia is an important cause of morbidity and mortality in older adults, with immunosenescence and inflammation being possible underlying mechanisms. We investigated the relationship between latent cytomegalovirus (CMV) infection, Interleukin 6 (IL-6) levels, muscle size and strength in a group of healthy older community-dwelling people.

**Methods:** Participants were healthy volunteers from the Lothian Birth Cohort 1936 study. Participants had IL-6 level and CMV antibody titre measured at age 70 years and grip strength and a volumetric T1-weighted MRI brain scan (allowing measurement of neck muscle cross-sectional area (CSA)) at age 73. Markers of childhood deprivation were adjusted for in the analysis due to correlations between childhood deprivation and latent CMV infection.

**Results:** 866 participants were studied; 448 men (mean age 72.48 years, sd 0.70) and 418 women (mean age 72.51 years, sd 0.72). In men, CMV seropositivity was associated with smaller neck muscle CSA ( $p = 0.03$ , partial eta squared = 0.01), even after adjustment for IL-6 levels. Neck muscle CSA was not associated with CMV seropositivity in women, or CMV antibody titre or IL-6 level in either sex. Grip strength associated negatively with IL-6 level (right grip strength  $p < 0.00001$ , partial eta squared 0.032 and left grip strength  $p < 0.00001$ , partial eta squared 0.027) with or without adjustment for CMV serostatus or antibody titre. CMV status and antibody titre were not significantly associated with grip strength in either hand.

**Conclusion:** These findings support the hypothesis that there is a relationship between markers of immunosenescence (i.e. CMV serostatus and IL6 level) and low muscle mass and strength and longitudinal studies in older cohorts are now required to investigate these relationships further.

**Keywords:** Sarcopenia, Grip strength, Cytomegalovirus, Interleukin-6, Immunosenescence

## Introduction

Sarcopenia refers to the decline in both muscle mass and function that occurs with age [1-3]. It is an important cause of morbidity and mortality in older adults [4-6]. Longitudinal studies have shown muscle mass to be lost at approximately 1% per annum and strength to be lost at 2-4% per annum in older adults [7,8]. Despite its clinical importance, current understanding of the mechanisms

underlying sarcopenia remains unclear. Immunosenescence and inflammation have both been identified as possible contributing factors. Immunosenescence is the age-associated impairment of immune function due to changes in both the innate and adaptive immune response [9]. These changes lead to a decreased ability to respond to pathogens, although an agreement on clinical biomarkers and associated clinical outcomes requires further research [9].

Seropositivity for cytomegalovirus (CMV, otherwise known as Human Herpes Virus 5), is common in older adults [10,11]. Most people are infected in childhood or young adulthood and become carriers of the virus in a

\* Correspondence: a.kilgour@ed.ac.uk

<sup>1</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, 7 George Square, Edinburgh EH8 9JZ, UK

<sup>2</sup>Geriatric Medicine Unit, University of Edinburgh, Edinburgh, UK

Full list of author information is available at the end of the article



latent state for the rest of their lives [12-14]. The role of CMV status in immunosenescence is a topic of current research, and it remains unclear whether the relationship is causal or associative. However, latent CMV infection has been linked to several clinical outcomes, including frailty and increased mortality. Several studies have found an association between CMV seropositivity and/or CMV antibody titre and the presence of atherosclerosis and coronary heart disease, with some studies also demonstrating a correlation with survival time [15-17]. Other studies have found associations between CMV and cognitive decline in older adults [18], and all cause mortality [19].

There is also evidence of an association between CMV infection and frailty. Aiello et al. [20] found that CMV antibody titre is negatively associated with the ability to carry out activities of daily living (ADLs) in elderly Latino subjects, after correcting for gender and age. However, this relationship became non-significant after adjusting for the total number of health conditions, body mass index, and household income. Schmaltz et al. found an association between frailty, defined using the Fried criteria, and CMV serostatus in older women [20]. Studies investigating frailty vary widely on the criteria used for diagnosis and not all frailty scores contain a measure of muscle mass [21,22]. Therefore, in order for clear conclusions to be drawn about the possible underlying mechanisms of sarcopenia, it is important to study it as an independent variable rather than as a component of a frailty score.

Interleukin 6 (IL-6) is a cytokine known to be part of the acute phase response, i.e. the initial immune system reaction to infection or trauma [23]. Increasing age is associated with latent low grade inflammation; levels of IL-6 appear to increase with age, particularly following the andropause or menopause [24,25]. In a large cross-sectional study of septuagenarians, raised IL-6 levels were associated with reduced muscle mass and strength [26]. However, in a further longitudinal cohort higher IL-6 levels were associated with loss of muscle strength, although no association was found with muscle mass [27].

Several studies investigating the effect of CMV on frailty and functional ability have adjusted for IL-6 to assess its role as a mediator, as CMV is known to increase IL-6 gene expression and production in peripheral blood mononuclear cells [28]. Indeed in the above mentioned study, Schmaltz et al. found that CMV positive subjects with high IL-6 levels had a significantly higher prevalence of frailty than those with a low IL-6 level [20]. Also, data from the Women's Health and Aging Studies found that IL-6 appeared to modulate the effect of CMV antibody titre on frailty as an outcome (measured using the Fried criteria), although the effect did not reach statistical significance [29].

We could find no previous studies which have looked at the association between muscle mass and CMV serostatus or antibody titre. Furthermore we found only one study which addressed the relationship between CMV serostatus and handgrip strength [21], an important marker of muscle function in older age, and that study only looked at women. As detailed above, IL-6 may play an important mediatory role in these relationships and it is therefore important to study this in tandem with CMV status. In this study we investigated the relationship between latent CMV infection, IL-6 level and markers of sarcopenia (muscle size and strength) in a healthy older cohort of community-dwelling men and women.

## Results

There were 866 participants in wave 2 of the LBC1936 study; 448 men (mean age 72.48 years, sd 0.70) and 418 women (mean age 72.51 years, sd 0.72). This represents 79.4% of the participants who attended at the first wave of testing aged 70 years (n=1091). Baseline data for neck muscle CSA, right and left grip strength, CMV serostatus, CMV antibody titre and IL-6 levels are shown in Table 1. Baseline data for measures of childhood deprivation are shown in Table 2.

We assessed associations between IL-6 and CMV antibody titre (using Spearman's rho correlations) and CMV serostatus (using the Wilcoxon independent samples test) with age, height, weight, the muscle variables and the

**Table 1 Muscle size and strength, CMV and IL-6 baseline data**

| Variable                                      | Statistic/group | Men         | Women       |
|---|-----------------|-------------|-------------|
| <b>Total neck muscle CSA (mm<sup>2</sup>)</b> | Mean            | 2576.6      | 1814.5      |
|   | SD              | 421.0       | 281.2       |
|   | n               | 343         | 298         |
| <b>Right grip strength (kg)</b>               | Mean            | 35.49       | 21.28       |
|   | SD              | 6.82        | 5.54        |
|   | n               | 448         | 416         |
| <b>Left grip strength (kg)</b>                | Mean            | 34.69       | 19.93       |
|   | SD              | 6.57        | 5.13        |
|   | n               | 447         | 416         |
| <b>CMV status</b>                             | Positive (%)    | 60.6        | 69.0        |
|   | Negative (%)    | 39.4        | 31.1        |
|   | n               | 439         | 409         |
| <b>CMV antibody titre</b>                     | Median          | 60.56       | 132.11      |
|   | IQ range        | 0.47-214.00 | 2.40-277.78 |
|   | n               | 437         | 406         |
| <b>IL-6 level</b>                             | Median          | 1.60        | 1.48        |
|   | IQ range        | 1.05-2.42   | 1.01-2.29   |
|   | n               | 425         | 390         |

**Table 2 Childhood deprivation baseline data**

| Variable                           | Statistic/group | Men       | Women     |
|------------------------------------|-----------------|-----------|-----------|
| Overcrowding index (age 11)        | Median          | 1.20      | 1.20      |
|                                    | IQ range        | 0.86-1.67 | 0.80-1.67 |
|                                    | n               | 447       | 416       |
| Indoor/outdoor toilet (age 11)     | Indoor (%)      | 87.5      | 90.0      |
|                                    | Outdoor (%)     | 12.5      | 10.0      |
|                                    | n               | 447       | 418       |
| Father's social class              | I (%)           | 6.9       | 7.3       |
|                                    | II (%)          | 21.5      | 17.2      |
|                                    | III (%)         | 56.7      | 54.7      |
|                                    | IV (%)          | 6.9       | 13.3      |
|                                    | V (%)           | 7.9       | 7.6       |
|                                    | n               | 404       | 384       |
| Years spent in full time education | Median          | 10        | 10        |
|                                    | IQ range        | 10-12     | 10-12     |
|                                    | n               | 448       | 418       |

measures of childhood deprivation (Table 3). The association between CMV serostatus and indoor/outdoor toilet was analysed using the chi-square test. In men, being CMV seropositive or having a high CMV titre is associated with all the markers of childhood deprivation. In women,

being CMV seropositive or having a high CMV titre is associated with a higher overcrowding index and lower social class of their father and in addition a high CMV titre is associated with fewer years of formal education. In men, IL-6 levels only significantly correlate with the number of years of full time education (i.e. the more years of education the lower the IL-6 level), whereas in women all the markers of childhood deprivation correlate with a higher IL-6 level, except for the indoor/outdoor toilet question. If IL-6 was acting as mediator for CMV infection (i.e. CMV infection causes inflammation, raising IL6 levels, which causes increased sarcopenia), we would expect a correlation between the two variables. However, the Spearman's rho correlation between IL-6 and CMV antibody titre was non-significant (rho=0.06, p=0.07), while a Wilcoxon independent samples test for IL-6 and CMV serostatus was also non-significant (test statistic 1.28, p=0.20).

General linear models (ANCOVAs) were then created for each muscle variable separately with each measure of immune status (ie CMV status, CMV titre and IL-6 level) before rerunning the models of CMV status and antibody titre adjusting for IL-6 status also. Tables 4 and 5 contain the results for the ANCOVA for CMV serostatus and neck muscle CSA, and IL-6 and grip strength. The p value gives the significance of the independent variable's association

**Table 3 Wilcoxon independent samples test (CMV serostatus) and Spearman's rho correlations (CMV antibody titre and IL-6 level) (p values)**

|  | Male                               |                                    |                                    | Female                             |                                  |                                   |
|--|------------------------------------|------------------------------------|------------------------------------|------------------------------------|----------------------------------|-----------------------------------|
|  | CMV status (pos/neg) <sup>a</sup>  | CMV titre <sup>b</sup>             | IL-6 titre <sup>b</sup>            | CMV status (pos/neg) <sup>a</sup>  | CMV titre <sup>b</sup>           | IL-6 titre <sup>b</sup>           |
| Age in days at wave 2                    | .68<br>(.50)                       | .09<br>(.06)                       | .09<br>(.08)                       | <b>2.32</b><br>(.02)               | .17<br>( <b>&lt;.01</b> )        | .08<br>(.14)                      |
| Height in cm                             | <b>-2.08</b><br>(.04)              | <b>-.11</b><br>(.02)               | <b>-.12</b><br>(.01)               | <b>-2.50</b><br>(.01)              | -.09<br>(.07)                    | .00<br>(.95)                      |
| Weight in kg                             | 1.44<br>(.15)                      | .07<br>(.15)                       | <b>.16</b><br>( <b>&lt;.01</b> )   | -1.06<br>(.29)                     | .02<br>(.71)                     | <b>.30</b><br>( <b>&lt;.001</b> ) |
| Total neck muscle CSA (mm <sup>2</sup> ) | -1.60<br>(.11)                     | -.06<br>(.26)                      | .09<br>(.10)                       | 1.02<br>(.31)                      | .09<br>(.12)                     | <b>.21</b><br>( <b>&lt;.001</b> ) |
| Grip strength right hand (kg)            | -.62<br>(.54)                      | -.05<br>(.34)                      | <b>-.25</b><br>( <b>&lt;.001</b> ) | <b>-2.81</b><br>(.01)              | <b>-.10</b><br>(.04)             | -.07<br>(.16)                     |
| Grip strength left hand (kg)             | -1.95<br>(.05)                     | <b>-.10</b><br>(.03)               | <b>-.25</b><br>( <b>&lt;.001</b> ) | <b>-2.04</b><br>(.04)              | -.08<br>(.12)                    | -.08<br>(.11)                     |
| Overcrowding index age 11                | <b>4.53</b><br>( <b>&lt;.001</b> ) | <b>.17</b><br>( <b>&lt;.001</b> )  | .03<br>(.56)                       | <b>3.56</b><br>( <b>&lt;.001</b> ) | <b>.16</b><br>( <b>&lt;.01</b> ) | .10<br>(.04)                      |
| Father's job class as a number           | <b>3.82</b><br>( <b>&lt;.001</b> ) | <b>.19</b><br>( <b>&lt;.001</b> )  | .03<br>(.55)                       | <b>2.51</b><br>(.01)               | <b>.14</b><br>( <b>&lt;.01</b> ) | .16<br>( <b>&lt;.01</b> )         |
| No. of years of full-time education      | <b>-3.07</b><br>( <b>&lt;.01</b> ) | <b>-.17</b><br>( <b>&lt;.001</b> ) | <b>-.13</b><br>(.01)               | -1.57<br>(.12)                     | <b>-.11</b><br>(.01)             | <b>-.10</b><br>(.04)              |
| Indoor=1 or outdoor=2 toilet at age 11   | <b>0.47*</b><br>(.02)              | <b>.10</b><br>(.03)                | .04<br>(.37)                       | 0.69*<br>(.33)                     | .06<br>(.18)                     | .05<br>(.30)                      |

<sup>a</sup>Wilcoxon independent samples test statistic (and associated p values) for CMV status and all predictor variables except indoor/outdoor toilet age 11, which was analysed using the chi-square test and shows \*the odds ratio for being CMV seropositive if indoor toilet age 11.

<sup>b</sup>The columns for CMV titre and IL-6 titre show the Spearman's rho correlation with the predictor variables (and the associated p value).

**Table 4 ANCOVA for CMV status and total neck muscle CSA**

| Source                                 | Sig.  | Partial eta squared |
|--|-------|---------------------|
| Age in days at wave 2                  | .38   | <.01                |
| Weight in kg                           | <.001 | .22                 |
| Sex (Male=1, Female=2)                 | <.001 | .42                 |
| Overcrowding index age 11              | .29   | <.01                |
| Indoor=1 or outdoor=2 toilet at age 11 | .88   | <.01                |
| Father's job class as a number         | .70   | <.01                |
| No. of years of full-time education    | .43   | <.01                |
| CMV serostatus (neg=1, pos=2)          | .10   | .01                 |
| Sex by CMV serostatus                  | .028  | .01                 |

and the partial eta squared gives a measure of effect size, for which Cohen [30] says the following: small  $\geq 0.0099$ ; medium  $\geq 0.0588$ ; large  $\geq 0.1379$ .

We found that CMV seropositivity was associated with a smaller neck muscle CSA in men but not in women ( $p = 0.028$ , partial eta squared = 0.01) (Table 4). In this model, lower weight and female sex were also associated with smaller neck muscle CSA. When this model was corrected for IL-6 level the effect remained significant ( $p = 0.047$ ). The model for neck muscle CSA and CMV antibody titre showed no significant association, both with and without adjustment for IL-6 level, nor did the model with IL-6 as predictor variable, without adjusting for CMV infection.

Weaker grip strength in both right and left hands was found to be associated with higher IL-6 level; right grip strength  $p < 0.00001$ , partial eta squared = 0.032 and left grip strength  $p < 0.00001$ , partial eta squared = 0.027 (Table 5). The associations remain strongly positive even after adjustment for CMV status ( $p < 0.0001$ ) and CMV antibody titre ( $p < 0.0001$ ). In these models (shown in Table 5), older age, shorter stature and female sex were

**Table 5 ANCOVA for IL-6 titre and grip strength right and left hands**

| Source                                 | Right hand |                     | Left hand |                     |
|--|------------|---------------------|-----------|---------------------|
|  | Sig.       | Partial eta squared | Sig.      | Partial eta squared |
| Age in days at Wave 2                  | .005       | .01                 | .004      | .01                 |
| Height                                 | <.001      | .08                 | <.001     | .07                 |
| Sex (Male=1, Female=2)                 | <.001      | .28                 | <.001     | .34                 |
| Overcrowding index age 11              | .72        | .00                 | .25       | .00                 |
| Indoor=1 or outdoor=2 toilet at age 11 | .75        | .00                 | .64       | .00                 |
| Father's job class as a number         | .95        | .00                 | .65       | .00                 |
| No. of years of full-time education    | .051       | .01                 | .10       | .00                 |
| IL-6 Level                             | <.001      | .03                 | <.001     | .03                 |

also significantly associated with weaker grip strength in both hands. The models using CMV status and antibody titre alone were not significantly associated with grip strength in either hand.

## Discussion

This report used data from waves 1 and 2 of a population-based elderly cohort study, to investigate the relationship between latent CMV infection, IL-6 levels and sarcopenia, measured using neck muscle CSA and grip strength in both hands. There was no significant group difference for sex, age or CMV status and titre between those who participated in wave 1 but not wave 2 ( $n=225$ ) and those who participated in both waves ( $n=866$ ) (independent t tests,  $p > 0.05$ ). We found that men who were seropositive for CMV antibody at age 70 years had a neck muscle CSA on average 4% smaller at age 73 than men who were seronegative. This effect remained positive whether adjusting for IL-6 level or not. It is well documented that muscle mass is lost at roughly 1% per year [7,8], therefore being a man who is CMV seropositive in your 70s confers the same risks of low muscle bulk as being 4 years older. We did not detect a significant association in women between CMV serostatus and neck muscle CSA, or between CMV serostatus and grip strength in either hand. Other studies have postulated that as CMV seropositivity is so common in older adults it may be more important to measure CMV antibody titre itself. However, we found no association between CMV antibody titre and either neck muscle CSA or grip strength. This result may indicate that latent CMV infection leads to increased muscle loss over an extended period, as CMV is commonly acquired in childhood, though there is the possibility of temporary reactivations of CMV throughout life, and that the titre reflects the current situation, which may have less impact on muscle bulk. Longitudinal studies will be able to explore these relationships further.

IL-6 levels were found to strongly predict grip strength in both right and left hands in men and women. IL-6 predicted 3.2% of the variance in right-sided grip strength and 2.7% of the variance in left-sided grip strength. These associations remained significant when adjusting for CMV serostatus or antibody titre. We found no significant association between IL-6 levels and neck muscle CSA. Therefore our findings do not support previous work that has found that IL-6 may act as a mediator by which latent CMV infection causes frailty [20,29].

It is widely accepted that muscle size and strength do not decline in a parallel manner [31,32], therefore they are not purely a function of each other and it may be that different factors cause decline in one parameter more than the other, as our results have shown. Similarly, a study looking at the effect of IL-6 levels on muscle found a

significant association with decline in grip strength but not muscle mass [27], again indicating parameter-specific effects. Also, these results are based on cross-sectional data and therefore do not necessarily reflect changes with age. Therefore it could be that individuals with latent CMV infection or lifelong raised IL6 levels have always had smaller/weaker muscles, rather than an increased rate of decline in muscle mass or function with age. However as sarcopenia is currently diagnosed using reference to peers or a healthy young population, having a lower peak muscle mass and function should still be considered risk factors for sarcopenia. Longitudinal studies may help elucidate these relationships further.

The sole previous study we found that investigated the relationship between CMV status and grip strength only included women [21]. They found no significant difference in grip strength between seropositive and seronegative women. We have replicated this finding, but found a sex specific effect between male sex and CMV serostatus. There is evidence that men lose more muscle mass than women with age even after correction for body stature [33], therefore differing factors may play more or less of a role between the genders. Additionally, their muscles are larger to start with and therefore a reduction may be easier to detect.

It is unclear how CMV might directly influence physical functioning, however latent CMV infection has been identified as an important component of an immune risk phenotype that is associated with immunosenescence, inflammation, and several latent health conditions observed with aging [34]. All these may represent possible contributory mechanisms to sarcopenia. For example, if CMV infection predisposes to cardiovascular disease this, in turn, might limit exercise and hence loss of muscle strength and size. Should our findings be confirmed, exploration of potential causal pathways will be warranted.

Raised plasma IL-6 levels are known to increase proteolysis within muscle, by upregulating the proteolytic UPP pathway [35], however it is not known if the degree of increase seen with ageing is enough to cause atrophy and it is thought that proteolysis is not a major factor in normal ageing muscle [36]. However, IL-6 may exert its effect through less direct routes. It is known that IL-6 causes anorexia, which would lead to decreased protein substrate. Also, IL-6 can both activate cortisol secretion and induce 11 $\beta$ -hydroxysteroid type 1 expression, so it may exert its effect via the steroid pathway [37,38]. Furthermore, animal studies have demonstrated that inflammatory cytokines can induce muscle apoptosis by DNA fragmentation [35], though such models may not represent low level inflammatory changes occurring over a prolonged period.

The narrow geographic, age and ethnic mix within the LBC1936 cohort means this study may not prove generalisable. However, the narrow age range helps to reduce

the powerful effect of advancing age on many of the parameters measured in this and other studies and which may have led to the impression of stronger direct relationships between co-associated variables than is actually the case. Additionally the size of the sample studied and the fact that we replicated results in some of our analyses found in other work is reassuring. The high correlation between the markers of childhood deprivation and CMV status were as predicted and raise the possibility that other correlates of childhood socioeconomic deprivation may be mediated by CMV infection, although this relationship may be less strong in other populations. Also, when studying sarcopenia it is important to consider rate of decline rather than solely cross-sectional measures. Therefore in the future, longitudinal studies will be crucial in developing an understanding of these relationships. Finally, the concept of a homogeneous model of sarcopenia, whereby all muscle throughout the body ages at the same rate, is proving increasingly unlikely to be valid with studies showing rates of muscle ageing to vary around the body [33,39,40]. Therefore whilst our measure of neck muscle CSA has previously been shown to correlate strongly to mid-thigh muscle CSA, it is important to consider that different factors may worsen or ameliorate muscle ageing in different muscle groups throughout the body.

## Conclusion

In a large population-based elderly cohort study we found the men who were seropositive for CMV had smaller neck muscle CSA than men who were seronegative, and this effect was independent of IL-6 level. We also found that higher IL-6 levels, but not CMV levels, were strongly associated with lower grip strength in both hands in men and women. These associations were not attenuated when the model was adjusted for CMV serostatus or antibody titre. These findings support the hypothesis that there is a relationship between immunosenescence and markers of sarcopenia and longitudinal studies are now required to investigate these relationships further.

## Methods

### Participants – The Lothian Birth Cohort 1936 (LBC1936)

The LBC1936 study consists of 1091 relatively healthy, age-homogeneous older people who, at the age of 11 years, participated in the Scottish Mental Survey of 1947, when they sat a general mental ability test, the Moray House Test number 12 (MHT). The cohort, including the imaging protocol, has been described previously in detail [41,42] At age 70 years they underwent a series of cognitive tests (including retaking the MHT), and physical and biochemical tests, at the Wellcome Trust Clinical Research Facility (WTCRF) at the Western General Hospital, Edinburgh, where 866 returned for further testing at age 73. All participants gave



written, informed consent to the study. All participants were Caucasian and almost all lived independently in the Lothian region (Edinburgh city and surrounding area) of Scotland.

#### **Neck muscle cross-sectional area**

In this study we use neck muscle cross-sectional area (CSA) as a validated measure of muscle size [32]. We have previously shown in a study of 24 subjects that neck muscle CSA is strongly correlated with thigh muscle CSA ( $R^2 = 0.77$ ), which is often used as a proxy for general muscle bulk. Neck muscle CSA is generally available in brain MRI studies, whereas thigh muscle CSA is not usually measured within longitudinal cognitive ageing studies.

Participants underwent a volumetric brain MRI scan as part of the LBC1936 study. MRI was performed with participants in a supine position using a 1.5 T clinical scanner (Signa HDxt, GE Healthcare, Milwaukee, USA) at the Brain Research Imaging Centre, University of Edinburgh ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)). A phased array eight channel head coil was used and inversion recovery prepared volumetric T1 weighted images were acquired in a coronal plane for each participant; the scan alignment was perpendicular to the long axis of the hippocampus determined from a preliminary T2-weighted sagittal sequence. The flip angle was 8°, bandwidth 15.63 KHz, echo time (TE) 4 to 13 ms, repetition time (TR) 9.6 ms and inversion or preparation time (TI) 500 ms. The field of view (FOV), fixed superiorly at the cranial vertex, was 25.6 × 25.6 cm, the acquisition matrix was 192×192, with 160 slices acquired with a slice thickness of 1.3 mm with no slice gap. These data took 8.13 minutes to acquire per patient.

Neck muscle CSA was measured using a validated technique as described previously [43]. In summary, the mid-point of the C2-vertebra was located in the sagittal slice of a 3D reconstructed image. The image was then converted to a transverse view and the posterior neck muscles were outlined using a cursor on a dedicated workstation. The software then calculated the contained area. The muscles groups measured were the semispinalis capitis, splenius capitis and trapezius (measured as a combined group), and the sternocleidomastoid. Each measurement was performed three times and the median for each value was used for the following analyses.

#### **Grip strength**

Grip strength was measured with a Jamar Hydraulic Hand Dynamometer, with all participants performing 3 trials with their right and left hands; the best of the 3 trials was used for the following analyses.

#### **CMV and IL-6 measures**

CMV was measured in plasma samples collected at age 70, using a CMV ELISA assay. Mock and viral-infected

lysate was coated onto ELISA plates and incubated overnight. Standards (a mixture of three CMV positive plasma samples) and plasma samples were added to the plates and incubated for one hour before washing. An anti-IgG horseradish peroxidase conjugated secondary antibody was then added to the plate to incubate for one hour. After washing, TMB substrate was added and the reaction stopped by addition of 1M HCL. The sample was assessed using an ELISA reader at 450 nm. To determine CMV titres, mock values were first subtracted from lysate values. The data were then analysed in PRISM, and CMV titres were calculated with reference to the standard curve. Values above 10 were considered to be seropositive. To ensure accuracy, all samples were tested in duplicate. IL-6 levels were analysed at the University of Glasgow using high sensitivity ELISA from R&D Systems. The minimum detectable dose ranged from 0.016-0.110 pg/mL (mean=0.039 pg/mL). The intra-assay CV ranged from 6.9 to 7.8%, while the inter-assay coefficient of variance ranged from 6.6 to 9.6%.

#### **Childhood deprivation**

At the age 70 assessment, participants were asked to provide background demographic and environmental information about their childhood, specifically for when they were aged about 11 years. Participants reported the number of people they lived with and the number of rooms in the house, which was used to calculate an overcrowding index (people/room). Participants also reported: whether their household had indoor or outdoor toilet facilities; their father's occupation to allow father's social class to be coded (categorised from I, professional, to V, unskilled); and the number of years they spent in full-time, formal education.

#### **Statistical analysis**

Descriptive statistics, exploratory analyses and general linear modeling (Analysis of Covariance; ANCOVA) were performed using SPSS version 18.0 for Windows (SPSS Inc, Chicago, Ill, USA). Missing values were excluded listwise for the ANCOVA analyses. For the ANCOVA, we constructed baseline models with the measures of neck muscle CSA and grip strength (right and left) as dependent (i.e. outcome) variables and CMV serostatus, CMV antibody titre and IL-6 level as independent variables, adjusting for age, gender and either height or weight, as a measure of body size, and the four measures of childhood deprivation. Total neck muscle CSA was found to correlate more strongly with weight ( $P < 0.001$ ), whereas both right and left grip strength correlated more strongly with height ( $p < 0.001$ ). Therefore we used the respective measures for adjustment in each of the analyses.

### Competing interests

The authors declared that they have no competing interests.

### Authors' contributions

AHMK and JMS proposed the hypotheses, performed the analyses and drafted the manuscript. AHMK performed the neck cross-sectional area measurements. IJD, JMS, MEB and JMW designed the LBC1936 study. CF, RH and PM designed and implemented the immunological measurements. All authors approved the final manuscript.

### Acknowledgements

Dr AHM Kilgour was funded during this research by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC), Economic and Social Research Council (ESRC) and Medical Research Council (MRC) is gratefully acknowledged. The LBC1936 studies have been funded by Age UK and the MRC. CMV materials were funded as part of a British Geriatrics Society start-up grant to Dr R Harrison. Ms C Firth is funded by an Age UK PhD studentship. JMW was part funded by the Scottish Funding Council through the Scottish Imaging Network, a Platform for Scientific Excellence (SINAPSE, www.sinapse.ac.uk) Initiative. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We thank A Gow, C Murray, J Corley, R Henderson and A Pattie at the Centre of Cognitive Aging and Cognitive Epidemiology, and MV Hernandez, SM Maniega, N Royle, E Sandeman, I Gerrish, of the Brain Research Imaging Centre, both of the University of Edinburgh, UK, for demographic and clinical data collection on the LBC1936. We would also like to thank the LBC1936 Study participants; CD Gray, S Semple, T MacGillivray and staff at the Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, UK; D Job, additional radiographers and other staff at The Brain Research Imaging Centre, Edinburgh, UK.

### Author details

<sup>1</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, 7 George Square, Edinburgh EH8 9JZ, UK. <sup>2</sup>Geriatric Medicine Unit, University of Edinburgh, Edinburgh, UK. <sup>3</sup>Brain Research Imaging Centre, Division of Neuroimaging Sciences, University of Edinburgh, Edinburgh, UK. <sup>4</sup>Department of Psychology, University of Edinburgh, Edinburgh, UK. <sup>5</sup>Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK. <sup>6</sup>School for Cancer Sciences, University of Birmingham, Birmingham B15 2TT, UK.

Received: 5 December 2012 Accepted: 12 August 2013

Published: 13 August 2013

### References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinkova E, Van-dewoude M, Zamboni M: **Sarcopenia: European consensus on definition and diagnosis.** *Age Ageing* 2010, **39**:412–423.
2. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, Abellan van Kan G, Andrieu S, Bauer J, Breuille D, Cederholm T, Chandler J, De-Meynard C, Donini L, Harris T, Kannt A, Keime Guibert F, Onder G, Papanicolaou D, Rolland Y, Rooks D, Sieber C, Souhami E, Verlaan S, Zamboni M: **Sarcopenia: An undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia.** *J Am Med Dir Assoc* 2011, **12**:249–256.
3. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, Fearon KC, Laviano A, Maggio M, Fanelli FR, Schneider SM, Schols A, Sieber CC: **Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by special interest groups (sig) "Cachexia-anorexia in chronic wasting diseases" and "Nutrition in geriatrics".** *Clin Nutr* 2010, **29**:154–159.
4. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di-Iorio A, Corsi AM, Rantanen T, Guralnik JM, Ferrucci L: **Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia.** *J Appl Physiol* 2003, **95**:1851–1860.
5. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD: **Epidemiology of sarcopenia among the elderly in New Mexico.** *Am J Epidemiol* 1998, **147**:755–763.
6. Rantanen T: **Muscle strength, disability and mortality.** *Scand J Med Sci Sports* 2003, **13**:3–8.
7. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, Newman AB, Hlth ABCS: **The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study.** *J Gerontol Biol Sci & Med Sci* 2006, **61**:1059–1064.
8. Visser M, Deeg DJH, Lips P: **Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the longitudinal aging study Amsterdam.** *J Clin Endocrinol Metab* 2003, **88**:5766–5772.
9. Pawelec G, Akbar A, Beverley P, Caruso C, Derhovanessian E, Fülöp T, Griffiths P, Grubeck-Loebenstien B, Hamprecht K, Jahn G, Kern F, Koch SD, Larbi A, Maier AB, Macallan D, Moss P, Samson S, Strindhall J, Trannoy E, Willis M: **Immunosenescence and cytomegalovirus: where do we stand after a decade?** *Immunity & Ageing* 2010, **7**:13.
10. Lubeck PR, Doerr HW, Rabenau HF: **Epidemiology of human cytomegalovirus (hcmv) in an urban region of Germany: what has changed?** *Med Microbiol Immunol* 2010, **199**:53–60.
11. Lopo S, Vinagre E, Palminha P, Paixao MT, Nogueira P, Freitas MG: **Seroprevalence to cytomegalovirus in the Portuguese population, 2002–2003.** *Euro Surveill* 2011, **16**:19896.
12. Soderberg-Naucler C, Nelson JY: **Human cytomegalovirus latency and reactivation - a delicate balance between the virus and its host's immune system.** *Intervirology* 1999, **42**:314–321.
13. Sinclair J: **Human cytomegalovirus: latency and reactivation in the myeloid lineage.** *J Clin Virol* 2008, **41**:180–185.
14. Slobedman B, Cao JZ, Avdic S, Webster B, McAllery S, Cheung AK, Tan JC, Abendroth A: **Human cytomegalovirus latent infection and associated viral gene expression.** *Future Microbiol* 2010, **5**:883–900.
15. Blankenberg S, Rupprecht HJ, Bickel C, Espinola-Klein C, Rippin G, Hafner G, Ossendorp M, Steinhagen K, Meyer J, Group ftA: **Cytomegalovirus infection with interleukin-6 response predicts cardiac mortality in patients with coronary artery disease.** *Circulation* 2001, **103**:2915–2921.
16. Muhlestein JB, Horne BD, Carlquist JF, Madsen TE, Bair TL, Pearson RR, Anderson JL: **Cytomegalovirus seropositivity and c-reactive protein have independent and combined predictive value for mortality in patients with angiographically demonstrated coronary artery disease.** *Circulation* 2000, **102**:1917–1923.
17. Strandberg TE, Pitkala KH, Tilvis RS: **Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease.** *Jama* 2009, **301**:380–382.
18. Aiello AE, Haan M, Blythe L, Moore K, Gonzalez JM, Jagust W: **The influence of latent viral infection on rate of cognitive decline over 4 years.** *J Am Geriatr Soc* 2006, **54**:1046–1054.
19. Roberts ET, Haan MN, Dowd JB, Aiello AE: **Cytomegalovirus antibody levels, inflammation, and mortality among elderly latinos over 9 years of follow-up.** *Am J Epidemiol* 2010, **172**:363–371.
20. Schmalz HN, Fried LP, Xue QL, Walston J, Leng SX, Semba RD: **Chronic cytomegalovirus infection and inflammation are associated with prevalent frailty in community-dwelling older women.** *J Am Geriatr Soc* 2005, **53**:747–754.
21. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA: **Cardiovascular Health study C: frailty in older adults: evidence for a phenotype.** *J Gerontol Sci & Med Sci* 2001, **56**:M146–M156.
22. Rockwood K, Mitnitski A: **Frailty in relation to the accumulation of deficits.** *J Gerontol A Biol Sci Med Sci* 2007, **62**:722–727.
23. Heinrich PC, Castell JV, Andus T: **Interleukin-6 and the acute phase response.** *Biochem J* 1990, **265**:621–636.
24. Hager K, Machein U, Krieger S, Platt D, Seefried G, Bauer J: **Interleukin-6 and selected plasma-proteins in healthy-persons of different ages.** *Neurobiol Aging* 1994, **15**:771–772.
25. Ershler WB, Keller ET: **Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty.** *Annu Rev Med* 2000, **51**:245–270.
26. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, Harris TB: **Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health Abc study.** *J Gerontol Sci & Med Sci* 2002, **57**:M326–M332.

27. Schaap LA, Pluijm SMF, Deeg DJH, Visser M: **Inflammatory markers and loss of muscle mass (sarcopenia) and strength.** *Am J Med* 2006, **119**:e9–e17.
28. Geist LJ, Dai LY: **Cytomegalovirus modulates interleukin-6 gene expression.** *Transplantation* 1996, **62**:653–658.
29. Wang GC, Kao WH, Murakami P, Xue QL, Chiou RB, Detrick B, McDyer JF, Semba RD, Casolaro V, Walston JD, Fried LP: **Cytomegalovirus infection and the risk of mortality and frailty in older women: A prospective observational cohort study.** *Am J Epidemiol* 2010, **171**:1144–1152.
30. Cohen J: *Statistical power analysis for the behavioral sciences*, ed *Second edition*. Hillsdale, New Jersey: Lawrence Erlbaum Associates; 1988.
31. Young A, Stokes M, Crowe M: **The size and strength of the quadriceps muscles of old and young men.** *Clin Physiol* 1985, **5**:145–154.
32. Skelton DA, Greig CA, Davies JM, Young A: **Strength, power and related functional ability of healthy people aged 65–89 years.** *Age Ageing* 1994, **23**:371–377.
33. Gallagher D, Visser M, De-Meersman RE, Sepulveda D, Baumgartner RN, Pierson RN, Harris T, Heymsfield SB: **Appendicular skeletal muscle mass: Effects of age, gender, and ethnicity.** *J Appl Physiol* 1997, **83**:229–239.
34. Pawelec G, Koch S, Franceschi C, Wikby A: **Human immunosenescence: does it have an infectious component?** *Ann N Y Acad Sci* 2006, **1067**:56–65.
35. Skipworth RJE, Stewart GD, Ross JA, Guttridge DC, Fearon KCH: **The molecular mechanisms of skeletal muscle wasting: Implications for therapy.** *Surgeon* 2006, **4**:273–283.
36. Tsujinaka T, Fujita J, Ebisui C, Yano M, Kominami E, Suzuki K, Tanaka K, Katsume A, Ohsugi Y, Shiozaki H, Monden M: **Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice.** *J Clin Invest* 1996, **97**:244–249.
37. Weber MM, Michl P, Auernhammer CJ, Engelhardt D: **Interleukin-3 and interleukin-6 stimulate cortisol secretion from adult human adrenocortical cells.** *Endocrinology* 1997, **138**:2207–2210.
38. Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, Strain A, Hewison M, Stewart PM: **Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: Tissue-specific induction by cytokines.** *Endocrinology* 2001, **142**:1982–1989.
39. Janssen I, Heymsfield SB, Wang ZM, Ross R: **Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr.** *J Appl Physiol* 2000, **89**:81–88.
40. Gallagher D, Heymsfield SB: **Muscle distribution: variations with body weight, gender, and age.** *Appl Radiat Isot* 1998, **49**:733–734.
41. Deary IJ, Gow AJ, Taylor MD, Corley J, Brett C, Wilson V, Campbell H, Whalley LJ, Visscher PM, Porteous DJ, Starr JM: **The Lothian Birth Cohort 1936: A study to examine influences on cognitive ageing from age 11 to age 70 and beyond.** *BMC Geriatr* 2007, **7**:28.
42. Deary IJ, Gow AJ, Pattie A, Starr JM: **Cohort profile: the Lothian Birth Cohorts of 1921 and 1936.** *Int J Epidemiol* 2012, **41**:1576–1584.
43. Kilgour A, Subedi D, Gray C, Deary I, Lawrie S, Wardlaw J, Starr J: **Design and validation of a novel method to measure cross-sectional area of neck muscles included during routine MR brain volume imaging.** *PLoS ONE* 2012, **7**:e34444.

doi:10.1186/1742-4933-10-33

**Cite this article as:** Kilgour et al.: Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly. *Immunity & Ageing* 2013 **10**:33.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit



# Increased Skeletal Muscle 11 $\beta$ HSD1 mRNA Is Associated with Lower Muscle Strength in Ageing

Alixé H. M. Kilgour<sup>1\*</sup>, Iain J. Gallagher<sup>2</sup>, Alasdair M. J. MacLulich<sup>1,2</sup>, Ruth Andrew<sup>3</sup>, Calum D. Gray<sup>4</sup>, Philippa Hyde<sup>2</sup>, Henning Wackerhage<sup>5</sup>, Holger Husi<sup>2</sup>, James A. Ross<sup>2</sup>, John M. Starr<sup>1</sup>, Karen E. Chapman<sup>3</sup>, Kenneth C. H. Fearon<sup>2</sup>, Brian R. Walker<sup>3</sup>, Carolyn A. Greig<sup>2</sup>

**1** Centre for Cognitive Ageing and Cognitive Epidemiology, Geriatric Medicine Unit, University of Edinburgh, Edinburgh, United Kingdom, **2** Department of Clinical and Surgical Sciences, Division of Health Sciences, School of Clinical Sciences, University of Edinburgh, Edinburgh, United Kingdom, **3** Endocrinology Unit, Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom, **4** Clinical Research Imaging Centre, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom, **5** School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

## Abstract

**Background:** Sarcopenia, the loss of muscle mass and function with age, is associated with increased morbidity and mortality. Current understanding of the underlying mechanisms is limited. Glucocorticoids (GC) in excess cause muscle weakness and atrophy. We hypothesized that GC may contribute to sarcopenia through elevated circulating levels or increased glucocorticoid receptor (GR) signaling by increased expression of either GR or the GC-amplifying enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1) in muscle.

**Methods:** There were 82 participants; group 1 comprised 33 older men (mean age 70.2years, SD 4.4) and 19 younger men (22.2years, 1.7) and group 2 comprised 16 older men (79.1years, 3.4) and 14 older women (80.1years, 3.7). We measured muscle strength, mid-thigh cross-sectional area, fasting morning plasma cortisol, quadriceps muscle GR and 11 $\beta$ HSD1 mRNA, and urinary glucocorticoid metabolites. Data were analysed using multiple linear regression adjusting for age, gender and body size.

**Results:** Muscle strength and size were not associated with plasma cortisol, total urinary glucocorticoids or the ratio of urinary 5 $\beta$ -tetrahydrocortisol +5 $\alpha$ -tetrahydrocortisol to tetrahydrocortisone (an index of systemic 11 $\beta$ HSD activity). Muscle strength was associated with 11 $\beta$ HSD1 mRNA levels ( $\beta$  -0.35,  $p$  = 0.04), but GR mRNA levels were not significantly associated with muscle strength or size.

**Conclusion:** Although circulating levels of GC are not associated with muscle strength or size in either gender, increased cortisol generation within muscle by 11 $\beta$ HSD1 may contribute to loss of muscle strength with age, a key component of sarcopenia. Inhibition of 11 $\beta$ HSD1 may have therapeutic potential in sarcopenia.

**Citation:** Kilgour AHM, Gallagher IJ, MacLulich AMJ, Andrew R, Gray CD, et al. (2013) Increased Skeletal Muscle 11 $\beta$ HSD1 mRNA Is Associated with Lower Muscle Strength in Ageing. PLoS ONE 8(12): e84057. doi:10.1371/journal.pone.0084057

**Editor:** Cedric Moro, INSERM/UMR 1048, France

**Received:** September 10, 2013; **Accepted:** November 18, 2013; **Published:** December 31, 2013

**Copyright:** © 2013 Kilgour et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The authors thank the British Heart Foundation and Chief Scientist Office of the Scottish Government for financial support. AHJK, AMJM and JMS are members of The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative. Funding from the BBSRC, EPSRC, ESRC and MRC is gratefully acknowledged. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** B.R.W. is an inventor on relevant patents owned by the University of Edinburgh and has consulted for several companies developing selective 11 $\beta$ HSD1 inhibitors. No other potential conflicts of interest relevant to this article were reported. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and material.

\* E-mail: a.kilgour@ed.ac.uk

## Introduction

Sarcopenia is the loss of muscle mass and function which accompanies even healthy ageing [1–3]. Both muscle mass and function (i.e., power and strength) begin to decline from the third decade with mass reducing by 1–2% per year and strength reducing by around 2% per year [4–8]. Sarcopenia is associated with an increased risk of falls and fractures, disability, loss of independence and mortality [9–11]. Despite this public health problem, current understanding of the mechanisms underlying sarcopenia is limited, hampering progress in the development of novel treatments for maintenance of muscle mass and physical

independence in old age. Theories underlying the development of sarcopenia include: inflammation, cellular senescence, hormones and growth factors and lifestyle factors (eg nutrition) [12,13].

One possible mechanism within the field of hormones and growth factors is glucocorticoid dysregulation. It is well known that glucocorticoids at pharmacological levels or in spontaneous Cushing's syndrome cause myopathy, with a combination of muscle atrophy and dysfunction. Glucocorticoids are believed to effect these changes on muscle through a combination of increased protein breakdown (particularly through the ubiquitin-proteasome system) [14], decreased protein synthesis (by inhibiting transport of amino acids into muscle and inhibiting the action of insulin and

IGF-1) [15] and decreasing production of IGF-1 and myostatin [14]. In the context of sarcopenia, this mechanism could occur via elevated circulating glucocorticoids due to age-related hypothalamic-pituitary-adrenal (HPA) axis dysregulation. Alternatively, it could occur selectively within the muscle, by increased activity of the glucocorticoid receptor (GR) or the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1). 11 $\beta$ HSD1 converts inactive cortisone to active cortisol and is known to be present and biologically active in human muscle as well as many other tissues [16–18]. Indeed a recent study by Tiganescu et al found that elevated 11 $\beta$ HSD1 activity was increased in skin biopsies from older adults compared to younger adults and that this increased activity was associated with markers of skin ageing (eg dermal atrophy and deranged collagen structural organization) [19]. Establishing links between GC and sarcopenia could lead to novel therapies, as several 11 $\beta$ HSD1 inhibitors are currently in clinical development for type 2 diabetes and other degenerative diseases, including cognitive dysfunction [20].

There is some evidence of an association between increased plasma and salivary cortisol and lower muscle mass and strength but these data are inconsistent [21–24]. Glucocorticoid metabolites in a 24 hour urine sample may be more informative than plasma cortisol levels since they reflect glucocorticoid status over the diurnal cycle. Additionally ratios of the metabolites can be used as an index of peripheral 11 $\beta$ HSD activity [25]. However, no studies to date have examined the relationship between urinary glucocorticoid metabolites and sarcopenia. Similarly, there are no published data examining the relationship between GR and 11 $\beta$ HSD1 expression and muscle loss and function in older adults. Importantly, expression of 11 $\beta$ HSD1 and GR mRNA has been shown to reflect glucocorticoid activity, for example there is a correlation between 11 $\beta$ HSD1 mRNA expression and enzyme function [26].

The aim of this study was to investigate the relationship between plasma and urinary glucocorticoid metabolites and levels of mRNA encoding GR and 11 $\beta$ HSD1 in skeletal muscle, with muscle size and strength. We hypothesized that increased glucocorticoid signaling in skeletal muscle acting through GR by, (a) elevated circulating cortisol, (b) increased expression of 11 $\beta$ HSD1 or (c) increased expression of GR, is associated with reduced muscle size and strength.

## Methods

### Participants

Participants were healthy volunteers recruited at two sites in Scotland: young and older men were recruited in Aberdeen (Group 1) and older men and women were recruited in Edinburgh (Group 2). This allowed us to test for possible age and gender effects. Participants were defined as healthy after applying previously published health selection criteria to the responses to a questionnaire [27]. Existing samples were available from two nearby cities in Scotland so these were used for analysis, rather than beginning a new de novo cohort collection. No comparisons were made between these two independent cohorts.

### Ethics Statement

Written informed consent was obtained and all procedures received local ethical committee approval. In Edinburgh this was by the Lothian Local Research Ethics Committee 02 and in Aberdeen this was by the North of Scotland Research Ethics Committees. The study conformed to the standards set by the Declaration of Helsinki.

### Anthropometry

Body weight was measured with participants in light clothing using a beam scale (Seca, UK). Height was measured using a wall mounted stadiometer.

### Muscle Function

Maximum voluntary isometric knee extensor strength was measured using an established method [28]. Following instruction, the participant made a maximum voluntary contraction (Newtons) which was held for 5 seconds. Three separate measurements were obtained and the highest value was used in subsequent analysis.

### Muscle Size

Mid-thigh quadriceps cross-sectional area (CSA) was measured using a 1.5T MR scanner (Phillips Gyroscan Intera). T1-weighted axial images were taken with the isocentre of the magnetic field located at the mid-femur point which was landmarked prior to the scan according to International Standards of Anthropometric Assessment (ISAK) guidelines 2001. Imaging parameters were: slice thickness 10 mm; acquisition matrix 512 $\times$ 512; echo time (TE) 15 ms; repetition time (TR) 425 ms; and flip angle 90°. The CSA of the quadriceps was quantified using Analyze 8.0 (Mayo Clinic, Rochester, USA) according to a previously published technique [29]. Two of the subjects from Group 2 did not undergo MRI due to claustrophobic symptoms.

### Plasma Cortisol

Blood samples were obtained from participants in the morning after overnight fast (mean time 0945h, range 0915–1030h). Plasma cortisol was measured by competitive immunoassay with direct chemiluminescent technology using the Bayer Advia Centaur method (see [http://labmed.ucsf.edu/labmanual/db/resource/Centaur\\_Cortisol.pdf](http://labmed.ucsf.edu/labmanual/db/resource/Centaur_Cortisol.pdf)).

### Quadriceps Muscle Biopsy

Quadriceps femoris samples were obtained from the region of vastus lateralis via percutaneous needle biopsy using a Bergstrom needle [30]. The biopsy was obtained in a sterile environment by sharp dissection under local anaesthetic using 1% lidocaine. The samples were then snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  before analysis [31].

### RNA Isolation

Total RNA was isolated from quadriceps muscle biopsies using the Qiazol reagent (Qiagen, Crawley, UK) and miRNeasy RNA isolation columns (Qiagen, Crawley, UK). Briefly biopsies were homogenised in 1400  $\mu\text{l}$  or 700  $\mu\text{l}$  Qiazol depending on the size of the tissue sample using a Polytron PT1200E (Kinematica AG). Total RNA was isolated from the homogenised muscle using miRNeasy columns with an on column DNase treatment step using the RNase-Free DNase Set (Qiagen, Crawley, UK). After elution from the column into 30  $\mu\text{l}$  nuclease free  $\text{H}_2\text{O}$ , RNA was quantified using the Nanodrop instrument (Labtech, UK) and quality assessed using the Bioanalyzer (Agilent, UK). All samples had 260/280 ratios above 1.8, and RIN scores above 7.5.

### cDNA Preparation and qPCR

RNA samples were converted to cDNA using the Ovation RNA Amplification kit (Nugen, Netherlands). RNA was diluted to 10 ng/ $\mu\text{l}$  and 50 ng total RNA was used in the amplification reaction carried out according to the manufacturer's instructions, yielding between 3  $\mu\text{g}$  and 11  $\mu\text{g}$  cDNA. For qPCR, cDNA was diluted to  $\sim 50$  ng/ $\mu\text{l}$ . Quantitative RT-PCR reactions were run,



**Table 1.** Group characteristics.

|  | Group 1 younger men (n = 19) | Group 1 older men (n = 33) | p-value <sup>a</sup> | Group 2 older men (n = 16) | Group 2 older women (n = 14) | p-value <sup>b</sup> |
|--|------------------------------|----------------------------|----------------------|----------------------------|------------------------------|----------------------|
| Age (years)                              | 22.2 (1.7)                   | 70.2 (4.4)                 | <0.001               | 79.1 (3.4)                 | 80.1 (3.7)                   | n/s                  |
| Height (cm)                              | 177.6 (6.7)                  | 171.9 (5.4)                | 0.001                | 171.3 (6.1)                | 157.6 (5.9)                  | <0.001               |
| BMI (kg/m <sup>2</sup> )                 | 24.0 (2.5)                   | 25.2 (2.5)                 | n/s                  | 25.3 (3.9)                 | 24.1 (3.1)                   | n/s                  |
| Muscle Size (cm <sup>2</sup> )           | 92.7 (11.5)                  | 67.3 (7.4)                 | <0.001               | 63.5 (7.3)                 | 43.8 (6.8)                   | <0.001               |
| Muscle Strength (Newton)                 | 774.9 (136.6)                | 525.2 (73.6)               | <0.001               | 364.7 (79.7)               | 273.4 (73.4)                 | 0.003                |
| Total Urinary GC* (microg/day)           | 9887 (7721–18372)            | 10224 (7841–17000)         | n/s                  | 8192 (5534–12506)          | 4925 (3699–6806)             | n/s                  |
| 11 $\beta$ HSD activity (urine THFs:THE) | 1.12 (0.37)                  | 1.15 (0.45)                | n/s                  | 1.28 (0.79)                | 0.81 (0.49)                  | n/s                  |
| Plasma cortisol (nmol/litre)             | –                            | –                          |                      | 349 (106)                  | 321 (65)                     | n/s                  |
| GR mRNA                                  | –                            | –                          |                      | 59.4 (24.5)                | 58.3 (18.8)                  | n/s                  |
| 11 $\beta$ HSD1 mRNA                     | –                            | –                          |                      | 25.3 (19.7)                | 32.2 (31.8)                  | n/s                  |

Data are mean (SD) except \*non-parametric data therefore median and IQ range shown.

a. Independent t test between younger and older men in Group 1.

b. Independent t test between men and women in Group 2.

n/s = not significant.

doi:10.1371/journal.pone.0084057.t001

in triplicate, on an Applied Biosystems Step One Plus system. The reaction mix was POWER SYBR Green x2 Master mix 12.5  $\mu$ l, forward primer (10  $\mu$ M) 1  $\mu$ l, reverse primer (10  $\mu$ M) 1  $\mu$ l, H<sub>2</sub>O 9.5  $\mu$ l and cDNA 1  $\mu$ l. Reaction conditions were 95°C for 10 mins, 95°C for 15s, 60°C for 60s (40 cycles) followed by melting curve generation from 60°C to 95°C. Ct values were examined and within triplicates any value greater than 0.3 Ct were removed before means were calculated. Data were then analysed using the delta Ct method with HPRT as a normaliser. After normalisation data were inverted and scaled such that the largest value for each gene was set to 100.

Primer sequences used were NR3C1 FP – CTGTCGCTTCTCAATCAGACTC; RP – GCATTGCT-TACTGAGCCTTTTG; 11 $\beta$ HSD1 FP – AGGCTGCTGCCTGCTTAGGA; RP – AGCCCCA-GAATGGGGAGGAGA; HPRT FP – TGACACTGGCAAAA-CAATGCA; RP- GGTCCCTTTTCACCAGCAAGCT. HPRT was chosen as a normaliser as preliminary analysis of housekeeping gene performance showed HPRT to be stable across samples and expressed at a similar level to genes of interest compared to b-actin, GAPDH, b2M and 18S.

### Urinary Glucocorticoid Metabolism

24 hour urine samples were collected to quantify urinary glucocorticoid metabolites using gas chromatography electron impact mass spectrometry following solid phase extraction, hydrolysis of conjugates and formation of their methoxime-trimethylsilyl derivatives, as described previously [25].

Two composites of the data were used in subsequent analyses. Firstly, total urinary steroids, comprising the sum of 5 $\beta$ -tetrahydrocortisol (5 $\beta$ THF), 5 $\alpha$ -tetrahydrocortisol (5 $\alpha$ THF), the main urinary metabolites of cortisol, and tetrahydrocortisone (THE), the main urinary metabolite of cortisone (total urinary GC = 5 $\beta$ THF + 5 $\alpha$ THF + THE). Secondly, an indirect indicator of systemic 11 $\beta$ HSD activity, comprising the ratio of 5 $\beta$ THF and 5 $\alpha$ THF to THE (ratio of cortisol to cortisone metabolites = (5 $\beta$ THF + 5 $\alpha$ THF)/THE).

### Statistical Analysis

Statistical analysis was performed using SPSS version 18.0. Bivariate correlations were performed using Spearman's rho to allow analysis of the non-parametric variables. Forced entry multiple linear regression was performed and the data from the

**Table 2.** Bivariate correlations including muscle size and strength.

|                         | Group 1 muscle size | Group 1 muscle strength | Group 2 muscle size | Group 2 muscle strength |
|-------------------------|---------------------|-------------------------|---------------------|-------------------------|
| Height                  | .34*                | .22                     | .68**               | .42*                    |
| BMI                     | -.04                | -.07                    | .32                 | .38*                    |
| Total urinary GC        | -.04                | .01                     | .61**               | .45*                    |
| 11 $\beta$ HSD activity | -.09                | -.05                    | .35                 | .16                     |
| Plasma cortisol         | –                   | –                       | -.20                | -.31                    |
| GR mRNA                 | –                   | –                       | .11                 | .04                     |
| 11 $\beta$ HSD1 mRNA    | –                   | –                       | -.15                | -.29                    |

Data are Spearman's Rho Correlation Coefficients.

\*\*p<0.01 (2-tailed).

\*p<0.05 (2-tailed).

doi:10.1371/journal.pone.0084057.t002

**Table 3.** Regression coefficients for the glucocorticoid measures in models predicting muscle size/strength (Group 1).

| Glucocorticoid Measure        | Muscle Size Beta (sig, n) | Muscle Strength Beta (sig, n) |
|-------------------------------|---------------------------|-------------------------------|
| Total Urinary GC <sup>a</sup> | -0.10 (p>0.05, 52)        | <-0.01 (p>0.05, 52)           |
| THFs:THE <sup>a</sup>         | <0.01 (p>0.05, 52)        | 0.04 (p>0.05, 52)             |

<sup>a</sup>adjusting for age and BMI.

doi:10.1371/journal.pone.0084057.t003

two groups were analysed separately, when possible, to test reproducibility. Due to the large difference in age between the older and younger groups, age was analysed as a binary variable in the multivariate regression. Group 1 (n = 52) had 80% power at the p = 0.05 level to detect a correlation of r = 0.38 and Group 2 (n = 30) had 80% power at the p = 0.05 level to detect a correlation of r = 0.49. In view of the power calculations and the exploratory nature of the study, adjusting for multiple hypotheses testing was not deemed to be appropriate.

## Results

In total, 82 participants were recruited. Table 1 shows numbers of participants and their age, height, BMI, and the main outcome variables for each group. Independent t tests found significant sex and age related differences for height, muscle size and muscle strength but not for BMI or any measure of glucocorticoid status (Table 1). Table 2 shows bivariate correlations, which confirmed that measures of body size (height and BMI) were significantly associated with muscle size and strength. Therefore in constructing multivariate models we adjusted for body size as well as age and gender, which had been selected *a priori* due to their accepted relationships with muscle size and strength. BMI correlated with total urinary GC (rho = 0.60, p = 0.0005) and plasma cortisol (rho = -0.52, p = 0.006), whereas height did not significantly correlate with total urinary GC or plasma cortisol (p > 0.05 for both). Therefore in multivariate analyses with urinary GC and plasma cortisol as predictor variables we adjusted for potential confounding by BMI, gender and age (see Tables 3 & 4). Neither BMI nor height correlated with the muscle GR or 11 $\beta$ HSD1 mRNA expression levels. Therefore because height correlated more significantly with muscle size and strength than BMI (Table 2), we adjusted for height and gender for the multivariate analyses with muscle GR and 11 $\beta$ HSD1 mRNA as predictor variables (Table 4).

Plasma cortisol was measured in Group 2 only. There were no significant association between fasting morning plasma cortisol and muscle size and a non-significant negative trend with muscle strength ( $\beta$  = -0.35, p = 0.08) (Table 4). In both groups neither total

urinary glucocorticoids nor the ratio of cortisol:cortisone metabolites were associated with muscle size or strength (Tables 3 & 4).

We used muscle biopsies from a subset of Group 2 to examine the relationships between GR and 11 $\beta$ HSD1 mRNA levels and muscle size and strength. Increased 11 $\beta$ HSD1 mRNA was significantly associated with lower muscle strength after adjustment for sex and height ( $\beta$  = -0.35, p = 0.039, n = 22:12 men mean age 79.8 (sd 3.6) and 10 women, mean age 80.5 (sd 4.1)). There were no significant relationships between GR mRNA and muscle size or strength, or between 11 $\beta$ HSD1 mRNA and muscle size (Table 4).

## Discussion

This study investigated the relationship between circulating and tissue indices of glucocorticoid status and muscle size and strength in two groups. Group 1 allowed comparison of older with younger men. There were no age differences in urinary cortisol metabolites, although muscle biopsies were not obtained in this group so we did not test the effect of ageing *per se* on muscle mRNA levels. Group 2 allowed comparison of older men with older women. There were no differences in plasma cortisol, urinary glucocorticoid metabolites or muscle GR or 11 $\beta$ HSD1 mRNA levels between the sexes in this relatively small sample. Within each group we explored associations between glucocorticoid variables and muscle size and strength after adjustment for potential confounding effects of age, gender and body size as appropriate. In these analyses, indices of HPA axis function, including morning plasma cortisol and 24 h urinary cortisol metabolite excretion, were not associated with muscle strength or size. Additionally urinary cortisol:cortisone metabolite ratios, which principally reflect 11 $\beta$ HSD activity in the major organs of liver and kidney, were not associated with muscle strength or size. However, in muscle itself, higher levels of mRNA encoding the cortisol-amplifying enzyme 11 $\beta$ HSD1 were associated with reduced muscle strength. This finding is consistent with the hypothesis that enhanced glucocorticoid signalling within muscle contributes to sarcopenia.

To our knowledge there have been no previous investigations of muscle glucocorticoid signaling in human sarcopenia. We

**Table 4.** Regression coefficients for the glucocorticoid measures in models predicting muscle size/strength (Group 2).

| Glucocorticoid Measure            | Muscle Size Beta (sig, n) | Muscle Strength Beta (sig, n) |
|-----------------------------------|---------------------------|-------------------------------|
| Plasma Cortisol <sup>a</sup>      | -0.12 (p>0.05, 25)        | -0.35 (p>0.05, 27)            |
| Total Urinary GC <sup>a</sup>     | 0.23 (p>0.05, 28)         | 0.18 (p>0.05, 30)             |
| THFs:THE <sup>a</sup>             | 0.08 (p>0.05, 28)         | 0.10 (p>0.05, 30)             |
| GR mRNA <sup>b</sup>              | 0.03 (p>0.05, 20)         | 0.04 (p>0.05, 22)             |
| 11 $\beta$ HSD1 mRNA <sup>b</sup> | -0.17 (p>0.05, 20)        | -0.35 (p = 0.04, 22)          |

<sup>a</sup>adjusting for gender and BMI.

<sup>b</sup>adjusting for gender and height.

doi:10.1371/journal.pone.0084057.t004

hypothesized that because 11 $\beta$ HSD1 and GR regulate the exposure of target tissues to glucocorticoids, increased expression of 11 $\beta$ HSD1 and GR could therefore contribute to sarcopenia in the absence of an increase in circulating GCs. We found that increased 11 $\beta$ HSD1 mRNA expression in muscle is associated with lower muscle strength. This is consistent with this hypothesis. We did not find a relationship between 11 $\beta$ HSD1 mRNA expression and muscle size, but in normal ageing, muscle strength is reported to deteriorate more rapidly than muscle size; suggesting a decline in force generating capacity with age [5,32]. A number of contributory mechanisms have been proposed to explain this (eg increased muscle fibre stiffness); our data suggest a possible role for increased GC action at the muscle level. GC may affect strength more than muscle mass by exacerbating glycation of the myosin molecule, which appears to slow the intrinsic shortening velocity of the muscle fibre, decrease force per cross-sectional area and increase intramuscular collagen cross-linking which can cause muscle stiffness [33,34]. In addition, GC may cause mitochondrial dysfunction and reduced oxidative capacity, which would similarly result in a decrease in force generating capacity [35]. 11 $\beta$ HSD1 is known to act locally within muscle, resulting in measurable production of cortisol in samples from veins draining human muscle, and therefore increased 11 $\beta$ HSD1 mRNA expression is likely to increase myocellular cortisol levels thereby mediating these effects [18]. More research with larger samples and with a wider range of severity of sarcopenia is required to investigate the relationship between 11 $\beta$ HSD1 expression and activity and muscle ageing.

We found no relationship between GR mRNA expression and muscle mass or strength. It is possible that polymorphisms of GR modulate the effect of GC on muscle, and that level of expression is less important than genotype. For example male carriers of the ER22/23EK polymorphism in GR, which is associated with relative GC resistance, have greater muscle mass and strength than non-carriers [36].

There are no published studies investigating the association between urinary GC and muscle size or strength. However, there are studies reporting associations between salivary and plasma GCs and muscle size and function. In a previous study of men and women >75 years higher salivary, but not serum, cortisol was associated with lower appendicular skeletal mass (ASM) measured using DEXA [21]. Similarly, in a large longitudinal ageing study higher salivary but not serum cortisol predicted loss of grip strength over 6 years, but there was no association of cortisol with baseline grip strength or ASM [22]. A smaller study including both young and older men found that increased serum cortisol correlated with lower knee extensor strength in both age groups and with quadriceps cross-sectional area only in the older group [23]. The Caerphilly Prospective Study, which included measurements of cortisol status and physical performance over 20 years, found that higher mid-life plasma cortisol predicted faster walking speeds in older age, although salivary cortisol did not correlate

with walking speed or balance in older age [24]. Collectively, these studies provide contradictory evidence relating salivary or plasma cortisol to muscle strength and mass. Taken with our data, there does not appear to be a consistent association between activation of the HPA axis and age-associated sarcopenia. These negative findings are important in excluding this plausible hypothesis.

Some limitations of this study should be acknowledged. We examined the effect of GC on ageing muscle using a younger and older group of volunteers separated in age by nearly 50 years and by many lifestyle factors; a problem inherent to cross-sectional studies. Longitudinal studies investigating rate of decline of muscle mass and function and measures of GC would be more informative but are difficult to conduct due to the slow decline of muscle mass and strength during ageing. The sample sizes were relatively modest, particularly with respect to muscle GC data which were obtained from only a subset of Group 2 who underwent muscle biopsy. It has also been shown that sarcopenia affects the upper and lower limbs differently and our study investigated only the lower limbs [4,37–39]. Also, our healthy older volunteers constituted a sample which may be not fully representative of the ageing population; this may influence the generalisability of our results. Finally, we used mRNA expression as a marker of activity rather than a direct measure of 11 $\beta$ HSD1 activity, however several studies have found correlations between mRNA expression and enzyme activity in rodents and humans, so we regard mRNA as an appropriate indicator of 11 $\beta$ -HSD1 activity [26].

## Conclusion

Sarcopenia is one of the major causes of frailty and disability in older people. It is associated with greatly increased risk of loss of independence and institutionalization. In this novel investigation of healthy old and young people we found a significant association between increased muscle 11 $\beta$ HSD1 expression and lower quadriceps strength. We found no significant associations between plasma cortisol, urinary GC metabolites or GR expression and muscle mass or strength. Longitudinal studies are now required to investigate these relationships and to further explore the possibility of 11 $\beta$ HSD1 inhibitors as a novel treatment for sarcopenia.

## Acknowledgments

We are grateful to staff of the Wellcome Trust Clinical Research Facility, Edinburgh for assistance in conducting the study.

## Author Contributions

Conceived and designed the experiments: AMJM HW JR BW KCHF CAG. Performed the experiments: IG PH HW CAG. Analyzed the data: AHMK IG RA CG PH HW JS BW CAG. Contributed reagents/materials/analysis tools: CG HH RA KC BW. Wrote the paper: AHMK JS KCHF BW CAG.

## References

- Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, et al. (2010) Sarcopenia: European consensus on definition and diagnosis. *Age and Ageing* 39: 412–423.
- Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, et al. (2011) Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc* 12: 249–256.
- Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, et al. (2010) Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by Special Interest Groups (SIG) “cachexia-anorexia in chronic wasting diseases” and “nutrition in geriatrics”. *Clinical Nutrition* 29: 154–159.
- Janssen I, Heymsfield SB, Wang ZM, Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology* 89: 81–88.
- Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, et al. (2006) The loss of skeletal muscle strength, mass, and quality in older adults: The health, aging and body composition study. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 61: 1059–1064.
- Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, et al. (2000) Aging of skeletal muscle: a 12-yr longitudinal study. *Journal of Applied Physiology* 88: 1321–1326.



7. Skelton DA, Greig CA, Davies JM, Young A (1994) Strength, power and related functional ability of healthy people aged 65–89 years. *Age and Ageing* 23: 371–377.
8. Starr JM, Deary IJ (2011) Socio-economic position predicts grip strength and its decline between 79 and 87 years: the Lothian Birth Cohort 1921. *Age Ageing* 40: 749–752.
9. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, et al. (2003) Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol* 95: 1851–1860.
10. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, et al. (1998) Epidemiology of sarcopenia among the elderly in New Mexico. *American Journal of Epidemiology* 147: 755–763.
11. Rantanen T (2003) Muscle strength, disability and mortality. *Scand J Med Sci Sports* 13: 3–8.
12. Welle S (2002) Cellular and molecular basis of age-related sarcopenia. *Canadian Journal of Applied Physiology-Revue Canadienne De Physiologie Appliquee* 27: 19–41.
13. Sayer A, Robinson S, Patel H, Shavlakadze T, Cooper C, et al. (2013) New horizons in the pathogenesis, diagnosis and management of sarcopenia. *Age and Ageing* 42: 145–150.
14. Schakman O, Gilson H, Thissen J (2008) Mechanisms of glucocorticoid induced myopathy. *Journal of Endocrinology* 197: 1–10.
15. Shah O, Kimball S, Jefferson L (2000) Acute attenuation of translation initiation and protein synthesis by glucocorticoids in skeletal muscle. *Am J Physiol Endocrinol Metab* 278: E76–82.
16. Jang C, Obeyesekere VR, Dilley RJ, Alford FP, Inder WJ (2006) 11 beta hydroxysteroid dehydrogenase type 1 is expressed and is biologically active in human skeletal muscle. *Clinical Endocrinology* 65: 800–805.
17. Whorwood CB, Donovan SJ, Wood PJ, Phillips DI (2001) Regulation of glucocorticoid receptor alpha and beta isoforms and type 1 11beta-hydroxysteroid dehydrogenase expression in human skeletal muscle cells: a key role in the pathogenesis of insulin resistance? *Journal of Clinical Endocrinology & Metabolism* 86: 2296–2308.
18. Hughes KA, Manolopoulos KN, Iqbal J, Cruden NL, Stimson RH, et al. (2012) Recycling between cortisol and cortisone in human splanchnic, subcutaneous adipose, and skeletal muscle tissues in vivo. *Diabetes* 61: 1357–1364.
19. Tiganescu A, Tahrani A, Morgan S, Otranto M, Desmoulière A, et al. (2013) 11 $\beta$ -Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest* 123: 3051–3060.
20. Hughes KA, Webster SP, Walker BR (2008) 11-Beta-hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) inhibitors in type 2 diabetes mellitus and obesity. *Expert Opin Investig Drugs* 17: 481–496.
21. Waters DL, Qualls CR, Dorin RI, Veldhuis JD, Baumgartner RN (2008) Altered growth hormone, cortisol, and leptin secretion in healthy elderly persons with sarcopenia and mixed body composition phenotypes. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences* 63: 536–541.
22. Peeters GMEE, van Schoor NM, van Rossum EFC, Visser M, Lips P (2008) The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. *Clinical Endocrinology* 69: 673–682.
23. Izquierdo M, Hakkinen K, Anton A, Garrues M, Ibanez J, et al. (2001) Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. *Med Sci Sports Exerc* 33: 1577–1587.
24. Gardner MP, Lightman SL, Gallacher J, Hardy R, Kuh D, et al. (2011) Diurnal cortisol patterns are associated with physical performance in the Caerphilly Prospective Study. *Int J Epidemiol* 40: 1693–1702.
25. Best R, Walker BR (1997) Additional value of measurement of urinary cortisone and unconjugated cortisol metabolites in assessing the activity of 11 beta-hydroxysteroid dehydrogenase in vivo. *Clinical Endocrinology* 47: 231–236.
26. Wake DJ, Rask E, Livingstone DEW, Soderberg S, Olsson T, et al. (2003) Local and systemic impact of transcriptional up-regulation of 11 beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. *Journal of Clinical Endocrinology & Metabolism* 88: 3983–3988.
27. Greig CA, Young A, Skelton DA, Pippet E, Butler FMM, et al. (1994) Exercise studies with elderly volunteers. *Age and Ageing* 23: 185–189.
28. Edwards RH, Hyde S (1977) Methods of measuring muscle strength and fatigue. *Physiotherapy* 63: 51–55.
29. Gray C, MacGillivray TJ, Eeley C, Stephens NA, Beggs I, et al. (2011) Magnetic resonance imaging with k-means clustering objectively measures whole muscle volume compartments in sarcopenia/cancer cachexia. *Clinical Nutrition* 30: 106–111.
30. Bergström J (1975) Percutaneous Needle Biopsy of Skeletal Muscle in Physiological and Clinical Research. *Scandinavian Journal of Clinical & Laboratory Investigation* 35: 609–616.
31. Stephens NA, Gallagher IJ, Rooyackers O, Skipworth RJ, Tan BH, et al. (2010) Using transcriptomics to identify and validate novel biomarkers of human skeletal muscle cancer cachexia. *Genome medicine* 2: 1.
32. Young A, Stokes M, Crowe M (1985) The size and strength of the quadriceps muscles of old and young men. *Clinical Physiology* 5: 145–154.
33. Hook P, Sriramoju V, Larsson L (2001) Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. *American Journal of Physiology-Cell Physiology* 280: C782–C788.
34. Haus JM, Carrithers JA, Trappe SW, Trappe TA (2007) Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *Journal of Applied Physiology* 103: 2068–2076.
35. Mitsui T, Azuma H, Nagasawa M, Iuchi T, Akaike M, et al. (2002) Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle. *Journal of Neurology* 249: 1004–1009.
36. van Rossum EFC, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, et al. (2004) The ER22/23EK Polymorphism in the Glucocorticoid Receptor Gene Is Associated with a Beneficial Body Composition and Muscle Strength in Young Adults. *Journal of Clinical Endocrinology & Metabolism* 89: 4004–4009.
37. Aniansson A, Sperling L, Rundgren A, Lehnberg E (1983) Muscle function in 75-year-old men and women - a longitudinal-study. *Scandinavian Journal of Rehabilitation Medicine*: 92–102.
38. Aniansson A, Hedberg M, Henning GB, Grimby G (1986) Muscle morphology, enzymatic-activity, and muscle strength in elderly men - a follow-up-study. *Muscle & Nerve* 9: 585–591.
39. Aniansson A, Grimby G, Hedberg M (1992) Compensatory muscle-fiber hypertrophy in elderly men. *Journal of Applied Physiology* 73: 812–816.

RESEARCH ARTICLE

Open Access

# A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse

Alixé HM Kilgour<sup>1,2\*</sup>, Oliver M Todd<sup>2</sup> and John M Starr<sup>1,2</sup>

## Abstract

**Background:** An association between cognition and physical function has been shown to exist but the roles of muscle and brain structure in this relationship are not fully understood. A greater understanding of these relationships may lead to identification of the underlying mechanisms in this important area of research. This systematic review examines the evidence for whether: a) brain structure is related to muscle structure; b) brain structure is related to muscle function; and c) brain function is related to muscle structure in healthy children and adults.

**Methods:** Medline, Embase, CINAHL and PsycINFO were searched on March 6th 2014. A grey literature search was performed using Google and Google Scholar. Hand searching through citations and references of relevant articles was also undertaken.

**Results:** 53 articles were included in the review; mean age of the subjects ranged from 8.8 to 85.5 years old. There is evidence of a positive association between both whole brain volume and white matter (WM) volume and muscle size. Total grey matter (GM) volume was not associated with muscle size but some areas of regional GM volume were associated with muscle size (right temporal pole and bilateral ventromedial prefrontal cortex). No evidence was found of a relationship between grip strength and whole brain volume however there was some evidence of a positive association with WM volume. Conversely, there is evidence that gait speed is positively associated with whole brain volume; this relationship may be driven by total WM volume or regional GM volumes, specifically the hippocampus. Markers of brain ageing, that is brain atrophy and greater accumulation of white matter hyperintensities (WMH), were associated with grip strength and gait speed. The location of WMH is important for gait speed; periventricular hyperintensities and brainstem WMH are associated with gait speed but subcortical WMH play less of a role. Cognitive function does not appear to be associated with muscle size.

**Conclusion:** There is evidence that brain structure is associated with muscle structure and function. Future studies need to follow these interactions longitudinally to understand potential causal relationships.

## Background

Maintaining good levels of brain and muscle function across the lifespan is crucial to achieving a good quality of life [1-3]. There is substantial evidence showing an association between cognition and muscle function [4-8], however the role of muscle and brain structure within this association is less well understood. A greater understanding of this role

will help to improve current knowledge of the mechanisms linking brain and muscle function over the lifecourse.

Several theories have been proposed as to why relationships between brain and muscle structure and function may exist. The common cause hypothesis postulates that there are core common underlying processes which drive ageing throughout the human body. The construct was originally described in a paper by Lindenberger and Baltes in 1994 who noted that measures of visual and auditory acuity accounted for variance in intelligence in old age [9]. Since then experiments in caloric restriction

\* Correspondence: [a.kilgour@ed.ac.uk](mailto:a.kilgour@ed.ac.uk)

<sup>1</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Room F2, 7 George Square, Edinburgh EH8 9JZ, UK

<sup>2</sup>Geriatric Medicine Unit, University of Edinburgh, Edinburgh, UK

have demonstrated that the ageing process can be slowed down in multiple systems throughout the body by one intervention [10,11]. However, environmental factors also impact on how tissues change across the life-course and another theory by Mitnitski et al. proposes that the number of environmental stressors experienced (e.g. disease, smoking) and the ability to recover from them, vary the level of deficit accumulation experienced in multiple organ systems, and hence how tissues like brain and muscle change with age [12]. Potential underlying mechanisms include: pro-inflammatory cytokines (e.g. TNF-alpha and IL-6); the role of glucocorticoids and their intracellular amplifier 11beta-hydroxysteroid dehydrogenase type 1 [13-15]; the role of vitamin D [16,17]; exercise as a way to improve cardiovascular fitness in addition to its beneficial effect through hormones and cytokines [18-20]; and cellular senescence (e.g. through oxidative stress) [21,22].

In view of these theories, there should be a correlation between the structure and function of brain and muscle throughout our lifetime in the absence of significant pathology. This systematic review will search for studies that test the hypotheses that brain structure is related to muscle structure and/or function and that muscle structure is related to brain function in healthy children and adults. Previous studies and reviews have looked at evidence relating brain function (e.g. MMSE score) to muscle function (e.g. walking speed) therefore this separate but closely related field of literature will not be included in this review [5,23-25].

## Methods

The study protocol was published online in December 2011 at: <http://www.ccace.ed.ac.uk/sites/default/files/Kilgouretal.pdf>.

## Inclusion criteria

### Population

All human subjects regardless of age were included in the study; from newborn babies to the oldest old, including post-mortem studies. This study is examining the relationship between brain and muscle in health, not within the effects of pathology therefore studies looking at how a disease affects brain or muscle were excluded. However studies which included a healthy control group, where the data from these subjects can be or was analysed separately were included. As morbidity increases in frequency with age it would be very restrictive to include solely those studies which include only participants who are free from any disease, therefore studies will be included provided the subjects have been recruited in a way that did not pre-dispose to morbidity being more prevalent than in the general population (e.g. from a diabetes clinic).

It was planned that subgroup analysis would be undertaken where possible and would include data being extracted to investigate the effects of gender, age, socioeconomic status and ethnicity.

### Interventions/Comparators

Not applicable as the study is investigating normal physiology.

### Outcomes

#### Brain structure

- Whole brain volume (WBV) or total brain volume (TBV)
- Volume or cross sectional area of regions within the brain (e.g. hippocampus, frontal lobes)
- White matter integrity (e.g. White matter hyperintensities (WMH) or white matter signal abnormalities (WMSA))
- Histological findings about brain structure on autopsy

#### Brain function

- Any recognised measure of cognitive function including: memory, attention, executive function, language and processing speed
- Reaction time will not be used as this is dependent on aspects of brain and muscle structure and function

#### Muscle structure

- Muscle cross sectional area on CT, MRI or USS
- Muscle volume (using CT or MRI)
- Whole body lean tissue mass using DEXA, giving: total lean mass (TLM) or appendicular lean mass (ALM)
- Bioimpedance analysis (BIA)
- Histological findings on muscle biopsy or on autopsy

#### Muscle function

- Any recognised test of muscle strength, including isometric, isotonic, isokinetic tests
- Any recognised test of muscle power
- Functional tests of muscle function (e.g. usual or maximum gait speed)

### Study design

As this review is studying a physiological relationship, intervention studies were not included, unless they contained either a control arm with extractable data with no placebo treatment or baseline data prior to the intervention.

Observational studies including cohort studies and cross sectional studies were included and the control arm of case control studies. Case reports were excluded as these would not contain evidence of normal physiological relationships out with pathology. The only other limiter used was "human" in Medline, Embase and PsycINFO but not Cinahl as it appears to screen out human studies erroneously.

### Search strategy

Database searches of Medline, Embase, CINAHL and PsycINFO were undertaken. All languages were included in the search. The Medline search strategy can be found in Appendix 1. The searches were all performed on 6th March 2014. A grey literature search was performed using Google and Google Scholar. Hand searching through citations and references of relevant articles was also undertaken.

### Study selection

The search was undertaken by two independent researchers. Titles +/- abstracts found using our search strategy were independently screened for relevance. The full text of the selected studies was reviewed against the inclusion criteria, and reasons for exclusion at this stage were recorded. At this point the two researchers met to discuss shortlists and discuss any articles which only one researcher had selected to decide if they should be included or not. Disagreements were resolved by consensus or adjudication by a third party (a Professor in Geriatric Medicine).

### Data extraction

The Clinical Fellow (AK) performed the data extraction using a data extraction sheet written by the Clinical Fellow and approved by the two co-authors (OT, JS).

### Contacting authors

Of the 84 studies found through our search, we wrote to 79 to request data or associations which were not given in the text. Five of the studies had given all the associations for the variables listed in the text. A letter was sent by email to either the corresponding author (after checking they were still working at the study location) or the last author (after the same checking process). Only one author was written to from each study (e.g. all articles arising from the Kansas Brain Aging Project, were grouped together when requesting extra data/associations). After the initial email a further email was sent around 2 weeks later to act as a reminder. Studies were given a minimum of around 1 month to reply.

Out of the 79 studies we wrote to: 25 studies (32%) sent either the requested data or associations; 22 (28%) replied stating they would try and send the data or associations to us but then never did; 12 studies (15%) replied stating they either no longer had access to the data

or did not want to send either the requested data or associations to us; and 20 (25%) never replied to either of the emails.

### Quality assessment and risk of bias

All papers included in the study had their inclusion and exclusion criteria reviewed to check for possible bias in the study selection. The topic of the review is not looking at an intervention, therefore the risk of reporting bias for an individual paper is small. Also, in most of the papers, the relationship between muscle and brain was not the primary topic of the paper, further decreasing the risk of reporting bias. However when contacting the authors, asking for either the data or the associations, it was considered that the studies which replied may show some bias. The authors may look at their data and only reply if an association was found, or if they found a strong relationship they may not want this to be initially reported within a systematic review, but rather in a paper in its own right. All summary measures were included (e.g. odds ratio, beta).

### Data analysis

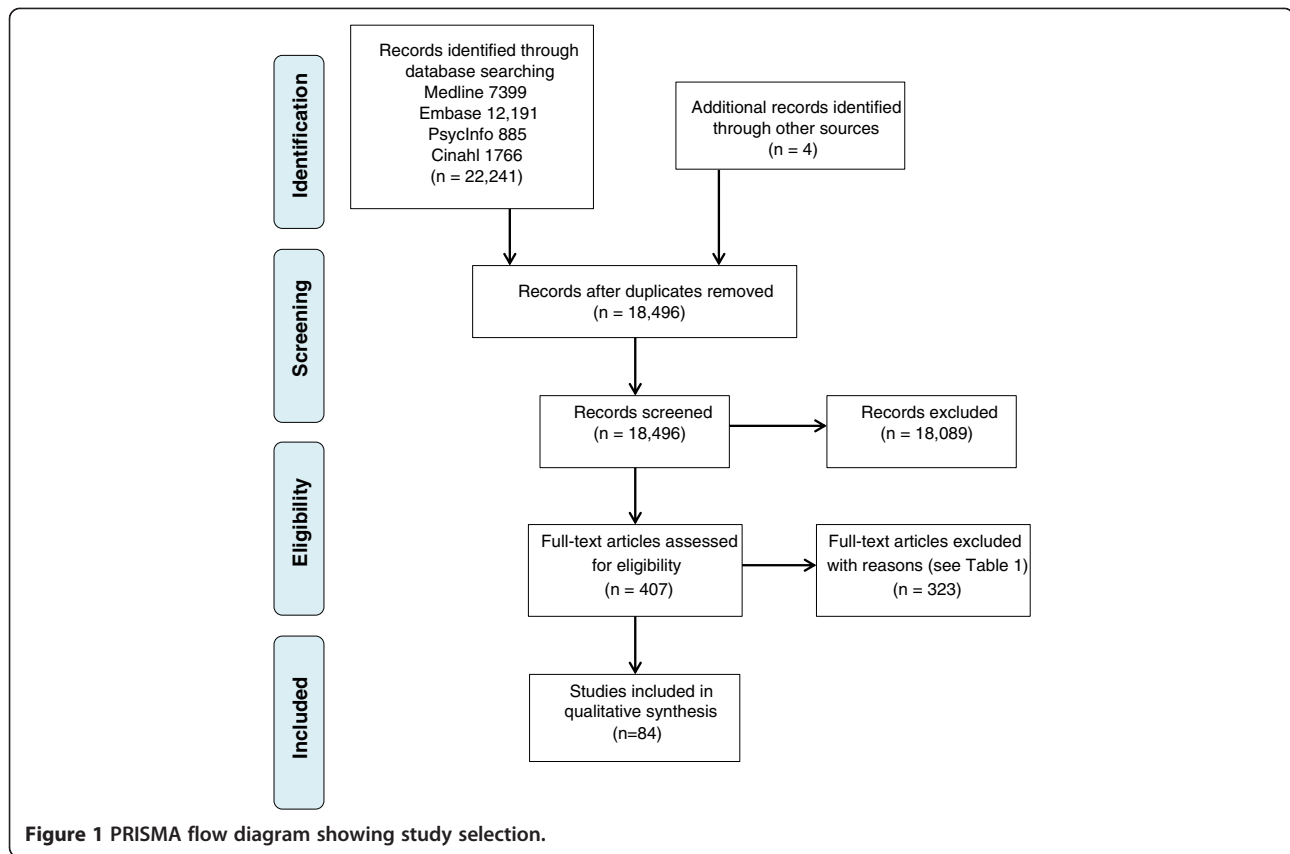
A narrative synthesis was completed. It was thought unlikely that the data would be comparable enough to allow meta-analysis (i.e. different measures of cognition, different muscle groups studied using different machines) and this proved correct. It was hoped that subgroup analysis would be undertaken, either in the form of a meta-analysis or more likely as a narrative synthesis for the reasons mentioned in the above paragraph.

### Results

The search results are presented in the PRISMA flow diagram in Figure 1. Reasons for exclusion of articles after reviewing the full text are reported in Table 1. After applying the inclusion and exclusion criteria 84 articles were identified; 53 articles either reported the appropriate associations or sent us the data or associations requested (Tables 2, 3 and 4), and 31 articles contained the required data but did not report the association between them and did not supply either the data or associations requested (Table 5). Out of the 53 articles which could be included in the review; 6 contained data on brain structure and muscle structure (Table 2); 33 contained data on brain structure and muscle function (Table 3); and 14 contained data on brain function and muscle structure (Table 4).

### Association of brain structure and muscle structure

Of the six articles which looked at the relationship between brain structure and muscle structure, three were from the Kansas Brain Aging Project [28-30], and the others were from Germany, UK and USA, Phoenix [26,27,31] (Table 2).



The Kansas Brain Aging Project was set up to determine the effects of exercise and cardiorespiratory fitness on age-related brain changes. Only one of the papers from this project reported the relationship between brain and muscle structure [29]: Burns et al. reported a positive relationship between WBV and TLM (beta 0.20,  $p < 0.001$ ) when control and subjects with Alzheimer’s disease (AD) were grouped together, adjusting for age, sex and intracranial volume (ICV), and they note that this was driven by WM volume [29]. They state that this relationship persists in just the control group but do not give any statistics for this relationship. A General Linear Model (GLM) was performed on the data from the non-demented group supplied to us by the study authors from the Kansas Brain Aging Project [28-30]. WBV, grey matter (GM) volume and hippocampal volume were not predicted by TLM adjusting for age, sex and ICV +/- education. White matter (WM) volume was predicted by TLM ( $t = 3.12$ ,  $p = 0.003$ , partial eta squared 14%) adjusting for age, sex and ICV. Adjusting for total years of formal education only slightly attenuated the results ( $t = 2.99$ ,  $p = 0.004$ , partial eta squared 13%).

Kilgour et al. also looked at older subjects however they used neck muscle CSA as a measure of muscle bulk [27]. They found that total neck muscle CSA predicted 17% of the variance in whole brain volume ( $p = 0.01$ ),

but they found no significant association between total neck muscle CSA and ventricular, hippocampal or cerebellar volumes ( $p > 0.05$ ), adjusting for age, sex, ICV and NART (a measure of childhood intelligence).

The other two studies looked at younger subjects. Heymsfield et al. specifically set out to investigate the

**Table 1 Full-text articles excluded, with reasons**

| Reason   | Number     |
|--|------------|
| Selected subjects (e.g. all had hip fracture, all had dementia etc.) | 73         |
| No measure brain or muscle structure                                 | 57         |
| Review article, no relevant references                               | 56         |
| No measure muscle structure or function                              | 47         |
| Abstract, no published results within timeframe or irrelevant        | 34         |
| No measure brain structure or function                               | 19         |
| Protocol paper, no published results within timeframe                | 12         |
| Anthropometry only measure of structure                              | 10         |
| Letter or editorial, no results                                      | 7          |
| Technique or theory paper  | 5          |
| Case Report  | 2          |
| No full text available   | 1          |
| <b>Total</b>   | <b>323</b> |

**Table 2 Studies identified with brain structure (+/- brain function) and muscle structure**

| Authors                           | Year | Country and dataset                     | n   | Study design  | Mean age (sd)                   | Male (%) | Brain structure/Function   | Muscle structure                                | Associations*  |
|-----------------------------------|------|---|-----|---|---------------------------------|----------|--|---|--|
| <b>1. Heymtsfield et al. [26]</b> | 2012 | Germany                                 | 260 | Cross-sectional study   | M 45.1 (14.9),<br>F 38.6 (13.7) | 43.1     | Structure: Brain volume transformed into mass using 1.036 g/cm <sup>3</sup><br><br>Function: not measured  | DEXA for FFM                                    | Study: Linear regression models found that after adjusting for age and fat mass, FFM predicted brain mass in men (beta 0.023, R <sup>2</sup> 5%, p = 0.01) and women (beta 0.003, R <sup>2</sup> 6%, p = <0.0001).   |
| <b>2. Kilgour et al. [27]</b>     | 2013 | UK, MacLulich Healthy Elderly Men Study | 51  | Longitudinal ageing study   | 73.8 (1.5)                      | 100      | Structure: Whole brain, hippocampal, ventricular, cerebellar volumes and ICV<br><br>Function: 9 tests of cognitive function reduced to 2 factors (cognitive processing factor and memory factor)   | Neck muscle CSA on MR head scan                 | Study: Total neck muscle CSA was found to predict 17% of the variance in whole brain volume (t = 2.86, p = 0.01). However, total neck muscle CSA did not significantly predict the variance in ventricular, hippocampal or cerebellar volumes (p > 0.05). Total neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor (p > 0.05), however, it did predict 10% of the variance in the NART score (t = -2.12, p < 0.05). Adjusting for age, sex, ICV and NART where appropriate. |
| <b>3. Wetmore et al. [28]</b>     | 2011 | USA, Kansas, Brain Aging Project        | 60  | 2 year observational case-control study (Alzheimer's dementia vs. non-dementia) | 73.0 (7.2)                      | 43.4     | Structure: MRI for WM, GM, CSF, WBV and ICV<br><br>Function: Logical Memory I & II, Free & Cued Selective Reminding Task, Boston naming test, Verbal fluency, Digit span forward and backward, Letter-number sequencing, Trail making A & B, Stroop color-word test and Block design, MMSE | DEXA for lean mass and ASM (just arms and legs) | Study: none<br><br>Calculated: Non-demented group only. WBV, GM volume and hippocampal volume not predicted by TLM adjusting for age, sex and ICV +/- education. WM volume was predicted by TLM (t 3.12, p = 0.003, partial eta squared 14%) adjusting for age, sex and ICV. TLM did not significantly predict global cognitive score or MMSE, adjusting for age and sex. Adjusting for height and education did not affect this.  |
| <b>4. Burns et al. [29]</b>       | 2010 | USA, Kansas, Brain Aging Project        | 70  | Cross-sectional case-control study (Alzheimer's dementia (AD) vs. non-dementia) | 73.3 (7.3)                      | 42.9     | Structure: MRI for WM, GM, CSF, WBV and ICV<br><br>Function: Logical Memory I & II, Free & Cued Selective Reminding Task, Boston naming test, Verbal fluency, Digit span forward and backward, Letter-number sequencing, Trail making A & B, Stroop color-word test and Block design, MMSE | DEXA for total lean mass                        | Study: Positive relationship between WBV and TLM when control and AD subjects grouped together (beta = 0.20, p < 0.001), adjusting for ICV, age and sex. This appears to be driven by WM (beta 0.19, p < 0.001) rather than GM (beta 0.06, p = 0.27) States this persists in just the control group but doesn't give any statistics for this. Positive relationship between MMSE and global cognitive score (composite   |

**Table 2 Studies identified with brain structure (+/- brain function) and muscle structure (Continued)**

|                             |      |                                  |                     |  |            |      |  |                                       |   |
|-----------------------------|------|----------------------------------|---------------------|--|------------|------|--|---------------------------------------|---|
|                             |      |                                  |                     |  |            |      |  |                                       | of the cognitive tests) and lean mass when grouping AD and control subjects together. States that controlling for dementia status attenuates these results, but no specific statistics given. Calculated: See Wetmore et al. (2011) for Kansas Brain Aging Project data analysis.   |
| <b>5. Honea et al. [30]</b> | 2009 | USA, Kansas, Brain Aging Project | 56 healthy controls | Cross-sectional case-control study (Alzheimer's dementia vs. non-dementia) | 73.3 (6.2) | 41.1 | Structure: MRI for GM, WM, CSF, WBV, hippocampal and parahippocampal volumes<br>Function: MMSE | DEXA for total lean mass              | Study: none<br><br>Calculated: See Wetmore et al. (2011) for Kansas Brain Aging Project data analysis.  |
| <b>6. Weise et al. [31]</b> | 2013 | USA, Phoenix                     | 76                  | Cross-sectional study  | 32.1 (8.8) | 31.6 | Structure: MRI brain volumes (GM, WM, CSF, regional GMV)<br>Function: not measured             | DEXA, FFMI (FFM/height <sup>2</sup> ) | Study: Fat-free mass index (FFMI) was negatively associated with GMV of the bilateral temporal lobes, ventromedial prefrontal cortex (vmPFC) (mainly subgenual portion of the ACC) and caudolateral orbitofrontal cortex and unilaterally with the left insular cortex (all $p < 0.01$ ). After adjusting for percentage body fat and fat mass, negative associations of FFM with GMV of the right temporal pole and bilateral vmPFC remained. All models adjusted for age, sex and handedness. |

\*Associations column key: Study = results published within the study; Calculated = study authors supplied raw data to us and we performed the analysis.



**Table 3 Studies identified with brain structure and muscle function**

| Author                                   | Year | Country and dataset                  | n           | Study design               | Mean age (sd)                    | Male (%)  | Brain structure <sup>#</sup>  | Muscle function               | Associations*   |
|--|------|--------------------------------------|-------------|----------------------------|----------------------------------|-----------|---|-------------------------------|---|
| <i>Brain structure and grip strength</i> |      |                                      |             |                            |                                  |           |   |                               |   |
| 1. Sachdev et al. [32]                   | 2009 | Australia, PATH through life project | 432         | Observational cohort study | M 62.61 (1.42)<br>F 62.62 (1.44) | 52.8      | Volumes of GM, WM and CSF, ICV and TBV (GM plus WM). Brain atrophy and subcortical atrophy, WMH | Grip strength in writing hand | Study: Total brain WMH volume predicted grip strength in men (beta -0.140, delta R <sup>2</sup> 0.019, p < 0.05) but not in women (beta -0.140, delta R <sup>2</sup> 0.018, p > 0.05).  |
| 2. Anstey et al. [33]                    | 2007 | Australia, PATH through life project | 432         | Observational cohort study | 62.63 (1.43)                     | 51.6      | Total, anterior, midbody and posterior corpus callosum (CC) area                                | Grip strength in writing hand | Study: Grip strength adjusted for sex and ICV was found to correlate with CC midbody area (r = 0.103, p < 0.05), however CC total area and anterior and posterior CC areas did not significantly correlate with grip strength (p > 0.05).   |
| 3. Sachdev et al. [34]                   | 2006 | Australia, PATH through life project | 469         | Observational cohort study | M 62.56 (1.44)<br>F 62.53 (1.47) | 51.8      | Volumes of GM, WM and CSF, ICV and TBV (GM plus WM). Brain atrophy and subcortical atrophy, WMH | Grip strength in writing hand | Study: None, see other articles from the PATH through life project for analysis using this dataset.   |
| 4. Sachdev et al. [35]                   | 2005 | Australia, PATH through life project | 478         | Observational cohort study | M 62.56 (1.44)<br>F 62.54 (1.47) | 52.3      | WMH, ICV  | Grip strength in writing hand | Study: Total brain WMH significantly predicted grip strength (beta -0.09, p = 0.002) adjusted for age, sex and depression. Correcting for comorbidity, cognition and brain atrophy did not attenuate the results (beta -0.13, p = 0.001).   |
| 5. Doi et al. [36]                       | 2012 | Japan                                | 110         | Cross-sectional study      | 75.4 (7.1)                       | 50        | GM, WM, CSF, brain atrophy (measured using healthy volunteers)                                  | Grip strength                 | Study: A MLR model found that grip strength is not related to brain atrophy (beta -0.082 (SE 0.005) p = 0.54). Adjusting for age, gender, BMI, education, MMSE, Tokyo Metropolitan Institute of Gerontology Index of Competence, geriatric depression scale and change in walking whilst dual tasking. No other associations given. |
| 6. Hardan et al. [37]                    | 2003 | USA, Philadelphia                    | 41 controls | Case-control study         | 18.6 (8.6)                       | Not given | Caudate, putamen and total brain volume   | Grip strength                 | Study: Non-significant trends showed a negative correlation between right grip strength and total caudate volume (r = -0.303, p = 0.05) and left grip strength (r = -0.28, p = 0.07) in the control group. Not corrected for age or sex. No relationships given for other measures.   |



**Table 3 Studies identified with brain structure and muscle function (Continued)**

| <i>Brain structure and gait speed</i> |      |   |      |   |                                  |      |   |   |  |
|---------------------------------------|------|---|------|---|----------------------------------|------|---|---|--|
| <b>7. Piguet et al. [38]</b>          | 2006 | Australia, Sydney Older Person's Study                    | 111  | Longitudinal observational cohort study | M 85.29 (2.89)<br>F 85.72 (3.41) | 54.5 | Cerebellar vermis area, (V1, V2 and V3 and total), Cerebellar volume, cerebral volume and ICV | Timed walk over 5 m, adjusted for lower limb arthritis    | Study: None of the brain size measures (cerebellar vermis area, cerebellar volume or cerebral volume) significantly predicted timed walk ( $p > 0.05$ ) after adjustment for age (but not sex, as was not deemed to be a significant contributor after univariate analyses).   |
| <b>8. Callisaya et al. [39]</b>       | 2013 | Australia, Tasmanian Study of Cognition and Gait (TASCOG) | 225  | Longitudinal cohort study               | 71.4 (6.8)                       | 56.4 | ICV, GM, WM-lesion free, hippocampal volume, WML  | 4.6 metre GaitRite computerized walkway (preferred speed) | Study: MLR were performed to investigate the relationship of longitudinal change in brain volumes and gait speed. They found that white matter atrophy (beta 0.25 (CI 0.09-0.40) $p = 0.001$ ), greater WML progression (beta $-0.89$ (CI $-1.75$ - $-0.02$ ) $p = 0.045$ ), grey matter atrophy (beta 0.25 (CI 0.00-0.19) $p = 0.06$ ) and hippocampal atrophy (beta 0.01 (CI 0.00-0.02) $p = 0.006$ ) were all associated with a greater decline in gait speed.  |
| <b>9. Srikanth et al. [40]</b>        | 2010 | Australia, TASCOG   | 385  | Longitudinal cohort study               | 72.2 (7.1)                       | 56   | WMLV, TBV   | Gait speed using 4.2 m GAITRite system                    | Study: none, see Callisaya et al. (2013) for analysis using the TASCOG dataset.  |
| <b>10. Srikanth et al. [41]</b>       | 2009 | Australia, TASCOG   | 294  | Longitudinal cohort study               | 72.3(7.0)                        | 55.4 | WMLV, TBV   | Gait speed using 4.2 m GAITRite system                    | Study: none, see Callisaya et al. (2013) for analysis using the TASCOG dataset.  |
| <b>11. Elbaz et al. [42]</b>          | 2013 | France, Three-city study                                  | 4010 | Cohort study                            | 73.4 (4.6)                       | 38.4 | WML volumes   | 6 metre walk speed (usual and maximum)                    | Study: Logistic regression stratified by education found that high WML volumes were not associated with slow walking speed among highly educated participants (OR = 0.72), but were associated with a 2-fold-increased risk of slow walking speed among those with low education (OR = 3.19/1.61 = 1.99) ( $p$ interaction = 0.026), adjusted for sex, age and total WM volume. Results remained unchanged after adjustment for height, BMI, and MMSE score.<br><br>Given: WM volume did not predict walking speed at baseline, adjusted for age, gender and ICV in a MLR ( $p > 0.05$ , $n = 1510$ ), or decline in walking speed over 7 years, adjusted for age, gender, ICV and |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                                  |      |                          |                               |              |                                    |                           |  |  |  |
|----------------------------------|------|--------------------------|-------------------------------|--------------|------------------------------------|---------------------------|--|--|--|
|                                  |      |                          |                               |              |                                    |                           |  |  | baseline walking speed, ( $p > 0.05$ , $n = 928$ ). A logistic regression found that WM volume was not significantly associated with an increased risk of being in the quartile with the highest walking speed decline ( $p > 0.05$ ).   |
| <b>12. Dumurgier et al. [43]</b> | 2012 | France, Three-city study | 1623                          | Cohort study | 73.3 (4.1)                         | 39.5                      | Regional grey matter volumes (sensorimotor cortex; frontal, parietal, temporal, occipital, and limbic lobes; insula; cerebellum; thalamus; basal ganglia nuclei, including the caudate nucleus, putamen and pallidum) and WMLs | Maximum walking speed over 6 metres                                      | Study: A linear regression found that only basal ganglia volume (beta 0.075 (SE 0.025) $p = 0.003$ ) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.114 (SE 0.024) $p < 0.001$ ). All other regional GM volumes were not significantly associated with walking speed. A semi-bayes model found again only the basal ganglia volume (beta 0.061 (SE 0.028) $p = 0.03$ ) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.050 (se 0.019) $p = 0.007$ ). There was found to be a linear relationship between quartiles of caudate nucleus volume and faster walking speed ( $p$ for linear trend (0.001). These relationships were attenuated slightly for total basal ganglia volume by adjusting for MMSE and comorbidity plus smoking but not for caudate nucleus volume. All models adjusted for; age, sex, BMI, education level, ICV, volume of WMLs and silent infarcts. Given: See Elbaz et al. (2013) for Three-City Study data analysis. |
| <b>13. Dumurgier et al. [44]</b> | 2010 | France, Three-city study | Baseline 3604, f/u at 4y 1774 | Cohort study | Baseline 73.4 (4.6) f/u 71.5 (3.6) | Baseline 38.1%, f/u 38.4% | WMH volume   | Maximum walking speed over 6 metres, 1st and 4th follow up, mean 7 years | Study: none<br>Given: See Elbaz et al. (2013) for Three-City Study data analysis.  |
| <b>14. Soumare et al. [45]</b>   | 2009 | France, Three-city study | 1702                          | Cohort study | 72.4 (4.1)                         | 39.4                      | PVH, deep WMH and total WMH and total WM and ICV   | Maximum walking speed over 6 metres, 1st and 4th follow up, mean 7 years | Study: A significantly lower mean walking speed was found in those with a total WMH volume above the 75th percentile compared to those below the 25th (Beta $-0.026$ , $p = 0.0003$ ). A similar relationship was found for both deep WMH and PVH. A WMH volume greater than the 90th percentile more than doubled the risk of decline   |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                        |      |  |                     |                                  |            |      |  |   |  |
|------------------------|------|--|---------------------|----------------------------------|------------|------|--|---|--|
| 15. Starr et al. [46]  | 2003 | UK, ABC1921 cohort study                                     | 97                  | Longitudinal cohort study        | 78-79years | 59.8 | WMH in deep/subcortical, PVH and brain stem, Fazekas score   | Self-paced time to walk 6metres               | in walking speed compared with subjects with lower volumes of WMH (OR 2.6 (1.5-4.5), p = 0.001). This finding was replicated when looking at PVH but not for deep WMH volume. Given: See Elbaz et al. (2013) for Three-City Study data analysis.   |
| 16. Manor et al. [47]  | 2012 | USA, Boston,   | 89 in control group | Case-control study               | 65.3 (8.2) | 48.3 | GM, WM, CSF, regional GM volumes; precentral and postcentral gyri, basal ganglia, cerebellum, and dorsolateral prefrontal cortex | 75 metre walk test at preferred pace          | Study: A slower 6metre walk test was associated with increased brain stem lesions (F 7.11, p = 0.009, partial eta <sup>2</sup> 0.070), but not with WMH (deep) (F 3.33, p = 0.071) or PVH (F 2.47, p = 0.12). Doesn't state if age and sex are adjusted for in these models. If HADS score and Raven's score are adjusted for, brainstem lesions are no longer significantly associated with walking time.                                 |
| 17. Hajjar et al. [48] | 2010 | USA, Boston, BP in stroke study (?overlap with Novak et al.) | Non-stroke group 43 | Case-control observational study | 68 (1)     | 44   | WM, GM (global and regional), CSF normalized for ICV   | Gait speed over 12mins at usual pace          | Study: Within linear regression models, global GM volume and all of the regional GM volumes were not associated with walking speed in the control group (p > 0.005, Bonferroni adjusted). Adjusted for age, sex and body mass.   |
| 18. Novak et al. [49]  | 2009 | USA, Boston (?overlap with Hajjar et al.)                    | 76                  | Observational study              | 64.7 (7.2) | 47.4 | GM, WM, CSF, WMH all as % brain tissue volume. WMH using Wahlund scale   | Gait speed over 12mins at normal walking pace | Study: Gait speed was not significantly associated with GM volume (p = 0.85), but was significantly associated with WM volume (B = 1.30, p = 0.03) adjusting for age, gender, BMI and antihypertensive use.  |
|                        |      |  |                     |                                  |            |      |  |   | Study: Gait speed was significantly associated with frontal WM normalized for brain tissue volume (R = 0.4, p = .003). Gait speed was significantly associated with frontal GM normalized for brain tissue volume (R = 0.3, p = .01). Adjusted for age and BMI (but not gender). Doesn't say about other regional brain volumes, ie temporal etc. WMH volumes and PVH and punctuate scores were not associated with gait speed (p > 0.05). |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                                |      |   |    |                                     |          |      |   |  |   |
|--------------------------------|------|---|----|-------------------------------------|----------|------|---|--|---|
| <b>19. Moscufo et al. [50]</b> | 2012 | USA, Boston, Moscufo study – 2 year f/u | 77 | Longitudinal cohort study           | 84 (3.9) | 40   | WMH volume as % of ICV and regional WMH burden expressed as % of ROI volume. At baseline and 2y f/u.                | Gait speed over 2.5 metres, maximum velocity and usual walking speed At baseline and 2y f/u. | <p>Study: Total WMH burden was significantly associated with usual walking speed at baseline but not at follow-up, and maximum walking speed was not associated with total WMH at baseline or follow up. At baseline, regional WMH burden in the splenium of corpus callosum and anterior and superior corona radiata, was significantly associated with both walking measures (<math>p &lt; 0.05</math>) and in addition the body of the corpus callosum was also associated with usual walking speed (<math>p &lt; 0.05</math>). At follow-up, WMH burden in the splenium was significantly associated with both walking measures (<math>p &lt; 0.05</math>) and in the body with maximum walking speed. Change in WMH burden, either total or in any of the 7 regional areas, over 2 years was not associated with a decline in usual walking speed (<math>p &gt; 0.1</math>).</p> <p>Given: WMH burden is significantly associated with lower gait speed after adjustment for age, sex and BMI (<math>\rho = -0.327</math>, <math>p = 0.0008</math>). WM/ICV is not significantly associated with gait speed with or without adjustment (<math>p &gt; 0.05</math>). GM/ICV is significantly associated with gait speed with adjustment for age, gender and BMI (<math>\rho = 0.232</math>, <math>p &lt; 0.05</math>). CSF/ICV is significantly associated with gait speed with adjustment for age, sex, BMI (<math>\rho = -0.285</math>, <math>p = 0.004</math>).</p> <p>Study: Total WMH burden (i.e. % of ICV) correlates with gait speed (<math>\rho = -0.288</math>, <math>p = 0.004</math>). Also all 9x regional burden measurements correlate with gait speed score too except sup. longitudinal fasciculus. No adjustment.</p> <p>Given: See Moscufo et al. (2012) for analysis using this dataset.</p> |
| <b>20. Moscufo et al. [51]</b> | 2011 | USA, Boston, Moscufo study - baseline   | 99 | Cross-sectional observational study | 83(4)    | 42.4 | WM, GM, WMH and CSF volumes all corrected for ICC. Brain atrophy. Regional WMH burden expressed as % of ROI volume. | Gait speed over 2.5metres (done as part of SPPB)   | <p>Study: Total WMH burden (i.e. % of ICV) correlates with gait speed (<math>\rho = -0.288</math>, <math>p = 0.004</math>). Also all 9x regional burden measurements correlate with gait speed score too except sup. longitudinal fasciculus. No adjustment.</p> <p>Given: See Moscufo et al. (2012) for analysis using this dataset.</p>   |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                                |      |  |                                       |   |  |      |   |   |  |
|--------------------------------|------|--|---------------------------------------|---|--|------|---|---|--|
| <b>21. Wolfson et al. [52]</b> | 2005 | USA, Boston, WML and mobility  | 28 at baseline, 14 at follow up       | Prospective longitudinal observational study      | SPPB 11 or 12 mean 81(1.7), SPPB = <8 mean 84(3.4) | 64.3 | GM, WM, WMSA, CSF, ICCV volumes                   | Gait velocity over 8metres                              | Study: Slower baseline gait velocity predicted more WMSA at visit 1 ( $p < 0.05$ ), but not change in WMSA volume between visit 1 and 2 ( $p < 0.07$ ). Significant negative relationship of between-visit change in gait velocity to CSF volume ( $r = 0.733$ , $p < 0.005$ ) and a positive relationship of between-visit change in gait velocity to WM volume ( $r = 0.558$ , $p < 0.05$ ). Betas not given. Brain volumes normalized for ICCV according to image processing section.   |
| <b>22. Guttman et al. [53]</b> | 2000 | USA, Boston, WML and mobility  | 28 (12 with SPPB score >10 and 16 <9) | Observational cross-sectional study               | SPPB > 10 79(5)<br>SPPB < 9 83(6)                  | 42.9 | WM, WMSA, GM, CSF (normalized for ICCV)           | Gait velocity over 8metres                              | Study: Gait velocity was not significantly predicted by age nor WMSA volume (no figures given or p value) adjusted with and without MMSE score.  |
| <b>23. Rosano et al. [54]</b>  | 2012 | USA, Cardiovascular health study   | 214                                   | Longitudinal observational study                  | 72.3 (3.8)   | 35.5 | Brain volumes (GM, WMH, Prefrontal area, WM, CSF) | Timed 15 ft walk at usual pace                          | Study: Prefrontal area volume significantly predicted time to walk in a stepwise forward model ( $\beta = -0.15$ , $p = 0.02$ ).   |
| <b>24. Barnes et al. [55]</b>  | 2009 | USA, Cardiovascular Health Cognition Study, nested within the CVS Health Study | 3375                                  | Prospective, population-based, longitudinal study | 75 (no sd)   | 41   | White matter disease and ventricular enlargement  | Gait speed over 15 ft                                   | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset  |
| <b>25. Rosano et al. [56]</b>  | 2006 | USA, Cardiovascular health study   | 321                                   | Longitudinal observational study mean f/u 4 years | 78.3 (no sd)                                       | 39.3 | WMAs, ventricular enlargement                     | Gait speed at usual pace over 4 metres using GaitMat II | Study: Gait speed was significantly correlated to total WMAs ( $r = -.18$ , $p < 0.0001$ ) and white matter lesions in the brainstem ( $r = -.18$ , $p = 0.01$ ). After adjusting for age, slower gait speed was still significantly associated with white matter grade ( $p = 0.02$ ). Logistic regression found that those in the lowest two quartiles of gait speed (ie < 1.02 m/s) had double the likelihood of having WMH graded 3 or above ( $p = 0.03$ ), after adjustment for age, race, gender, and prevalent clinical CVD. VE graded >4 was not found to be significantly predicted by gait speed, however VE graded > 5 was, independent of age, gender, race and presence of CVD (OR = 2.91 for 1st vs. 4th quartile, OR 3.82 for 2nd vs 4th quartile) |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                         |      |                                  |      |   |            |      |  |   |  |
|-------------------------|------|----------------------------------|------|---|------------|------|--|---|--|
| 26. Rosano et al. [57]  | 2005 | USA, Cardiovascular health study | 2450 | Longitudinal observational study mean f/u 4 years | 74.4 (4.7) | 43   | WMH and ventricular enlargement (graded as minimal, moderate and severe)         | Gait speed over 15 ft at usual pace, starting from standing still | Study: Grade of ventricular enlargement was associated with baseline gait speed and mean change in gait speed/year. Gait speed decline was 2.5x that for those with severe VE than minimal VE. (p < 0.001). Grade of WMH was associated with baseline gait speed and mean change in gait speed/year (p = 0.003). In both analyses adjustment had been made for age, sex, race and education and CV risk factors (BMI, systolic BP, antihypertensive meds, internal carotid wall thickness, and ETOH intake) and prevalent CV disease.  |
| 27. Silbert et al. [58] | 2008 | USA, Oregon Brain Aging Study    | 104  | Longitudinal cross-sectional study                | 85.1 (5.6) | 38.5 | PV WMH and s/c WMH, total WMH, brain volume, CSF volume, hippocampal volume, ICV | Gait speed over 9 m. Self-selected pace.                          | Study: Adjusted for age and ICV, higher baseline total WMH vol. was associated with increased rate of change in timed walking in seconds ( $r^2 = 0.08$ , $p = 0.0052$ ). This relationship became non-significant after adjustment for multiple comparisons to threshold p value. PVH volume is associated with increased rate of change in timed walk in seconds ( $r^2 = 0.12$ , $p = .0039$ ). However, baseline subcortical WMH vol. was not related to change in gait performance over time. Higher rate of PVH accumulation is associated with increased rate of change of time to walk 9 m ( $r^2 = 0.15$ , $p = .0453$ ). Adjusted for age, ICV and baseline WMH volume:<br><br>Calculated: In an unadjusted GLM, gait speed was predicted by total brain, WMH and hippocampal volume ( $p < 0.001$ ). The relationship remained significant after adjusting for sex, age, ICV and height, for total brain volume ( $t = 3.61$ , $p = .004$ , partial eta squared 4.3%) and WMH ( $t = -2.80$ , $p = 0.006$ , partial eta squared 4.4%) but not for hippocampal volume. |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|  |      |                               |     |                                    |  |      |   |   |   |
|--|------|-------------------------------|-----|------------------------------------|--|------|---|---|---|
| <b>28. Marquis et al. [59]</b>   | 2002 | USA, Oregon Brain Aging Study | 108 | Longitudinal cross-sectional study | 83.2 (7.9)                                 | 37   | Total brain volume, hippocampal volume, ICV   | Gait speed over 9 m. Self-selected pace.                                      | Study: Negative correlation between hippocampal volume and time to walk 30 ft ( $r = -.12$ ). No p value given.<br><br>Calculated: See Silbert et al. (2008) for Oregon Brain Aging Study data analysis.  |
| <b>Brain structure and gait speed plus grip strength or isometric knee extension strength (IKES)</b> |      |                               |     |                                    |  |      |   |   |   |
| <b>29. Rosano et al. [60]</b>  | 2010 | Iceland, AGES-Reykjavik study | 795 | Longitudinal cohort study          | M 75.6 (5.4)<br>F 75.6 (5.7)               | 41.1 | MTR, ICV, brain parenchyma volume, semiquantitative subcortical WMH and PVH and total WMH volume, brain atrophy index | Gait speed over 6 m usual speed and maximal isometric knee extension strength | Study: In men: Time to walk 6metres predicted by WMH volume (beta 0.13, $p = 0.02$ ) but not brain atrophy or peak height MTR (adjusted for age and brain size as includes measure of brain atrophy). In women: Usual walking speed predicted by lower MTR height (i.e. indicating abnormal brain tissue) (beta $-0.14$ ( $p = 0.01$ ), increased WMH (beta 0.12, $p = 0.003$ ) and greater brain atrophy (beta 0.15, $p = 0.01$ ) (adjusted for age and brain size). Lower muscle strength associated with peak height MTR ( $p < 0.005$ , beta not given).  |
| <b>30. Arribasala et al. [61]</b>  | 2013 | UK, LBC 1936 study            | 694 | Longitudinal cohort study          | 69.5 (0.7) wave 1<br>and 72.5 (0.7) wave 2 | 52.9 | TBV, ventricular volume, GM, NAWM and WML at wave 2   | 6 metre walk (normal walking pace) and grip strength at wave 1 and 2          | Study: Grip strength at wave 1 significantly predicts ventricular volume at wave 2 (standardized beta $-0.10$ ), however there was no significant association with other brain volumes. 6metre walk at wave 1 predicted TBV ( $-0.07$ ), ventricular volume (0.09), NAWM ( $-0.07$ ) and WML (0.11) all $p < 0.05$ . Grip strength at wave 2 was associated with ventricular volume ( $-0.11$ ) and NAWM (0.08). 6 MW at wave 2 was associated with TBV ( $-0.07$ ), NAWM ( $-0.09$ ) and WML (0.11) all $p < 0.05$ . Change in physical function between wave 1 and 2 (i.e. decrease in grip strength or increase in 6 MW) was not significantly associated with any brain volume measure. GM volume did not significantly associate with any of the physical function variables at wave 1 or 2. All analyses were adjusted for age, ICV, age 11 IQ, years of education, social class, comorbidity and smoking status. Corrected for false discovery rate. |

**Table 3 Studies identified with brain structure and muscle function (Continued)**

|                                   |      |                                  |      |   |                                       |                                     |   |  |  |
|-----------------------------------|------|----------------------------------|------|---|---------------------------------------|-------------------------------------|---|--|--|
| <b>31. Rosano et al. [62]</b>     | 2011 | USA, Cardiovascular health study | 643  | Longitudinal observational study                  | 72.1-72.6 broken down by BP diagnosis | 31-42.7 broken down by BP diagnosis | WMH scale 0-9   | Gait speed over 15 ft, starting from standstill. Grip strength of dominant hand. | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset.   |
| <b>32. Rosano et al. [63]</b>     | 2008 | USA, Cardiovascular health study | 3156 | Longitudinal observational study mean f/u 4 years | 74 (4.6)                              | 43.2                                | White matter disease score, brain atrophy score (ventricular enlargement) | Gait speed over 15 ft and grip strength in dominant hand                         | Study: none, see Rosano (2012), Rosano (2006), Rosano (2005) and Longstreth (1996) for analysis using the Cardiovascular Health study dataset.   |
| <b>33. Longstreth et al. [64]</b> | 1996 | USA, Cardiovascular health study | 3658 | Longitudinal observational study                  | 70.7 (no sd)                          | 41.7                                | MR WMSA graded 0-9  | Time to walk 15feet, grip strength in dom and non-dom hand                       | Study: Time to walk 15 ft correlated with white matter grade (0-9) ( $r = 0.153$ , $p < 0.001$ ), with adjustment for age, sex and presence of clinically silent stroke on MRI. Same model showed no significant associated between grip strength in dom hand or non-dom hand and white matter grade ( $p > 0.05$ ). |

#All brain structure variables performed using MRI.

\*Associations column key: Study = results published within the study; Given = associations calculated by study authors and supplied to us for this review; Calculated = study authors supplied raw data to us and we performed the analysis.



**Table 4 Studies identified with brain function and muscle structure**

| Author                  | Year | Country and dataset                      | n    | Study design   | Mean age (sd)                     | Male (%) | Brain function              | Muscle structure               | Associations*   |
|-------------------------|------|--|------|--|-----------------------------------|----------|-----------------------------|--------------------------------|---|
| 1. Berryman et al. [65] | 2013 | Canada, Training Intervention Study      | 48   | Baseline characteristics from a large physical training intervention study | 70.8 (5.4)                        | 41.67    | MMSE & modified Stroop test | LBM (DEXA)                     | Study: none<br><br>Calculated: A GLM showed no association between LBM and MMSE, Stroop naming, reading or inhibition tasks, adjusted for sex and age. However there was an association between the Stroop flexibility task and LBM (t 2.126, p = 0.039, partial eta squared 9.3%), however after adjusting for education and height the effect was attenuated (p > 0.05).  |
| 2. Bites et al. [66]    | 2013 | Chile                                    | 306  | Retrospective study  | M 74.9 (61–91),<br>F 75.5 (69–90) | 24.5     | MMSE                        | TLM, Arm LM and Legs LM (DEXA) | Study: none<br><br>Calculated: Authors sent one data sheet for this study and Bunout et al., as there is a large amount of overlap between the studies. N = 401, mean age 75.3 (sd 4.8), males 28.7%. GLM performed adjusting for sex and gender. Total LM (t 2.38, p = 0.018, partial eta squared 1.4%) and Leg LM (t 3.53, p < 0.001, partial eta squared 3.1%) were both associated with MMSE score but Arm LM is not. After adjusting for height the relationship between total LM and MMSE is non-significant and between leg LM and MMSE is attenuated (t 2.09, p = 0.038, partial eta squared 1.1%). |
| 3. Bunout et al. [67]   | 2005 | Chile                                    | 298  | RCT  | M 75.4 (4.8)<br>F 75.8 (4.7)      | 29.2     | MMSE                        | TLM, Arm LM and Legs LM (DEXA) | Study: none<br><br>Calculated: See Bites et al. 2013 for analysis using this dataset  |
| 4. Auyeung et al. [68]  | 2013 | Chinese University of Hong Kong - 4y f/u | 3153 | Prospective observational study  | M 71.76 (4.67)<br>F 72.03 (5.07)  | 49.7     | CSI-D and MMSE              | ASM, LLMM, FFM (DEXA)          | Study: none<br><br>Given: CS-CSID did not predict TLM or ASM at baseline or at 4 years (all p > 0.05). However baseline MMSE was associated with baseline TLM (rho = 0.058, p = 0.002) and ASM (rho = 0.061, p = 0.001) and at follow-up (TLM rho = 0.058, p = 0.002, ASM rho = 0.054, p = 0.005). MMSE at follow up was not associated with TLM or ASM at baseline or follow-up (p > 0.05).  |

**Table 4 Studies identified with brain function and muscle structure (Continued)**

|                                |      |  |             |                                    |  |      |  |                       |   |
|--------------------------------|------|--|-------------|------------------------------------|--|------|--|-----------------------|---|
| <b>5. Auyeung et al. [69]</b>  | 2011 | Chinese University of Hong Kong - 4y f/u   | 2737        | Prospective observational study    | M 71.6 (4.58)<br>F 71.5 (4.85)         | 55.3 | CSI-D and MMSE   | ASM (DEXA)            | Study: In men, low baseline ASM predicted lower MMSE score after 4 years ( $B = 0.246$ , $p < 0.01$ ) however after adjustment for age, years of education and baseline MMSE it no longer did ( $p > 0.05$ ). In women, ASM did not significantly predict MMSE after 4 years, either before adjustment or after ( $p > 0.05$ ).<br><br>Given: see Auyeung et al. (2013) for analysis using this dataset   |
| <b>6. Lee et al. [70]</b>      | 2011 | Chinese University of Hong Kong            | 4000        | Prospective observational study    | M 72.3 (5.0)<br>F 72.5 (5.3)           | 50   | CSI-D and MMSE   | ASM, LLMM, FFM (DEXA) | Study: none<br><br>Given: see Auyeung et al. (2013) for analysis using this dataset   |
| <b>7. Auyeung et al. [71]</b>  | 2008 | Chinese University of Hong Kong - baseline | 4000        | Prospective observational study    | M 72.3 (5.0)<br>F 72.5 (5.3)           | 50   | CSI-D and MMSE   | ASM (DEXA)            | Study: none<br><br>Given: see Auyeung et al. (2013) for analysis using this dataset   |
| <b>8. Pedersen et al. [72]</b> | 2012 | Denmark                                    | 72 controls | Cross-sectional study              | Median 53 (48–60 inter quartile range) | 46   | DART, WAIS-III information subtest, TMT-A&B, Rey Auditory Verbal Learning Test (RAVLT), Symbol Digit Modalities Test (SDMT), and fluency tests | FFM (DEXA)            | Study: None<br><br>Calculated: FFM did not predict the cognitive z score with or without adjusting for BMI and childhood intelligence (Danish Adult Reading Test, DART). The six individual cognitive tests were then analysed: FFM did not predict RAVLT, SDMT, category fluency (using animals) or TMT-b test, with or without adjusting for BMI and childhood intelligence (DART). Unadjusted, there was no significant association between the letter fluency test (using "s") and FFM ( $P > 0.05$ ), however after adjustment for BMI and DART, letter fluency was significantly associated with FFM ( $t 2.34$ , $p = 0.02$ , partial eta squared 7.7%). TMT-a test did significantly predict FFM ( $t 3.08$ , $p = 0.003$ , partial eta squared 12.3%). After adjusting for BMI and DART the relationship became non-significant. |
| <b>9. Magri et al. [73]</b>    | 2006 | Italy                                      | 27 controls | Cross-sectional case-control study | Controls 33.3 (7.15)                   | 0    | MMSE   | FFM (BIA)             | Study: none<br><br>Calculated: FFM did not significantly predict MMSE ( $p > 0.05$ ), adjusting for age. Adjustments for BMI and educational level did not significantly affect the results.  |

**Table 4 Studies identified with brain function and muscle structure (Continued)**

|                                |      |  |                     |   |                    |      |  |   |   |
|--------------------------------|------|--|---------------------|---|--------------------|------|--|---|---|
| <b>10. Lasaite et al. [74]</b> | 2009 | Lithuania                                  | 29 healthy controls | Observational case-control study  | 66.2(6.3)          | 0    | TMT-A and B and digit span   | FFM (BIA)   | Study: none<br><br>Calculated: FFM does not significantly predict TMT-A or B adjusting for age +/- height (p > 0.05). Trend with FFM predicting digit span (t 1.96, p = 0.06, partial eta squared 13%) but attenuated when adjusted for height in addition to age (p = 0.37).   |
| <b>11. Liu et al. [75]</b>     | 2014 | Taiwan, I-Lan Longitudinal Aging Study     | 983                 | Population based ageing cohort study  | 65.2 (9.3)         | 50.6 | MMSE   | LBM and Relative ASM (=ASM/height <sup>2</sup> ) (DEXA) | Study: A t test comparing mean MMSE in those with normal RASM and those within the lowest 20 % of RASM found a significant difference in men and women of all ages (p < 0.05).<br><br>Given: In a MLR, RASM did not predict MMSE after adjusting for age and sex (beta -0.003, p = 0.940). Adjusting for education in addition did not affect the results.                    |
| <b>12. Moore et al. [76]</b>   | 2012 | USA, Baltimore Longitudinal Study of Aging | 786                 | Longitudinal cohort study   | 66.3 (range 26-96) | 51.9 | California Verbal Learning Test (CVLT), digit-span test, TMT A & B | Mid-femur thigh CSA (CT)                                | Study: none<br><br>Given: In a linear regression, none of the cognitive tests predicted thigh CSA, adjusting for age and gender. After adjusting for age, gender and height, the digit-span backward test became significantly associated with thigh CSA (beta -1.55, p = 0.024).   |
| <b>13. Kamijo et al. [77]</b>  | 2014 | USA, FITKids Study                         | 37 (healthy weight) | Cross-sectional study (case-control substudy comparing obese and healthy weight children) | 8.8 (0.6)          | 46   | Kaufman Brief Intelligence Test (K-BIT)                            | TLM (DEXA)  | Study: none<br><br>Calculated: Authors sent one data sheet for the FITKids study as there is considerable overlap in subjects between the two Kamijo et al. papers [77,78], (n = 139, mean age 8.8 (sd 0.6), male 51.1%). A GLM found that TLM did not predict K-BIT after adjustment for age and gender (p > 0.05). Adjusting for BMI in addition did not alter the results. |
| <b>14. Kamijo et al. [78]</b>  | 2012 | USA, FITKids Study                         | 126                 | Cross-sectional study   | 8.9 (0.5)          | 50   | Kaufman Brief Intelligence Test (K-BIT)                            | TLM (DEXA)  | Study: none<br><br>Calculated: as per Kamijo et al. [77]  |

\*Associations column key: Study = results published within the study; Given = associations calculated by study authors and supplied to us for this review; Calculated = study authors supplied raw data to us and we performed the analysis.

**Table 5 Studies identified with measures of brain structure or function and muscle structure or function but no associations given in paper or on request**

| Authors  | Year | Country and dataset  | n                                | Study design  | Mean age (sd)                              | Male (%) | Brain structure or function  | Muscle structure or function                 |
|--|------|--|----------------------------------|---|--|----------|--|--|
| <i>Studies with brain structure and muscle structure (re: Table 2)</i> |      |  |                                  |   |  |          |  |  |
| <b>1. Chowdhury et al. [79]</b>  | 1994 | Sweden   | 8                                | Methodology paper   | 35 (8)                                     | 100      | Brain volume (CT)  | Calculated skeletal muscle volume (CT)       |
| <i>Studies with brain structure and muscle function (re: Table 3)</i>  |      |  |                                  |   |  |          |  |  |
| <b>2. Liu-Ambrose et al. [80]</b>                                      | 2010 | Canada, Exercise RCT in Vancouver                                  | 155                              | RCT, prospective over 52 weeks                            | 69.6 (2.9)                                 | 0        | Whole brain volume (MRI)   | Gait speed, quads strength and muscle power  |
| <b>3. Nadkarni et al. [81]</b>   | 2012 | Canada, Sunnybrook Dementia Study                                  | 20 controls                      | Cross-sectional substudy of longitudinal study            | 75 (9)                                     | 40       | Score on Age-Related White Matter Change Scale (MRI)   | Self-selected speed on a treadmill           |
| <b>4. Sullivan et al. [82]</b>   | 2005 | USA, California, Stanford  | 51                               | Case-control study  | 45.2 (13.9)                                | 100      | Caudate, putamen, nucleus accumbens and medial septal / diagonal band volumes and ICV (MRI)                      | Bilateral grip strength                      |
| <i>Studies with brain function and muscle structure (re: Table 4)</i>  |      |  |                                  |   |  |          |  |  |
| <b>5. Guthrie et al. [83]</b>  | 2004 | Australia, The Melbourne Women's Midlife Health Project            | 1897                             | 9 year prospective, observational population based sample | Median 50                                  | 0        | Episodic verbal memory using a 10 word recall task (CERAD)   | Body composition (DEXA)                      |
| <b>6. Ellis et al. [84]</b>  | 2009 | Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging | 768 healthy controls             | Longitudinal case control study (AD vs MCI vs normal)     | 70.0 (7.0)                                 | 43       | CVLT-II, Logical memory, RCFT, digit span, digit symbol coding, D-KEFS verbal fluency, BNT, clock, WTAR, Stroop. | Body composition (DEXA) in subgroup in Perth |
| <b>7. Dao et al. [85]</b>  | 2013 | Canada, Exercise RCT in Vancouver                                  | 114                              | Secondary analysis of RCT data                            | 69.4 (2.9)                                 | 0        | Stroop test, MMSE  | Sub-total lean mass (DEXA)                   |
| <b>8. Schwartz et al. [86]</b>   | 2013 | Canada, Saguenay Youth Study                                       | 983                              | Longitudinal cohort study                                 | M 14.9 (1.8), F 15.1 (1.9)                 | 48.8     | Executive function and Memory  | FFM (BIA)                                    |
| <b>9. Bagger et al. [87]</b>   | 2004 | Denmark, PERF study  | 5607                             | Prospective, observational cohort study                   | 71.1 (6.6)                                 | 0        | Short Blessed Test   | TLM (DEXA)                                   |
| <b>10. Abellan van Kan [88]</b>  | 2013 | France, EPIDOS study   | 3025                             | Prospective multi-centre cohort study                     | 80.51(3.9)                                 | 0        | SPMSQ  | Lean mass and ALM (DEXA)                     |
| <b>11. Nourhashemi et al. [89]</b>                                     | 2002 | France, EPIDOS study   | 7105                             | Cross-sectional study                                     | 80.3 (3.65) (SPMSQ > =8)                   | 0        | SPSMQ for orientation, concentration and memory  | FFM (DEXA)                                   |
| <b>12. Nourhashemi et al. [90]</b>                                     | 2001 | France, EPIDOS study   | 7364                             | Prospective multicentre study                             | Broken down by ADLs; means 79.9-82.7 years | 0        | Pfeiffer's test (aka SPMSQ)  | Body composition (DEXA)                      |
| <b>13. Paolisso et al. [91]</b>  | 1997 | Italy, Naples  | 30 (>50y), 30 (75-99y) 19 (>99y) | Observational study                                       | 44.5(1.8), 78(0.7), 102(0.8)               | 46.8     | MMSE   | FFM (BIA)                                    |

**Table 5 Studies identified with measures of brain structure or function and muscle structure or function but no associations given in paper or on request (Continued)**

|                                      |      |  |                               |   |  |                                 |   |   |
|--------------------------------------|------|--|-------------------------------|---|--|---------------------------------|---|---|
| <b>14. Malaguarnera et al. [92]</b>  | 2007 | Italy, Sicily                                  | 66                            | Placebo controlled, randomized, double-blind, 2-phase study         | 101(1.3) treatment, 101(1.4) placebo   | 31.8                            | MMSE  | Total muscle mass (BIA)   |
| <b>15. Jacobsen et al. [93]</b>      | 2012 | Netherlands                                    | 318                           | RCT   | Mean for each arm given range 73.4-74.0                                      | 0                               | 15 words test and Trails B test                   | BIA and DEXA  |
| <b>16. Genton et al. [94]</b>        | 2011 | Switzerland                                    | 213 in 1999 and 112 in 2008   | Cross-sectional study with 9 year f/u visit                         | 1999 M 71.7(5.2)<br>2008 M 80.3(5.2)<br>1999 F 73.2(5.5)<br>2008 F 82.2(5.6) | 1999 49.3<br>2008 49.1          | MMSE  | FFM (BIA), ASMM (DEXA) and BCM (total body potassium)   |
| <b>17. Donaldson et al. [95]</b>     | 1996 | USA, Baltimore                                 | 73                            | Cross-sectional study   | 68.8 (7.2)   | 31.5                            | MMSE  | FFM (DEXA)  |
| <b>18. Bove et al. [96]</b>          | 2013 | USA, Boston, Harvard                           | 12                            | Cross-sectional study   | 31.6 (6.4)   | 0                               | Multiple tests broken down to 5 cognitive domains | Cross sectional area of mid-thigh (CT)  |
| <b>19. Papadakis et al. [97]</b>     | 1995 | USA, California, San Francisco                 | 104                           | Cross-sectional study   | 75.5(4.9)  | 100                             | MMSE, Trails B and DSST                           | Lean tissue mass (DEXA)   |
| <b>20. Janssen [98]</b>              | 2006 | USA, Cardiovascular health study               | Baseline 5036                 | Longitudinal observational study (over 8 years)                     | 65-70 (42.7%), 71-76 (32.7%), 83-89 (18.2%), ≥90 (6.4%)                      | 43.6                            | MMSE  | Whole body muscle mass (BIA) and normalized for height to the skeletal muscle index (SMI, kg/m <sup>2</sup> ) |
| <b>21. Masley et al. [99]</b>        | 2008 | USA, Florida                                   | 56                            | RCT   | Controls 43.5 (11.2), Intervention 47.1 (9.4)                                | Control 39.3, Intervention 53.6 | CNS vital signs battery                           | FFM (BIA)   |
| <b>22. Houston et al. [100]</b>      | 2012 | USA, Health, Aging, and Body Composition study | 2641                          | Longitudinal cohort study   | 74.7 (2.9)   | 48.9                            | MMSE  | Lean mass (DEXA)  |
| <b>23. Middleton et al. [101]</b>    | 2011 | USA, Health, Aging, and Body Composition study | 197                           | Cross-sectional study from a 9 year longitudinal cohort study       | Separated into tertile of activity, means range from 73.9-75.8               | Not given                       | 3MS   | FFM (DEXA)  |
| <b>24. Koster et al. [102]</b>       | 2010 | USA, Health, Aging, and Body Composition study | 2949                          | Cross-sectional study from a 9 year longitudinal cohort study       | Age 70-79 at baseline  | 48.5                            | 3MS   | Total bone-free lean mass, trunk lean mass, appendicular lean mass (DEXA)                                     |
| <b>25. de Rekeneire et al. [103]</b> | 2003 | USA, Health, Aging, and Body Composition study | 2926                          | Baseline data from a 9 year longitudinal cohort study               | Diabetes mellitus (DM) 73.6 (2.9) and non-DM 73.6 (2.9)                      | DM 55.9<br>Non-DM 46.9          | MMSE and DSST                                     | Lean mass and lean soft tissue mass (i.e. lean mass minus bone) (DEXA)  |
| <b>26. de Rekeneire et al. [104]</b> | 2003 | USA, Health, Aging, and Body Composition study | Fallers 652, non-fallers 2398 | Baseline data from a 9 year longitudinal cohort study               | Range 70-79  | Fallers 41.7, non-fallers 50.3  | Teng Mini-mental State Examination and DSST       | Total muscle mass and skeletal muscle mass in the legs (DEXA)   |
| <b>27. Watts et al. [105]</b>        | 2013 | USA, Kansas, Brain Aging Project               | 74 healthy controls           | Longitudinal case-control study (Alzheimer's dementia vs. controls) | 74.0 (7.2)   | 43                              | MMSE  | Lean mass (DEXA)  |

**Table 5 Studies identified with measures of brain structure or function and muscle structure or function but no associations given in paper or on request (Continued)**

|                                |      |  |  |                                      |                          |      |  |                                    |
|--------------------------------|------|--|--|--------------------------------------|--------------------------|------|--|------------------------------------|
| <b>28. Canon et al. [106]</b>  | 2011 | USA, National Health and Nutrition Examination Survey (NHANES) | 867  | Cross-sectional longitudinal study   | Range 60-85              | 44.8 | Digit-symbol coding test   | Lean tissue mass (DEXA)            |
| <b>29. Garry et al. [107]</b>  | 2007 | USA, New Mexico Aging Process Study                            | 809 rolling participants (average 302 seen per year) | Longitudinal Aging study (1979–2003) | 60+ Varied between years | 40   | 3MS (annual), WAIS R digit span, Fuld object memory evaluation, Color Trails 1 and 2, clock drawing (all less than annual) | Annual skeletal tissue mass (DEXA) |
| <b>30. Haren et al. [108]</b>  | 2008 | USA, St Louis, African-American Health Study                   | 124  | Population based longitudinal study  | 56.1(4.4)                | 100  | MMSE, TMT A&B  | TLM and ASM (DEXA)                 |
| <b>31. Dvorak et al. [109]</b> | 1998 | USA, Vermont   | 30   | Case-control study                   | 73(7)                    | 43.3 | MMSE   | ASM and FFM (DEXA)                 |

relationship between brain mass and body composition [26]. They performed multiple linear regression and found that after adjusting for age and fat mass, FFM predicted brain mass in men (beta 0.023, R<sup>2</sup> 5%,  $p = 0.01$ ) and women (beta 0.003, R<sup>2</sup> 6%,  $p < 0.0001$ ). Fat mass or bone mineral content did not significantly predict brain mass in either sex. So they conclude that it is FFM that drives the relationship between body size and brain size not bone or fat mass. Weise et al. investigated the associations between regional grey matter volume and fat free mass index (FFMI = FFM/height<sup>2</sup>) [31]. They found several areas of grey matter volume that were significantly associated with FFMI ( $p < 0.01$ , see Table 2), however after adjusting for percentage body fat or fat mass only two areas remained significant (the right temporal pole and bilateral ventromedial prefrontal cortex).

#### **Association of brain structure and muscle function**

Thirty three studies which included measures of brain structure and muscle function were identified (Table 3). The muscle function variables most commonly studied were grip strength and gait speed. Only one study was identified which used a different measure of muscle function and that was maximal isometric knee extension strength (IKES) [60]. The brain structure variables include: corpus callosum area, and volumes for total and regional GM and WM, cerebrospinal fluid (CSF), cerebellum, hippocampus, basal ganglia and whole brain volume and measures of prevalence of WMH, either volume or scoring systems (e.g. Fazekas).

#### **Brain structure and grip strength**

The PATH through life project [32-35], the Cardiovascular Health Study [55-57,62-64], the Lothian Birth Cohort 1936 study [61], a study from Japan [36] and a study from Philadelphia [37] all looked at the relationship between grip strength and brain structure.

There are four papers identified by our search strategy from the PATH through life project, which was set up to track and define the lifespan course of depression, anxiety, substance use and cognitive ability. In one paper from this project, Anstey et al. (2007) studied the relationship between the area of the corpus callosum (CC) (measured in three sections: anterior, midbody and posterior; and total area) and grip strength [33]. They used the grip strength from the hand the subject wrote with and adjusted for age, sex and ICV. They found no significant relationship between total, anterior or posterior CC area and grip strength however they found a positive relationship between midbody CC area and grip strength (beta  $-0.09$ ,  $p < 0.05$ ). They conclude that this is due to the association between midbody CC and the motor cortices. Another paper from the PATH through life project studied the association between grip strength and the

percentage of WM occupied by WMH in different brain areas [35]. They found that a larger percentage of WMH per WM volume is associated with decreased grip strength for both the total brain and several brain areas (frontal, temporal, parietal, anterior horn and periventricular body (all  $p < 0.01$ )). However, the amount of WMH in the occipital lobe, the cerebellum and the posterior horn was not associated with grip strength. The 2009 paper from this study further investigated the relationship between WMH and grip strength [32]. This time they looked at the relationship in men and women separately. They found that larger amounts of WMH was associated with reduced grip strength, adjusting for age, depression severity and brain atrophy index, in men ( $p < 0.05$ ) but not in women (n/s). However they comment that they feel that the relationship between WMH volume and motor function is likely to be the same in both sexes and that their finding may be due to the difference in WMH amount between men and women in their study population. Sachdev's 2006 paper from this study did not look at the relationship between motor function and brain structure and the authors did not respond to our data request [34].

The Cardiovascular Health Study (CHS) is a large, longitudinal, observational study of risk factors for cardiovascular disease in adults 65 years or older, which commenced in 1989 [52]. The CHS measured grip strength and gait speed and WMSA, however only one paper from this study looked at the relationship between grip strength and WMSA [64]. In this paper Longstreth et al. (1996) performed a partial correlation which found no significant association between grade of WMSA (graded on a scale of 0-9) and grip strength in either the dominant or non-dominant hand ( $p > 0.05$ ) after adjusting for age, sex and presence of clinically silent stroke on MRI [64].

The Lothian Birth Cohort 1936 study measured grip strength at baseline and 3 years later at which point brain volumes were also measured [61]. It is the only study to look at longitudinal changes in muscle strength and brain structure. Grip strength at wave 1 predicted ventricular volume at wave 2 (standardized beta  $-0.10$ ), however there was no significant association with other brain volumes and grip strength at wave 2 predicted ventricular volume ( $-0.11$ ) and NAWM (0.08). Therefore, increased grip strength was associated with less brain atrophy in this wave. However, decreased grip strength over 3 years was not significantly associated with any brain volume measure.

The paper by Doi et al. used multiple linear regression to show that grip strength is not related to brain atrophy (beta  $-0.082$  (SE 0.005)  $p = 0.54$ ) [36]. They measured brain atrophy by mapping the MR brain scans from their subjects to those from healthy controls. Most studies



used an index to intracranial volume to calculate degree of brain atrophy. No associations with the other measured brain volumes were included in the paper.

The paper by Hardan et al. looked at the association between caudate volume and grip strength in both hands in children and young adults [37]. They found non-significant statistical trends using Pearson's correlation between total caudate volume and mean grip strength in the right ( $r = -0.303$ ,  $p = 0.05$ ) and left ( $r = -0.28$ ,  $p = 0.07$ ) hands. The relationships are negative, therefore there is a trend that those with larger caudate nuclei were found to have lower grip strength in both hands.

### **Gait speed and brain structure**

The Sydney Older Person's Study [38], the TASCOC study [39-41], the Three-City Study [42-45], the AGES-Reykjavik study [60], ABC1921 study [46], WML and mobility study [52,53], further studies from Boston [47-51], the Cardiovascular Health Study [54-57,62-64], the Oregon Brain Aging Study [58,59], the LBC1936 study [61] all looked at the relationship between structural brain measures and gait speed. There were 27 studies identified to include in this section, making it the most researched association in our review. The measurement of gait speed varied considerably, with studies variously using maximum speed or usual pace, and some studies requiring a turn halfway through the measurement and others not. The distance used for the measurement also varied from 2.5 to 75 metres, however the most commonly used measure was usual pace over 6 metres.

The Sydney Older Person's Study was set up to investigate the environmental, biological and social determinants of healthy ageing. Within it Piguet et al. looked at the relationship between timed walk over 5 meters, adjusted for lower limb arthritis, and cerebellar vermis area (broken down into V1, V2, V3 and total), and total cerebellar volume. None of the measures of cerebellar size/volume significantly predicted the timed walk [38].

The Tasmanian Study of Cognition and Gait was set up to examine the role of age-related brain changes in causing problems with walking, balance and cognitive abilities in the general community. It measured brain volumes and usual walking speed over 4.6 metres at baseline and 31 months [39]. They found that a greater decline in gait speed over this period was associated with more WM atrophy and hippocampal atrophy and greater accumulation of WML ( $p < 0.05$ ). There was a non-significant trend with GM atrophy and decline in gait speed ( $p = 0.06$ ).

The Three-City study is a longitudinal study of the relation between vascular diseases and dementia in persons aged 65 years and older in France, which includes measures of WM volume and maximum walking speed

over 6 metres and a repeat walking speed test at the fourth follow up assessment (i.e. roughly 7 years after the first). There were four papers identified from this study which contained reference to these variables.

Soumare et al. looked at the association between WMH volume and both baseline walking speed and decline in walking speed over the 7 year follow up period [45]. They adjusted for age, gender, education and brain white matter volume. They found a significantly lower mean walking speed in those with a total WMH volume above the 75th percentile compare to those below the 25th. They found similar relationships for both deep WMH and periventricular hyperintensities (PVH), however further analyses revealed that PVH may have more of an effect on walking speed than deep WMH. They also looked at WMH volume and the decline in walking speed over the follow up period. They found that having a WMH volume greater than the 90th percentile, more than doubled the risk of decline in walking speed compared with subjects with lower volumes of WMH. This finding was replicated when looking at PVH but not for deep WMH volume. Elbaz et looked at this association further and found that large WMH volumes were not associated with slow walking speed among highly educated participants ( $OR = 0.72$ ), but were associated with a 2-fold-increased risk of slow walking speed among those with low education ( $OR = 3.19/1.61 = 1.99$ ) ( $p$  interaction = 0.026) [42]. Results remained unchanged after adjustment for height, BMI, and MMSE score.

Dumurgier et al. looked at GM volumes and gait speed in the same cohort and found that only basal ganglia volume (beta 0.075 (SE 0.025)  $p = 0.003$ ) was significantly associated with walking speed; driven by caudate nucleus volume (beta 0.114 (SE 0.024)  $p < 0.001$ ) [43]. All other regional GM volumes were not significantly associated with walking speed.

The authors from the Three-City study provided further associations between the variables of interest on written request [39-41]. They looked at the relationship between WM volume and maximal walking speed at baseline, and walking speed decline over 31 months using a multiple linear regression (MLR) and found no significant association. Finally they performed a logistic regression between a one standard deviation increase in WM volume and the risk of having the highest walking speed decline, which was again not significant.

The AGES-Reykjavik study is a longitudinal cohort study which includes an MRI brain and usual walking pace over 6 metres [60]. The MR brain imaging included a magnetization transfer imaging sequence, which can be used to calculate the magnetisation transfer ratio (MTR), which can detect normal and diseased brain tissue by looking at the homogeneity of the brain tissue



being studied. They found that in men usual walking speed was predicted by WMH volume (beta 0.13,  $p = 0.02$ ) but not by degree of brain atrophy or peak MTR height (both  $p > 0.05$ ) (adjusted for age and brain size) [60]. However in women slower walking speed was associated with: lower MTR height (i.e. indicating abnormal brain tissue) (beta  $-0.14$  ( $p = 0.01$ ); increased WMH (beta 0.12,  $p = 0.003$ ); and greater brain atrophy (beta 0.15,  $p = 0.01$ ) [60]. Additionally they comment that isometric knee extension strength was found to positively correlate with peak height MTR ( $p < 0.005$ ) however they do not give the strength of the correlation or say what it was adjusted for.

The Aberdeen Birth Cohort 1921 study is a longitudinal study which includes a measure of gait speed (self-paced walk time over 6 metres) and a MR brain scan, which was assessed for WMH. Lower gait speed was significantly associated with increased WMH in the brainstem ( $p = 0.009$ , partial eta squared 7%), but not in the cerebral white matter or with PVHs [46].

Seven studies were identified which met the inclusion criteria from the Boston area in the United States. These include two papers from the WML and mobility observational follow up study [52,53], two papers looking at mobility, brain changes and cardiovascular risk factors at baseline [51] and follow up at 2 years [50], two papers conducted at the Beth Israel Deaconess Medical Centre, where it seems there may be overlap between the study volunteers [48,49] and a case-control study about diabetic peripheral neuropathy [47]. The two studies from the WML and mobility study recorded variables at baseline [53] and after a period of follow up (19–22 months) [52]. The baseline paper comments that gait velocity was not significantly predicted by WMSA corrected for ICV, however does not give any specific figure for this analysis [53]. The follow up paper found a significant negative relationship between gait velocity and WMSA at baseline ( $p < 0.05$ ) [52], however this is in contrast to the baseline paper and only 14 of the original 28 subjects consented for this study. Change in gait speed between visit 1 and 2 did not predict WMSA volume ( $p = 0.07$ ). They also state they found a significant negative relationship between change in gait speed between visits and CSF volume ( $r = 0.733$ ,  $p < 0.005$ ) and a positive relationship between change in gait speed and WM volume ( $r = 0.558$ ,  $p < 0.05$ ) [52]. However both the quoted correlations are positive.

Moscufo et al. recruited 99 subjects to a longitudinal study about mobility, brain changes and cardiovascular risk factors [50,51]. Gait speed was measured using time to walk 2.5 metres as part of the Short Physical Performance Battery (SPPB). This is a considerably shorter distance than most other measures of gait speed used. The authors supplied Spearman partial correlations between

the brain volumetric variables and gait speed, which were not described in the paper. Greater WMH burden ( $\rho = -0.365$ ,  $p = 0.0002$ ) and CSF volumes ( $\rho = -0.284$ ,  $p = 0.004$ ) are associated with slower gait speed. White matter was not found to significantly predict gait speed, however larger GM volume did predict faster gait speed ( $\rho = 0.232$ ,  $p = 0.020$ ) [51].

An analysis was made in the baseline paper, to investigate whether location of WMH affected gait speed [51]. They selected 10 regions of interest (ROI), which were neural pathways involved in sensory input or motor response and performed a Spearman's correlation with a corrected significance threshold of  $\leq 0.005$  (calculated using the Bonferroni method to adjust for multiple comparisons). All 10 ROI were found to significantly correlate with the walking speed score at  $p < 0.005$  ( $\rho$  values between 0.279 and 0.426), except in the superior longitudinal fasciculus ( $p = 0.035$ ) [51].

The follow up paper in this study, performed after 2 years, found that total WMH burden was significantly associated with usual walking speed at baseline but not at follow-up, and maximum walking speed was not associated with total WMH at baseline or follow up [50]. At baseline, regional GM WMH burden in the splenium of corpus callosum and anterior and superior corona radiata, was significantly associated with both usual and maximum walking speed ( $p < 0.05$ ) and in addition the body of the corpus callosum was also associated with usual walking speed ( $p < 0.05$ ). At follow-up, WMH burden in the splenium was significantly associated with both walking measures ( $p < 0.05$ ) and in the body with maximum walking speed. Change in WMH burden, either total or in any of the 7 regional areas, over 2 years was not associated with a decline in usual walking speed ( $p > 0.1$ ). However decline in walking speed was entered as a binary variable for this analysis (i.e. decline or no decline in walking speed over 2 years), which may have missed a relationship between greater WMH burden and greater declines in walking speed.

Two papers carried out their studies at the Beth Israel Deaconess Medical Centre. One paper looked at healthy volunteers [49] and the other looked at stroke patients in comparison to healthy volunteers [48]. It does not explicitly state the healthy volunteers are the same for each study, but the exclusion criteria, time period and author list would indicate this. The first study measured gait speed over 12 minutes at normal walking speed. MR brain images were analysed for WMH burden and brain volumes corrected for ICV. They found that gait speed was correlated to frontal WM volume ( $r = 0.4$ ,  $p = 0.003$ ) and frontal grey matter volume ( $r = 0.3$ ,  $p = 0.01$ ) [49]. However total WMH burden was not associated with gait speed. It is not exactly clear why they looked at frontal brain volumes and gait speed and not other

regions of the brain or total brain volume. The second paper also measured gait speed over 12 minutes and used MR brain images. In the non-stroke group, white matter volume was found to predict gait speed (B 1.30,  $p = 0.03$ ) but not grey matter ( $p > 0.05$ ). They comment that greater brain atrophy is associated with slower gait speed, but this is for the whole group, so includes stroke patients.

The final study from Boston was by Manor et al. and quoted results from the control group [47]. They found no association between total GM volume or regional GM volumes and walking speed over 75 metres ( $p > 0.005$ , Bonferroni adjusted). They were being compared to subjects with diabetic peripheral neuropathy in this study. No results for WM or CSF were reported in the study.

Seven studies from the Cardiovascular Health Study (CHS) met our criteria for inclusion. However three of the studies did not contain any associations between the variables of interest and the study authors did not supply the raw data or correlations [55,62,63]. The first study used gait speed measured over 15 feet, and MR brain images were used to measure ventricular enlargement (VE) and WMH both of which were recorded on a 10 point scale (0–9). Both greater ventricular enlargement ( $p < 0.001$ ) and greater WMH burden ( $p = 0.003$ ) were associated with slower baseline gait speed and greater decline in gait speed over the 4 year follow up period [57]. Indeed, after adjusting for baseline performance, those with severe VE were found to have 2.5x the decline in gait speed compared to those with minimal VE at baseline. The model included adjustment for age, sex, race and education.

The next study looked at a subset of the CHS who had undergone two MR brain scans, separated by roughly 5 years, and a MMSE and had undergone assessment on the GaitMat, a 4 metre long instrumented walking surface [56]. THE MR brain scans were classified as above, but given binary cutoffs for the analysis of WMH grade  $\geq 3$  or  $< 3$  and VE  $> 4$  or  $< 4$  for some of the analyses. Gait speed was correlated with WMH grade ( $r = -0.18$ ,  $p < 0.0001$ ) and with WMH in the brainstem ( $r = -0.18$ ,  $p < 0.01$ ). Logistic regression was used to analyse the relationship further and gait speed was separated into quartiles. This showed that those in the lowest two quartiles of gait speed (i.e.  $< 1.02$  m/s) had double the likelihood of having WMH graded 3 or above ( $p = 0.03$ ). VE graded  $> 4$  was not found to be significantly predicted by gait speed, however VE graded  $> 5$  was significantly predicted by gait speed (OR = 2.91 for 1st vs. 4th quartile, OR 3.82 for 2nd vs 4th quartile) [56].

Longstreth et al. is mentioned in the above section on grip strength and brain structure, as this was also studied in this paper [64]. Gait speed was again measured

over 15 feet and WMH burden was scored 0–9 on MR brains scans. Time to walk 15 feet was found to correlate with WMH grade (partial correlation coefficient 0.153,  $p < 0.001$ , adjusting for age, sex and presence of clinical silent stroke on MR brain) [64]. The population in this study and the study by Rosano et al. [57] overlap considerably and only appear to differ in the time they were still in the study and the particular inclusion and exclusion criteria for that part of the study.

In a separate paper, Rosano et al. found that prefrontal area volume significantly predicted time to walk in a stepwise forward model (beta  $-0.15$ ,  $p = 0.02$ ) [54]. This relationship was attenuated when adjustment was made for DSST score, which is a measure of processing speed. They conclude that smaller prefrontal area volume may contribute to slower gait speed through slower information processing.

The final two studies identified are both from the Oregon Brain Aging Study (OBAS) [58,59]. OBAS I is a prospective study commenced in 1989 of healthy older adults age 65 years or older at the initial assessment, a second arm was added in 2004, OBAS II, with less stringent exclusion criteria and these subjects were 85 or older at the start of the study. The first paper by Marquis et al. (2002), looked at the correlation of timed walk, measured at self-selected pace over 9 metres, against brain volumes. Hippocampal volume was found to negatively correlate with timed walk (partial  $r = -0.12$ ), however no significance value was given and it did not explicitly state what was adjusted for in the correlation [59]. The correlation between TBV and timed walk was  $< 0.1$ . The other OBAS paper, by Silbert et al. (2008), found that a higher baseline total WMH volume was associated with a greater increase in timed walk over follow up ( $R^2 = 0.08$ ,  $p = 0.0052$ ), the average follow up was 9.1 years [58]. They then looked at whether location of WMH mattered and found that whilst periventricular (PV) WMH volume was associated with a greater change in timed walk over follow up ( $R^2 = 0.12$ ,  $p = 0.0039$ ), a higher subcortical WMH volume was not. These analyses were adjusted for age and ICV. They next looked at change in WMH volume with time and found that a higher rate of accumulation of PV WMH was associated with a greater increase in timed walk ( $R^2 = 0.15$ ,  $p = 0.0453$ ). However there was no relationship described between subcortical or total WMH accrual and change in timed walk. Further data from the study was requested, which was kindly provided. Baseline data from all subjects from OBAS I and II who had had a MRI brain scan and a timed walk at baseline was used to perform our analysis ( $n = 176$ ).

GLMs were performed to investigate the relationship between brain structures and gait speed, calculated in metres per second for the analysis. In an unadjusted model, gait speed was predicted by TBV, hippocampal

volume and WMH volume, all  $p < 0.001$ . Upon adjusting for age, sex, ICV and height, TBV ( $t = 3.61$ ,  $p = 0.004$ , partial eta squared 4.3%) and WMH ( $t = -2.80$ ,  $p = 0.006$ , partial eta squared 4.4%) significantly predicted gait speed, but hippocampal volume did not ( $p > 0.05$ ).

#### Association of brain function and muscle structure

Fifteen papers were identified which looked at brain function and muscle structure: DEXA was used in eleven of the studies; BIA in two; and CT for thigh muscle CSA and MRI for neck muscle CSA in the final two papers (Table 4). Measures of brain function were the MMSE, the Community Screening Instrument of Dementia (CSI-D), Trail Making Test (TMT) A and B, digit span and a measure of global cognitive performance (using  $z$  scores from multiple cognitive tests). The studies included the Kansas Brain Aging Project [28-30] and the MHEM study [27], both mentioned in the above section, and studies from Canada [65], Chile [67], the Chinese University of Hong Kong [69,71], Denmark [72], Italy [73], Lithuania [74], Taiwan (the I-Lan Longitudinal Aging Study, ILAS) [75], and from the USA, the Baltimore Longitudinal Study of Aging [76] and the FITKids Study [77,78].

From the Kansas Brain Aging Project, Burns et al. (2010) found a relationship between both MMSE (beta 0.11,  $p = 0.009$ ) and global cognitive performance (beta 0.12,  $p = 0.007$ ) and TLM, again grouping AD and control subjects together [29]. They state that in this relationship if the AD subjects are removed from the analysis the results are attenuated, but do not show any results for this. A GLM was performed on the data from the non-demented group supplied to us by the study authors, and we found that neither the global cognitive performance score nor MMSE was predicted by TLM adjusting for age and sex. Adjusting for height and education did not affect this.

The MHEM study used 9 different cognitive tests, which they reduced to two factors using principal components analysis [27]. Total neck muscle CSA did not significantly predict variance in either the memory factor or the cognitive processing factor ( $p > 0.05$ ), however, it did predict 10% of the variance in the NART score ( $t = -2.12$ ,  $p < 0.05$ ). The NART score is a measure of childhood intelligence and the authors comment that the finding is the opposite of what they hypothesized, as they found that lower childhood intelligence is associated with larger neck muscle size in old age.

Berryman et al. supplied the baseline data from their physical training intervention trial. Subjects had performed a MMSE and modified Stroop test and underwent a DEXA scan at baseline [65]. A GLM was performed which showed no association between LBM and MMSE or the Stroop naming, reading or inhibition tasks. However there was an association between the Stroop flexibility task and

LBM ( $t = 2.126$ ,  $p = 0.039$ , partial eta squared 9.3%), however after adjusting for education and height the effect was attenuated ( $p > 0.05$ ). This effect was in the opposite direction than might be expected, i.e. bigger muscle mass is associated with a worse score (the Stroop test is measured in seconds to perform the task).

The two papers from Chile are a study by Bunout et al. which includes baseline data for a randomized controlled trial (RCT) investigating an exercise intervention in the elderly [67] and a paper by Bites et al. which used baseline trial data from several studies, including the study by Bunout et al., held on their University database [66]. The authors sent one data sheet for both studies as there is a large amount of overlap between the studies (overlap  $n = 203$ ). A GLM was performed which showed total LM ( $t = 2.38$ ,  $p = 0.018$ , partial eta squared 1.4%) and leg LM ( $t = 3.53$ ,  $p < 0.001$ , partial eta squared 3.1%) were both associated with MMSE score but arm LM is not. After adjusting for height the relationship between TLM and MMSE became non-significant and between leg LM and MMSE is attenuated ( $t = 2.09$ ,  $p = 0.038$ , partial eta squared 1.1%). Therefore it seems that leg LM is driving the relationship between total LM and MMSE.

Four papers from the Chinese University of Hong Kong were identified which used data from a large prospective longitudinal study looking at bone mineral density in older Chinese adults to assess the relationship between physical and cognitive function [68-71]. They used two measures of cognitive function; the MMSE and the cognitive score from the Community Screening Instrument for Dementia (CS-CSID). Only one of the papers included the associations between the cognitive tests and muscle mass [69]. They found that in men, but not in women, lower appendicular skeletal mass (SM) at baseline predicted lower MMSE at follow up (for a 2.54 kg increase in appendicular SM, there would a 0.246 change improvement in MMSE,  $p < 0.001$ ). However after adjustment for age, years of education and baseline MMSE, the relationship became non-significant ( $P > 0.05$ ) [69]. The authors from this study kindly supplied further analyses of their data upon our request.

They performed Spearman's partial correlations, adjusting for age and sex. There was no significant relationship between baseline CS-CSID and total LM or appendicular LM at baseline or 4 years. However baseline MMSE predicted both baseline total LM (partial rho 0.058,  $p = 0.002$ ) and appendicular LM (partial rho 0.061,  $p = 0.001$ ) and 4 years follow up total LM (partial rho 0.058,  $p = 0.002$ ) and appendicular LM (partial rho 0.054,  $p = 0.005$ ). However, the effect size is small. They also looked at the whether those with lower MMSE at follow up had lower muscle mass at baseline or follow up but found no significant associations ( $p > 0.05$ ) [69]. Unfortunately the study authors did not supply data



for the relationship between change in cognition and change in muscle mass over the four year follow up which would be very interesting in such a large study population.

Pedersen et al. investigated cognition and physical fitness in normal controls, subjects with impaired glucose tolerance and type 2 diabetes [72]. They supplied the raw data for their control group to be analysed. Subjects underwent DEXA for FFM and six cognitive tests (a cognitive z score was computed as a marker of general cognition). FFM did not predict the cognitive z score with or without adjusting for BMI and childhood intelligence (Danish Adult Reading Test, DART). The six individual cognitive tests were then analysed. There was no association between FFM and most of the individual cognitive tests. Also, unadjusted there was no significant association between the letter fluency test (using "s") and FFM ( $P > 0.05$ ), however after adjustment for BMI and DART, letter fluency was significantly associated with FFM ( $t$  2.34,  $p = 0.02$ , partial eta squared 7.7%). Letter fluency is a test of executive function and this may indicate that change in this type of cognition with age is associated with FFM in older age. TMT-A test did significantly predict FFM ( $t$  3.08,  $p = 0.003$ , partial eta squared 12.3%), but after adjusting for BMI and DART the relationship became non-significant. The TMT-A test is a measure of processing speed.

Magri et al. (2006) performed a cross-sectional study looking at postmenopausal women and HRT, however their study also contained a control group of young healthy women which were used for our analyses after the study authors kindly supplied their data [73]. A GLM was performed with MMSE as the outcome/dependent variable and FFM as the predictor/independent variable as measured by BIA. FFM did not significantly predict MMSE after adjustment for age ( $p > 0.05$ ) [73]. Adjustments for BMI and educational level did not further affect these results.

Lasaitte et al. performed an observational case-control study which looked at women with osteoporosis and healthy controls [74]. The study data for the healthy controls was supplied to us on request. The cognitive measures were the TMT-A and B and a digit span test. FFM was measured using BIA. A GLM was performed on the available data. FFM did not predict TMT-A or B adjusting for age +/- height ( $p > 0.05$ ). There was a non-significant trend with FFM predicting digit span adjusting for age ( $t$  1.96,  $p = 0.06$ , partial eta squared 13%), however when adjusted for height too, the relationship was attenuated ( $p = 0.37$ ).

The I-Lan Longitudinal Aging Study is an ageing cohort study in Taiwan [75]. Within the study they performed a t test comparing mean MMSE in those with a normal relative appendicular skeletal mass ( $RASM = ASM/height^2$ ) with those in the lowest 20% for RASM,

and they found a significant difference in both men and women. They also supplied the results of a linear regression on our request for further data, which showed that RASM did not predict MMSE after adjusting for age and sex (beta  $-0.003$ ,  $p = 0.940$ ). This may mean there is a non-linear relationship between cognition and muscle mass.

The Baltimore Longitudinal Aging Study is a large longitudinal cohort study, in which the subjects underwent four cognitive tests and had a mid-femur CT for thigh CSA [76]. No associations between the cognitive tests and thigh CSA were included in the study, but the authors sent the results of a MLR they had performed. They found that none of the cognitive tests predicted thigh CSA, adjusting for age and gender. After adjusting for age, gender and height, the digit-span backward test became significantly negatively associated with thigh CSA (beta  $-1.55$ ,  $p = 0.024$ ), meaning those with bigger thigh muscles perform better on the test (a higher score is better in the digit span tests).

The final study which looked at cognition and muscle structure is the FITKids study based in Illinois, USA [77,78]. Two papers from this study were identified; however there were no relevant associations in the papers and the study authors kindly provided us with the raw data on which to perform an analysis. As the subjects were all from the same study the authors provided us with one dataset for the study. We performed a GLM which found that TLM did not predict the Kaufman Brief Intelligence Test, used to assess IQ.

## Discussion

This systematic review looked at the evidence for whether: a) brain structure is related to muscle structure, b) brain structure is related to muscle function and c) brain function is related to muscle structure in healthy humans over the life course.

### Brain volumes and muscle mass

The relationship between brain structure and muscle structure was first reviewed (see Table 6 for summary). Three studies tested for an association between whole brain volume and muscle mass; the three papers from the Kansas Brain Aging Project are treated as one study [26-30]. Two studies found a positive association between WBV and muscle mass [26,27] and one study found no significant association [28-30]. However, this study found a significant positive association between WM volume and FFM but no association between GM volume and FFM [28-30]. A different study looked at regional GM volume and found four areas negatively associated with FFM but found most areas to have no association with FFM [31]. Two studies found no association between hippocampal volume and muscle mass

**Table 6 Number of studies of brain structure and muscle structure, direction of effect and number of subjects**

|                                     | Negative association (n) | No association (n) | Positive association (n) | References |
|-------------------------------------|--------------------------|--------------------|--------------------------|------------|
| Whole brain size and muscle size    | -                        | 1 (70)             | 2 (311)                  | [26-30]    |
| White matter volume and muscle size | -                        | -                  | 1 (70)                   | [28-31]    |
| Grey matter volume and muscle size  | 1 (76)*                  | 2 (146)*           | -                        | [28-31]    |
| Hippocampal volume and muscle size  | -                        | 2 (121)            | -                        | [27-30]    |
| Cerebellar volume and muscle size   | -                        | 1 (51)             | -                        | [27]       |
| Ventricular volume and muscle size  | -                        | 1 (51)             | -                        | [27]       |

\*This study found negative associations between some areas of grey matter volume (the right temporal pole and bilateral vmPFC) and muscle size but found the majority of GM areas had no association.

[27-30]. One study looked at ventricular volume and cerebellar volume and muscle size and found no association either [27]. Four of the studies were of older adults and two were of younger adults, and it may be that the relationship between brain and muscle structure varies over the life course. Furthermore if there is a relationship between whole brain volume and muscle size it looks like it may be regional brain volume that drives this relationship rather than total volume. The studies are all cross-sectional and a large longitudinal study is needed to explore these relationships further.

#### Brain structure and muscle function

Next evidence for an association between muscle function and brain structure was reviewed. Muscle function was either grip strength (5 studies, see Table 7 for summary) or gait speed (13 studies, see Table 8 for summary) apart from in one paper where isometric knee extensor strength (IKES) was used [60].

#### Brain structure and grip strength

Only one study looked at the relationship between whole brain, GM or WM volume and grip strength [61]. There were no significant associations except for a positive relationship between WM volume and grip strength at wave 2 (age 73) [61]. This could mean that the relationship between WM volume and grip strength only becomes important with age, once a volumetric threshold is passed. Another study found no association between caudate volume and grip strength [37]. However the basal ganglia may be expected to play less of a role in grip strength than in gait speed. Two studies found a negative association with markers of brain atrophy and grip strength [36,61], however one of these studies also looked at change in grip strength over 3 years and found no association with ventricular volume (a marker of brain atrophy) [61]. This means that whilst cerebral atrophy and grip strength appear to be associated, decline in grip strength does not predict cerebral atrophy. A

**Table 7 Number of studies of brain structure and grip strength, direction of effect and number of subjects**

|   | Negative association (n) | No association (n)   | Positive association (n) | References |
|---|--------------------------|----------------------|--------------------------|------------|
| Grip strength and whole brain volume                    | -                        | 1 (694)              | -                        | [61]       |
| Grip strength and WM volume                             | -                        | 1 (694) <sup>a</sup> | 1 (694) <sup>a</sup>     | [61]       |
| Grip strength and GM volume                             | -                        | 1 (694)              | -                        | [61]       |
| Grip strength and caudate volume                        | -                        | 1 (41)               | -                        | [37]       |
| Grip strength and ventricular volume/brain atrophy      | 2 (804)                  | -                    | -                        | [36,61]    |
| Grip strength and WMH                                   | 1 (478)                  | 2 (4352)             | -                        | [35,61,64] |
| Change in grip strength over f/u and whole brain volume | -                        | 1 (694)              | -                        | [61]       |
| Change in grip strength over f/u and WM volume          | -                        | 1 (694)              | -                        | [61]       |
| Change in grip strength over f/u and GM volume          | -                        | 1 (694)              | -                        | [61]       |
| Change in grip strength over f/u and ventricular volume | -                        | 1 (694)              | -                        | [61]       |
| Change in grip strength over f/u and WMH volume         | -                        | 1 (694)              | -                        | [61]       |

<sup>a</sup>At wave 1 in this study there was no association and at wave 2 there was a positive association [61].

**Table 8 Number of studies of brain structure and gait speed, direction of effect and number of subjects**

|  | Negative association (n) | No association (n)     | Positive association (n) | References                   |
|--|--------------------------|------------------------|--------------------------|------------------------------|
| Gait speed and whole brain volume                          | -                        | -                      | 2 (885)                  | [58,59,61]                   |
| Gait speed and WM volume                                   | -                        | 2 (1587)               | 3 (813) <sup>b</sup>     | [42,48-50,61]                |
| Gait speed and GM volume                                   | -                        | 4 (2449) <sup>c</sup>  | 3 (367) <sup>b,d</sup>   | [43,47-50,54,61]             |
| Gait speed and hippocampal volume                          | -                        | 1 (191)                | -                        | [58,59]                      |
| Gait speed and cerebellar volume                           | -                        | 1 (111)                | -                        | [38]                         |
| Gait speed and WMH volume                                  | 7 (7145) <sup>e</sup>    | 4 (278) <sup>e,f</sup> | -                        | [45,46,49,50,52,53,56-61,64] |
| Gait speed and CSF volume/ventricular volume/brain atrophy | 3 (3221) <sup>g</sup>    | 2 (1489) <sup>g</sup>  | -                        | [50,56,57,60,61]             |
| Gait speed and WMH progression over f/u                    | -                        | 1 (14)                 | -                        | [52]                         |
| Change in gait speed over f/u and whole brain volume       | -                        | 1 (694)                | -                        | [61]                         |
| Change in gait speed over f/u and WM volume                | -                        | 2 (1622)               | -                        | [42,61]                      |
| Change in gait speed over f/u and GM volume                | -                        | 1 (694)                | -                        | [61]                         |
| Change in gait speed over f/u and CSF/ventricular volume   | -                        | 1 (694)                | 1 (2450)                 | [57,61]                      |
| Change in gait speed over f/u and WMH volume               | -                        | 1 (694)                | 2 (4152)                 | [45,57,61]                   |
| Change in gait speed over f/u and WM atrophy               | -                        | -                      | 1 (225)                  | [39]                         |
| Change in gait speed over f/u and GM atrophy               | -                        | 1 (225)                | -                        | [39]                         |
| Change in gait speed over f/u and hippocampal atrophy      | -                        | -                      | 1 (225)                  | [39]                         |
| Change in gait speed over f/u and WMH progression          | -                        | 1 (77)                 | 1 (225)                  | [39,50]                      |

<sup>b</sup>one study only looked at frontal WM/GM volume not total WM volume [49].

<sup>c</sup>basal ganglia volume was positively associated with gait speed in this study [43].

<sup>d</sup>one study only looked at prefrontal area volume within GM [54].

<sup>e</sup>this study found usual walking speed was negatively associated with WMH but that maximum walking speed was not [50].

<sup>f</sup>except brainstem WMH which were negatively associated with gait speed in this study [46].

<sup>g</sup>at wave 1 there was a negative association and at wave 2 there was no association in this study [61].

longitudinal study including both measures would help explain this relationship further.

One study found an association between WMH and grip strength [35]. They found that location of the WMH is important, with some brain areas correlating with grip strength and others not [35]. On looking at the data separated by sex, this relationship persisted in men, but not women, but the study authors think this is due to a sex difference present in their study population, with the men having higher volumes of WMH [32]. Two larger studies found no association between WMH and grip strength, however one of these studies used a visual rating scale from 0–9 to measure WMH, which may lead to differing results than using WMH volumes [61,64]. The other study also looked at change in grip strength over 3 years and WMH volume at follow up and found no association [61]. WMH are known to predict dementia and

cerebrovascular disease but their relationship to physical function is less well understood [110].

#### **Brain structure and gait speed**

Two studies found a positive association between WBV and gait speed [58,59,61], whereas studies investigating the relationship between WM and GM volume and gait speed found less unanimous results. Three studies found a positive association between WM volume and gait speed [48,49,61] and two studies found no association [42,50]. Four studies found no association between GM volume and gait speed [43,47,48,61] but three studies found a positive relationship [49,50,54]. There was no evidence that hippocampal volume or cerebellar volume were associated with gait speed [38,58,59]. It may be that specific sub-regions of the white and grey matter are associated with gait speed, for example one paper found

an association between basal ganglia volume and gait speed but no association with total GM and gait speed. Further studies looking at regional brain areas will help to clarify these relationships. Five studies looked at markers of brain atrophy and gait speed; two found a negative association (i.e. more atrophy associated with a slower gait speed) [50,56,57] and one found no association [60], with one finding an association at wave 1 but not at wave 2 [61].

No association was found between change in gait speed over follow up and whole brain, WM and GM volume (mean length of follow up in each study, 3 and 7 years) [42,61]. However one large study did find an association between ventricular volume and change in gait speed over follow up (mean 4 years) [57] but another study found no association (mean follow up 3 years) [61]. Only one study looked at the relationship between change in gait speed and change in brain structure over time (mean follow up 30.6 months) [39]. They found a positive association between change in gait speed and WM and hippocampal atrophy but no association with GM atrophy. The well-established relationship between cognitive decline and gait speed and cognitive decline and brain atrophy could underpin the possible relationship between brain atrophy and gait speed [4-8]. It is interesting that the only study to look at both variables in a longitudinal study found significant associations between brain structure and gait speed and further studies like this are needed.

Eleven studies were found which looked at gait speed and WMH, making it the most studied relationship in our review. Seven of these studies found that greater levels of WMH were associated with slower gait speed [45,50,52,56-61,64], but four other smaller studies found

no association [46,49,50,53]. Two of these studies found that this is primarily due to the volume of PVH and not subcortical WMH lesions [45,58] and two papers found that volume of brainstem WMH was associated with gait speed [46,56]. One small study (n = 14) found no association between gait speed and WMH progression over follow up (19–22 months) [52]. However, change in gait speed was found to be associated with WMH volume in two large studies [45,57] with another study showing no association [61]. Two studies looked at change in both variables; one found that greater decline in gait speed was associated with greater WMH progression [39], whereas the other found no association [50]. Further studies looking not just at total WMH volume but their rate of accumulation and location within the brain, and their association with gait speed should help clarify this area.

#### Cognitive function and muscle mass

Nine studies were found which looked at cognitive function and muscle structure. Table 9 shows the main results from these studies. Three studies looked at a measure of global cognitive performance (a composite score of several tests used in their study) and muscle size and all 3 found no association [27,28,72]. The Kaufman Brief Intelligence Test can also be used as a marker of general cognition and it too found no association with muscle size [77,78]. Seven studies looked at muscle size and MMSE score, which is a useful screening tool for dementia but is not a robust test of cognitive function. Four of the studies found no association between MMSE and muscle mass [28,65,73,75] and one found an association but with a very small effect size [68]. However in one study which showed no association between

**Table 9 Number of studies of cognition and muscle size, direction of effect and number of subjects**

|   | Negative association (n) | No association (n)      | Positive association (n) | References          |
|---|--------------------------|-------------------------|--------------------------|---------------------|
| Muscle size and global cognitive score          | -                        | 3 (193)                 | -                        | [27,28,72]          |
| Muscle size and MMSE                            | -                        | 5 (1434) <sup>h,i</sup> | 2 (3459) <sup>h</sup>    | [28,65,66,68,73,75] |
| Muscle size and California Verbal Learning Test | -                        | 1 (786)                 | -                        | [76]                |
| Muscle size and CSI-D                           | -                        | 1 (3153)                | -                        | [68]                |
| Muscle size and digit span                      | -                        | 2 (815) <sup>j</sup>    | -                        | [74,76]             |
| Muscle size and Kaufman Brief Intelligence Test | -                        | 1 (139)                 | -                        | [77,78]             |
| Muscle size and modified Stroop test            | -                        | 1 (48)                  | -                        | [65]                |
| Muscle size and NART                            | 1 (51)                   | -                       | -                        | [27]                |
| Muscle size and TMT-a                           | -                        | 3 (887)                 | -                        | [72,74,76]          |
| Muscle size and TMT-b                           | -                        | 3 (887)                 | -                        | [72,74,76]          |

<sup>h</sup>Leg LM was associated with muscle size but not total LM or arm LM in this study [66].

<sup>i</sup>In an MLR there was no association between MMSE and FFM but when comparing subjects with normal RASM and those within the lowest 20% of RASM this study found a significant difference [75].

<sup>j</sup>Digit span forwards unadjusted and adjusted was not associated with thigh muscle CSA, but adjusted backwards digit span was negatively associated with thigh muscle CSA in this study [76].

MMSE and FFM, when comparing subjects with normal RASM and those within the lowest 20% of RASM this study found a significant difference in mean MMSE [75]. Several of the included studies did not include those with cognitive impairment and it may be that an association does exist between MMSE and muscle size but in a non-linear relation, affecting the frailer older adult more, but that it was not picked up in these studies due to the method of analysis in a linear regression. Overall though in healthy individuals it seems that no such association exists. The final study found an association between leg LM and MMSE but not between total or arm LM [66]. Sarcopenia is known to affect leg and arm muscles differently which perhaps explains this effect [111]. Another screening tool for dementia, the cogscore part of the CSI-D, also found no association with muscle mass [68]. It is well established that gait speed and cognition are associated in older age and these results appear to show that muscle size is not a driving force behind this relationship [4-6].

With regard to the individual cognitive tests (which measure processing speed and executive function), there were no significant associations [65,72,74,76], except for the NART (a measure of childhood IQ, which showed a negative association with neck muscle CSA [27]. The authors comment that perhaps subjects with higher cognition are more likely to have sedentary jobs and therefore more likely to lose their muscle mass over time. None of the studies looking at cognition and muscle size contained longitudinal associations therefore whilst these results appear to support no association between muscle mass and cognition; it may be that longitudinal data would show an association, whereby those that lose more muscle with age have a sharper slope of decline in their cognition also. Longitudinal studies will help to elucidate these complex relationships further.

### Limitations

In the review protocol the decision was made to write to study authors for relevant associations or data that were not given in the study but could be calculated using the recorded variables. This expanded the number of articles included in the review and the scope that they covered, however this may have led to some bias in which articles responded to the request and therefore what was reported, as study authors who found an association may have been more likely to reply. Of the 79 articles written to 59 replied therefore 25% did not respond. However the studies that did respond included both those which showed a significant association and those that did not. The associations which were sent to us and the associations performed by us have not undergone peer review (e.g. for variable selection when adjusting the models), however we

have included this information in our review and the statistical technique used to remain as transparent as possible.

The studies included in the review used a wide variety of techniques to record the variables of interest which means it is difficult to compare them (e.g. in a meta-analysis). Gait speed for example was recorded over multiple lengths, using automated and manual techniques and different levels of speed (i.e. maximum or usual pace). The large differences in how gait speed was measured combined with the fact that over longer distances it can become a test of cardiovascular fitness etc. more than a test of muscle function, makes it difficult to compare the results of these studies directly. Hopefully more standardized testing will come about in the wake of resources like the NIH toolbox which includes a protocol for measuring gait speed [112].

When looking at the relationship between brain size and muscle size or function it is important to make sure that the size of the individual being studied is not acting as a confounding factor (i.e. that large people have large brains and large muscles). This meant that it was important to ensure that some measure of body size had been adjusted for in each association (e.g. ICV, height, BMI). In most of the studies we looked at this occurred but in some it did not and this may lead to a false relationship being reported.

A relatively wide range of ethnicities are represented in the study, however Caucasian subjects were by far the most commonly studied and there were no studies including those of Arabic or Indian ethnicity. Also most of the studies used subjects in their sixties, seventies or eighties, meaning the validity of our findings for other age groups, particularly children and adolescents is limited.

Finally, while a few of the studies included longitudinal data, it would be very useful to have more studies looking at the relationships over time as these may be able to highlight potential modifiable factors.

### Conclusions

An increasing body of research has now linked brain function (cognition) and muscle function (e.g. gait speed) [4-8], however less well studied is the role of muscle and brain structure in this relationship. This systematic review looks at the evidence for whether: brain structure is related to muscle structure; brain structure is related to muscle function; and brain function is related to muscle structure in healthy humans across the lifecourse.

The review found evidence of a positive association between whole brain volume and total white matter volume with muscle size and evidence that some areas of regional grey matter volume (right temporal pole and bilateral vmPFC) are negatively associated with muscle size [26-31].



The review found no evidence of a relationship between grip strength and whole brain volume, however there was some evidence of a positive association between grip strength and WM volume. Markers of brain ageing, that is brain atrophy and greater WMH accumulation, were associated with grip strength [35,36,61]. Unlike grip strength, there is evidence that gait speed is positively associated with whole brain volume; this relationship may be driven by total WM volume or regional GM volumes, specifically the hippocampus [58,59,61]. Like grip strength, gait speed is also associated with markers of brain aging; WMH accumulation, brain atrophy and WM atrophy all show evidence of either a temporal association with gait speed or change in gait speed with time, with PVH and brainstem WMHs playing a particularly important role, but not subcortical WMH [45,57].

The evidence overwhelmingly points to no association between cognition and muscle size, except in the case of MMSE where it is mixed, but MMSE is more a screening tool for dementia than a true marker of cognitive function [27,28,65,66,68,72,73,75]. Longitudinal studies are now needed to explore these relationships over time, which will allow a better understanding of the potential causal relationships.

## Appendix 1 Medline Search

1. brain/ or exp brain stem/ or exp cerebral ventricles/ or exp limbic system/ or exp mesencephalon/ or exp prosencephalon/ or exp rhombencephalon/
2. (brain adj3 volume).tw.
3. white matter.tw.
4. mental processes/ or cognition/ or cognitive reserve/ or comprehension/ or executive function/ or higher nervous activity/ or maze learning/ or exp memory/ or thinking/ or decision making/ or judgment/ or problem solving/
5. Intelligence/
6. exp aptitude tests/ or exp neuropsychological tests/
7. cognitive function.tw.
8. muscles/ or muscle, skeletal/ or abdominal muscles/ or rectus abdominis/ or deltoid muscle/ or neck muscles/ or pectoralis muscles/ or psoas muscles/ or quadriceps muscle/ or rotator cuff/
9. Body Composition/
10. muscle cross sectional area.tw.
11. exp Muscular Atrophy/
12. exp Muscle Strength/
13. exp Walking/
14. muscle power.tw.
15. Physical Fitness/
16. physical performance.tw.
17. 1 or 2 or 3
18. 4 or 5 or 6 or 7

19. 8 or 9 or 10 or 11
20. 12 or 13 or 14 or 15 or 16
21. 17 and 19
22. 17 and 20
23. 18 and 19
24. 21 or 22 or 23
25. limit 24 to humans
26. limit 25 to case reports
27. 25 not 26

## Abbreviations

6 MW: Six metre walk test; AD: Alzheimer's disease; ALM: Appendicular lean mass; ASM: Appendicular skeletal mass; BIA: Bioimpedance analysis; BMI: Body mass index; CC: Corpus callosum; CSF: Cerebrospinal fluid; CSI-D: Community screening instrument of dementia; CVD: Cardiovascular disease; FFM: Fat free mass; GLM: General Linear Model; GM: Grey matter; ICV: Intracranial volume; IKES: Isometric knee extension strength; K-BIT: Kaufman Brief Intelligence Test; LM: Lean mass; LLMM: Lower limb muscle mass; MLR: Multiple linear regression; MMSE: Mini mental state examination; MTR: Magnetisation transfer ratio; NAWM: Normal appearing white matter; PVH: Periventricular hyperintensities; RASM: Relative appendicular skeletal mass; RCT: Randomized controlled trial; ROI: Region(s) of interest; SM: Skeletal mass; SPPB: Short physical performance battery; TBV: Total brain volume; TLM: Total lean mass; TMT: Trail making test; VE: Ventricular enlargement; WBV: Whole brain volume; WM: White matter; WMH: White matter hyperintensities; WML: White matter lesions; WMSA: White matter signal abnormalities.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

AHMK and JMS proposed the hypotheses and drafted the manuscript. AHMK and OMT performed the longlisting and shortlisting of the included studies. AHMK wrote the data extraction sheet, compiled the tables and performed the data extraction and data analysis. All authors approved the final manuscript.

## Acknowledgements

AHMK was funded during this research by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). We gratefully acknowledge the help of the study authors who replied to our request for raw data or associations not included in their original paper. The OBAS project requested that we included details of their funding: NIA-funded Oregon Alzheimer Disease Center (OADC): P30 AG008017 Oregon Brain Aging Study (OBAS): Office of Research Development, Clinical Sciences Research & Development Service, Department of Veterans Affairs Dementia Prevention Study—DPS: P50 AT00066 DPS (subjects rolled into OBAS II after DPS ended). We are very grateful for the help of Mrs Sheila Fiskien, University of Edinburgh, who helped perform the original database searches.

Received: 19 November 2013 Accepted: 1 July 2014

Published: 10 July 2014

## References

1. Salthouse T: **Consequences of age-related cognitive declines.** *Annu Rev Psychol* 2012, **63**:201–226.
2. Johansson MM, Marcusson J, Wressle E: **Cognition, daily living, and health-related quality of life in 85-year-olds in Sweden.** *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2012, **19**:421–432.
3. Sayer AA, Syddall HE, Martin HJ, Dennison EM, Roberts HC, Cooper C: **Is grip strength associated with health-related quality of life? Findings from the Hertfordshire Cohort Study.** *Age Ageing* 2006, **35**:409–415.
4. van Kan Abellan G, Rolland Y, Andrieu S, Bauer J, Beauchet O, Bonnefoy M, Cesari M, Donini LM, Gillette Guyonnet S, Inzitari M, Nourhashemi F, Onder G, Ritz P, Salva A, Visser M, Vellas B: **Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International**

- Academy on Nutrition and Aging (IANA) Task Force. *J Nutr Health Aging* 2009, **13**:881–888.
5. Atkinson HH, Rosano C, Simonsick EM, Williamson JD, Davis C, Ambrosius WT, Rapp SR, Cesari M, Newman AB, Harris TB, Rubin SM, Yaffe K, Satterfield S, Kritchevsky SB: **Cognitive function, gait speed decline, and comorbidities: the health, aging and body composition study.** *J Gerontol A Biol Sci Med Sci* 2007, **62**:844–850.
  6. Scherder E, Eggermont L, Swaab D, van Heuvelen M, Kamsma Y, de Greef M, van Wijck R, Mulder T: **Gait in ageing and associated dementias; its relationship with cognition.** *Neurosci Biobehav Rev* 2007, **31**:485–497.
  7. Drago V, Babiloni C, Bartres-Faz D, Caroli A, Bosch B, Hensch T, Didic M, Klafki HW, Pievani M, Jovicich J, Venturi L, Spitzer P, Vecchio F, Schoenknecht P, Wiltfang J, Redolfi A, Forloni G, Blin O, Irving E, Davis C, Hårdemark HG, Frisoni GB: **Disease tracking markers for Alzheimer's disease at the prodromal (MCI) stage.** *J Alzheimers Dis* 2011, **26**(Suppl 3):159–199.
  8. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Munch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V: **Brain atrophy in type 2 diabetes: regional distribution and influence on cognition.** *Diabetes Care* 2013, **36**:4036–4042.
  9. Lindenberger U, Baltes PB: **Sensory functioning and intelligence in old-age - a strong connection.** *Psychol Aging* 1994, **9**:339–355.
  10. Speakman J, Mitchell S: **Caloric restriction.** *Mol Asp Med* 2011, **32**:159–221.
  11. Park SK, Prolla TA: **Lessons learned from gene expression profile studies of aging and caloric restriction.** *Ageing Res Rev* 2005, **4**(1):55–65.
  12. Mitnitski A, Song X, Rockwood K: **Assessing biological aging: the origin of deficit accumulation.** *Biogerontology* 2013, **14**:709–717.
  13. Het S, Ramlow G, Wolf O: **A meta-analytic review of the effects of acute cortisol administration on human memory.** *Psychoneuroendocrinology* 2005, **30**:771–784.
  14. Meaney MJ, Odonnell D, Rowe W, Tannenbaum B, Steverman A, Walker M, Nair NPV, Lupien S: **Individual-differences in hypothalamic-pituitary-adrenal activity in later life and hippocampal aging.** *Exp Gerontol* 1995, **30**:229–251.
  15. Cooper MS, Rabbitt EH, Goddard PE, Bartlett WA, Hewison M, Stewart PM: **Osteoblastic 11beta-hydroxysteroid dehydrogenase type 1 activity increases with age and glucocorticoid exposure.** *J Bone Miner Res* 2002, **17**:979–986.
  16. Roth SM, Zmuda JM, Cauley JA, Shea PR, Ferrell RE: **Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men.** *Journals of Gerontology Series A-Biological Sciences and Medical Sciences* 2004, **59**:10–15.
  17. Geusens P, Vandevyver C, Vanhoof J, Cassiman JJ, Boonen S, Raus J: **Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women.** *J Bone Miner Res* 1997, **12**:2082–2088.
  18. Adams GR, Haddad F: **The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy.** *J Appl Physiol* 1996, **81**:2509–2516.
  19. Adams GR, McCue SA: **Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats.** *J Appl Physiol* 1998, **84**:1716–1722.
  20. Davis CL, Tomporowski PD, McDowell JE, Austin BP, Miller PH, Yanasak NE, Allison JD, Naglieri JA: **Exercise improves executive function and achievement and alters brain activation in overweight children: a randomized, controlled trial.** *Health Psychol* 2011, **30**:91–98.
  21. Deary IJ, Harris SE, Fox HC, Hayward C, Wright AF, Starr JM, Whalley LJ: **KLOTHO genotype and cognitive ability in childhood and old age in the same individuals.** *Neurosci Lett* 2005, **378**:22–27.
  22. Jang YC, Lustgarten MS, Liu YH, Muller FL, Bhattacharya A, Liang HY, Salmon AB, Brooks SV, Larkin L, Hayworth CR, Richardson A, Van Remmen H: **Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration.** *Faseb Journal* 2010, **24**:1376–1390.
  23. Angevaren M, Aufdemkampe G, Verhaar HJJ, Aleman A, Vanhees L: **Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment.** *Cochrane Database Syst Rev* 2008, **3**:CD005381. doi: 10.1002/14651858.CD005381.pub3.
  24. Duff K, Mold JW, Roberts MM: **Walking speed and global cognition: results from the OKLAHOMA Study.** *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2008, **15**:31–39.
  25. Alfaro-Acha A, Al Snih S, Raji MA, Kuo Y-F, Markides KS, Ottenbacher KJ: **Handgrip strength and cognitive decline in older Mexican American's.** *Journals of Gerontology Series A-Biological Sciences and Medical Sciences* 2006, **61**:859–865.
  26. Heymsfield SB, Muller MJ, Bosy-Westphal A, Thomas D, Shen W: **Human brain mass: similar body composition associations as observed across mammals.** *Am J Hum Biol* 2012, **24**:479–485.
  27. Kilgour AH, Ferguson KJ, Gray CD, Deary IJ, Wardlaw JM, MacLulich AM, Starr JM: **Neck muscle cross-sectional area, brain volume and cognition in healthy older men: a cohort study.** *BMC Geriatr* 2013, **13**:20.
  28. Wetmore JB, Honea RA, Vidoni ED, Almehtmi A, Burns JM: **Role of lean body mass in estimating glomerular filtration rate in Alzheimer disease.** *Nephrol Dial Transplant* 2011, **26**:2222–2231.
  29. Burns JM, Johnson DK, Watts A, Swerdlow RH, Brooks WM: **Reduced lean mass in early Alzheimer disease and its association with brain atrophy.** *Arch Neurol* 2010, **67**:428–433.
  30. Honea RA, Thomas GP, Harsha A, Anderson HS, Donnelly JE, Brooks WM, Burns JM: **Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer disease.** *Alzheimer Dis Assoc Disord* 2009, **23**:188–197.
  31. Weise CM, Thiyyagura P, Reiman EM, Chen K, Krakoff J: **Fat-free body mass but not fat mass is associated with reduced gray matter volume of cortical brain regions implicated in autonomic and homeostatic regulation.** *NeuroImage* 2013, **64**:712–721.
  32. Sachdev PS, Parslow R, Wen W, Anstey KJ, Easteal S: **Sex differences in the causes and consequences of white matter hyperintensities.** *Neurobiol Aging* 2009, **30**(6):946–956.
  33. Anstey KJ, Mack HA, Christensen H, Li S-C, Reglade-Meslin C, Maller J, Kumar R, Dear K, Easteal S, Sachdev P: **Corpus callosum size, reaction time speed and variability in mild cognitive disorders and in a normative sample.** *Neuropsychologia* 2007, **45**:1911–1920.
  34. Sachdev PS, Anstey KJ, Parslow RA, Wen W, Maller J, Kumar R, Christensen H, Jorm AF: **Pulmonary function, cognitive impairment and brain atrophy in a middle-aged community sample.** *Dement Geriatr Cogn Disord* 2006, **21**(5–6):300–308.
  35. Sachdev PS, Wen W, Christensen H, Jorm AF: **White matter hyperintensities are related to physical disability and poor motor function.** *J Neurol Neurosurg Psychiatry* 2005, **76**(3):362–367.
  36. Doi T, Makizako H, Shimada H, Yoshida D, Ito K, Kato T, Ando H, Suzuki T: **Brain atrophy and trunk stability during dual-task walking among older adults.** *Journals of Gerontology Series A-Biological Sciences & Medical Sciences* 2012, **67**:790–795.
  37. Hardan AY, Kilpatrick M, Keshavan MS, Minschew NJ: **Motor performance and anatomic magnetic resonance imaging (MRI) of the basal ganglia in autism.** *J Child Neurol* 2003, **18**(5):317–324.
  38. Piguet O, Cramsie J, Bennett HP, Kriil JJ, Lye TC, Corbett AJ, Hayes M, Creasey H, Broe G: **Contributions of age and alcohol consumption to cerebellar integrity, gait and cognition in non-demented very old individuals.** *Eur Arch Psychiatry Clin Neurosci* 2006, **256**:504–511.
  39. Callisaya ML, Beare R, Phan TG, Blizzard L, Thrift AG, Chen J, Srikanth VK: **Brain structural change and gait decline: A longitudinal population-based study.** *J Am Geriatr Soc* 2013, **61**:1074–1079.
  40. Srikanth V, Phan TG, Chen J, Beare R, Stapleton JM, Reutens DC: **The location of white matter lesions and gait—A voxel-based study.** *Ann Neurol* 2010, **67**:265–269.
  41. Srikanth V, Beare R, Blizzard L, Phan T, Stapleton J, Chen J, Callisaya M, Martin K, Reutens D: **Cerebral white matter lesions, gait, and the risk of incident falls: a prospective population-based study.** *Stroke* 2009, **40**:175–180.
  42. Elbaz A, Vicente-Vytopilova P, Tavernier B, Sabia S, Dumurgier J, Mazoyer B, Singh-Manoux A, Tzourio C: **Motor function in the elderly: evidence for the reserve hypothesis.** *Neurology* 2013, **81**:417–426.
  43. Dumurgier J, Crivello F, Mazoyer B, Ahmed I, Tavernier B, Grabli D, Francois C, Tzourio-Mazoyer N, Tzourio C, Elbaz A: **MRI atrophy of the caudate nucleus and slower walking speed in the elderly.** *NeuroImage* 2012, **60**:871–878.
  44. Dumurgier J, Elbaz A, Dufouil C, Tavernier B, Tzourio C: **Hypertension and lower walking speed in the elderly: the Three-City study.** *J Hypertens* 2010, **28**:1506–1514.
  45. Soumare A, Elbaz A, Zhu Y, Maillard P, Crivello F, Tavernier B, Dufouil C, Mazoyer B, Tzourio C: **White matter lesions volume and motor performances in the elderly.** *Ann Neurol* 2009, **65**:706–715.
  46. Starr J, Leaper S, Murray A, Lemmon H, Staff R, Deary I, Whalley L: **Brain white matter lesions detected by magnetic resonance imaging are associated with balance and gait speed.** *J Neurol Neurosurg Psychiatry* 2003, **74**:94–98.
  47. Manor B, Abduljalil A, Newton E, Novak V: **The relationship between brain volume and walking outcomes in older adults with and**

- without diabetic peripheral neuropathy. *Diabetes Care* 2012, **35**:1907–1912.
48. Hajjar I, Zhao P, Alsop D, Abduljalil A, Selim M, Novak P, Novak V: **Association of blood pressure elevation and nocturnal dipping with brain atrophy, perfusion and functional measures in stroke and nonstroke individuals.** *Am J Hypertens* 2010, **23**(1):17–23.
49. Novak V, Haertle M, Zhao P, Hu K, Munshi M, Novak P, Abduljalil A, Alsop D: **White matter hyperintensities and dynamics of postural control.** *Magn Reson Imaging* 2009, **27**(6):752–759.
50. Moscufo N, Wolfson L, Meier D, Liguori M, Hildenbrand PG, Wakefield D, Schmidt JA, Pearson GD, Guttmann CR: **Mobility decline in the elderly relates to lesion accrual in the splenium of the corpus callosum.** *Age* 2012, **34**:405–414.
51. Moscufo N, Guttmann CRG, Meier D, Csapo I, Hildenbrand PG, Healy BC, Schmidt JA, Wolfson L: **Brain regional lesion burden and impaired mobility in the elderly.** *Neurobiol Aging* 2011, **32**:646–654.
52. Wolfson L, Wei X, Hall CB, Panzer V, Wakefield D, Benson RR, Schmidt JA, Warfield SK, Guttmann CRG: **Accrual of MRI white matter abnormalities in elderly with normal and impaired mobility.** *J Neurol Sci* 2005, **232**:23–27.
53. Guttmann CR, Benson R, Warfield SK, Wei X, Anderson MC, Hall CB, Abu-Hasaballah K, Mugler JP 3rd, Wolfson L: **White matter abnormalities in mobility-impaired older persons.** *Neurology* 2000, **54**:1277–1283.
54. Rosano C, Studenski SA, Aizenstein HJ, Boudreau RM, Longstreth WT Jr, Newman AB: **Slower gait, slower information processing and smaller prefrontal area in older adults.** *Age Ageing* 2012, **41**:58–64.
55. Barnes DE, Covinsky KE, Whitmer RA, Kuller LH, Lopez LH, Yaffe K: **Predicting risk of dementia in older adults: The late-life dementia risk index.** *Neurology* 2009, **73**:173–179.
56. Rosano C, Brach J, Longstreth WT Jr, Newman AB: **Quantitative measures of gait characteristics indicate prevalence of underlying subclinical structural brain abnormalities in high-functioning older adults.** *Neuroepidemiology* 2006, **26**(1):52–60.
57. Rosano C, Kuller LH, Chung H, Arnold AM, Longstreth WT Jr, Newman AB: **Subclinical brain magnetic resonance imaging abnormalities predict physical functional decline in high-functioning older adults.** *J Am Geriatr Soc* 2005, **53**(4):649–654.
58. Silbert LC, Nelson C, Howieson DB, Moore MM, Kaye JA: **Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline.** *Neurology* 2008, **71**(2):108–113.
59. Marquis S, Moore MM, Howieson DB, Sexton G, Payami H, Kaye JA, Camicioli R: **Independent predictors of cognitive decline in healthy elderly persons.** *Arch Neurol* 2002, **59**:601–606.
60. Rosano C, Sigurdsson S, Siggeirsdottir K, Phillips CL, Garcia M, Jonsson PV, Eiriksdottir G, Newman AB, Harris TB, van Buchem MA, Gudnason V, Launer LJ: **Magnetization transfer imaging, white matter hyperintensities, brain atrophy and slower gait in older men and women.** *Neurobiol Aging* 2010, **31**(7):1197–1204.
61. Aribisala BS, Gow AJ, Bastin ME, Valdes Hernandez MDC, Murray C, Royle NA, Maniega SM, Starr JM, Deary IJ, Wardlaw JM: **Associations between level and change in physical function and brain volumes.** *PLoS ONE* 2013, **8**(11):e80386. doi:10.1371/journal.pone.0080386.
62. Rosano C, Longstreth WT Jr, Boudreau R, Taylor CA, Du Y, Kuller LH, Newman AB: **High blood pressure accelerates gait slowing in well-functioning older adults over 18-years of follow-up.** *J Am Geriatr Soc* 2011, **59**(3):390–397.
63. Rosano C, Newman AB, Katz R, Hirsch CH, Kuller LH: **Association between lower digit symbol substitution test score and slower gait and greater risk of mortality and of developing incident disability in well-functioning older adults.** *J Am Geriatr Soc* 2008, **56**(9):1618–1625.
64. Longstreth WJ, Manolio T, Arnold A, Burke G, Bryan N, Jungreis C, Enright P, O'Leary D, Fried L: **Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people.** *The Cardiovascular Health Study Stroke* 1996, **27**:1274–1282.
65. Berryman N, Bherer L, Nadeau S, Lauziere S, Lehr L, Bobeuf F, Kergoat MJ, Vu TTM, Bosquet L: **Executive functions, physical fitness and mobility in well-functioning older adults.** *Exp Gerontol* 2013, **48**:1402–1409.
66. Bites AC, Bunout D, Barrera G, Hirsch S, Leiva L, De La Maza MP: **Association between functional measures and mortality in older persons.** *International Journal of Gerontology* 2013, **7**:17–21.
67. Bunout D, Barrera G, Avendano M, Gattas V, Leiva L, Hirsch S: **Results of a community-based weight-bearing resistance training programme for healthy Chilean elderly subjects.** *Age & Ageing* 2005, **34**:80–83.
68. Auyeung TW, Lee JSW, Leung J, Kwok T, Woo J: **Adiposity to muscle ratio predicts incident physical limitation in a cohort of 3,153 older adults—an alternative measurement of sarcopenia and sarcopenic obesity.** *Age* 2013, **35**:1377–1385.
69. Auyeung T, Lee J, Kwok T, Woo J: **Physical frailty predicts future cognitive decline - A four-year prospective study in 2737 cognitively normal older adults.** *Journal of Nutrition, Health & Aging* 2011, **15**:690–694.
70. Lee JS, Auyeung TW, Leung J, Kwok T, Leung PC, Woo J: **Physical frailty in older adults is associated with metabolic and atherosclerotic risk factors and cognitive impairment independent of muscle mass.** *Journal of Nutrition, Health & Aging* 2011, **15**:857–862.
71. Auyeung TW, Kwok T, Lee J, Leung PC, Leung J, Woo J: **Functional decline in cognitive impairment—The relationship between physical and cognitive function.** *Neuroepidemiology* 2008, **31**:167–173.
72. Pedersen M, Pedersen KK, Bruunsgaard H, Krabbe KS, Thomsen C, Faerch K, Pedersen BK, Mortensen EL: **Cognitive functions in middle aged individuals are related to metabolic disturbances and aerobic capacity: a cross-sectional study.** *PLoS ONE [Electronic Resource]* 2012, **7**:e51132.
73. Magri F, Gabellieri E, Busconi L, Guazzoni V, Cravello L, Valdes V, Sorrentino AR, Chytris S, Ferrari E: **Cardiovascular, anthropometric and neurocognitive features of healthy postmenopausal women: Effects of hormone replacement therapy.** *Life Sci* 2006, **78**(22):2625–2632.
74. Lasaite L, Krasauskiene A: **Psychological state, quality of life, and body composition in postmenopausal women with osteoporosis in Lithuania.** *Arch Osteoporos* 2009, **4**(1–2):85–90.
75. Liu LK, Lee WJ, Chen LY, Hwang AC, Lin MH, Peng LN, Chen LK: **Sarcopenia, and its association with cardiometabolic and functional characteristics in Taiwan: Results from I-Lan Longitudinal Aging Study.** *Geriatr Gerontol Int* 2014, **14**:36–45.
76. Moore AZ, Caturegli G, Metter EJ, Makrogiannis S, Resnick SM, Harris TB, Ferrucci L: **Difference in muscle quality over the adult life span and biological correlates in the baltimore longitudinal study of aging.** *J Am Geriatr Soc* 2014, **62**:230–236.
77. Kamijo K, Pontifex MB, Khan NA, Raine LB, Scudder MR, Drollette ES, Evans EM, Castelli DM, Hillman CH: **The negative association of childhood obesity to cognitive control of action monitoring.** *Cereb Cortex* 2014, **24**:654–662.
78. Kamijo K, Khan NA, Pontifex MB, Scudder MR, Drollette ES, Raine LB, Evans EM, Castelli DM, Hillman CH: **The relation of adiposity to cognitive control and scholastic achievement in preadolescent children.** *Obesity* 2012, **20**:2406–2411.
79. Chowdhury B, Sjoström L, Alpsten M, Kostantý J, Kvist H, Lofgren R: **A multicompartiment body composition technique based on computerized tomography.** *International Journal of Obesity & Related Metabolic* 1994, **18**:219–234.
80. Liu-Ambrose T, Nagamatsu LS, Graf P, Beattie BL, Ashe MC, Handy TC: **Resistance training and executive functions: a 12-month randomized controlled trial.** *Arch Intern Med* 2010, **170**:170–178.
81. Nadkarni NK, Levine B, McIlroy WE, Black SE: **Impact of subcortical hyperintensities on dual-tasking in Alzheimer disease and aging.** *Alzheimer Dis Assoc Disord* 2012, **26**:28–35.
82. Sullivan EV, Deshmukh A, De Rosa E, Rosenbloom MJ, Pfefferbaum A: **Striatal and forebrain nuclei volumes: Contribution to motor function and working memory deficits in alcoholism.** *Biol Psychiatry* 2005, **57**(7):768–776.
83. Guthrie JR, Dennerstein L, Taffe JR, Lehert P, Burger HG: **The menopausal transition: a 9-year prospective population-based study.** *The Melbourne Women's Midlife Health Project Climacteric* 2004, **7**:375–389.
84. Ellis KA, Bush AI, Darby D, De Fazio D, Foster J, Hudson P, Lautenschlager NJ, Lenzo N, Martins RN, Maruff P, Masters C, Milner A, Pike K, Rowe C, Savage G, Szoëke C, Taddei K, Villemagne V, Woodward M, Ames D: **The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease.** *Int Psychogeriatr* 2009, **21**:672–687.
85. Dao E, Davis JC, Sharma D, Chan A, Nagamatsu LS, Liu-Ambrose T: **Change in body fat mass is independently associated with executive functions in older women: a secondary analysis of a 12-month randomized controlled trial.** *PLoS ONE [Electronic Resource]* 2013, **8**:e52831.
86. Schwartz DH, Leonard G, Perron M, Richer L, Syme C, Veillette S, Pausova Z, Paus T: **Visceral fat is associated with lower executive functioning in adolescents.** *Int J Obes* 2013, **37**:1336–1343.



87. Bagger YZ, Tanko LB, Alexandersen P, Qin G, Christiansen C: **The implications of body fat mass and fat distribution for cognitive function in elderly women.** *Obes Res* 2004, **12**:1519–1526.
88. van Kan Abellan G, Cesari M, Gillette-Guyonnet S, Dupuy C, Nourhashemi F, Schott AM, Beauchet O, Annweiler C, Vellas B, Rolland Y: **Sarcopenia and cognitive impairment in elderly women: results from the EPIDOS cohort.** *Age & Ageing* 2013, **42**:196–202.
89. Nourhashemi F, Andrieu S, Gillette-Guyonnet S, Reynish E, Albaredo JL, Grandjean H, Vellas B: **Is there a relationship between fat-free soft tissue mass and low cognitive function? Results from a study of 7,105 women.** *J Am Geriatr Soc* 2002, **50**:1796–1801.
90. Nourhashemi F, Andrieu S, Gillette-Guyonnet S, Vellas B, Albaredo JL, Grandjean H: **Instrumental activities of daily living as a potential marker of frailty: a study of 7364 community-dwelling elderly women (the EPIDOS study).** *Journals of Gerontology Series A-Biological Sciences & Medical Sciences* 2001, **56**:M448–M453.
91. Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riondino M, Tagliamonte MR, Rizzo MR, Carella C, Varricchio M: **Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function.** *Journal of Clinical Endocrinology & Metabolism* 1997, **82**:2204–2209.
92. Malaguarnera M, Cammalleri L, Gargante MP, Vacante M, Colonna V, Motta M: **L-Carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial.** *Am J Clin Nutr* 2007, **86**:1738–1744.
93. Jacobsen DE, Melis RJF, Verhaar HJJ, Olde Rikkert MGM: **Raloxifene and tibolone in elderly women: A randomized, double-blind, double-dummy, placebo-controlled trial.** *Journal of the American Medical Directors Association* 2012, **13**:189.e181-189.e187.
94. Genton L, Karsegard VL, Chevalley T, Kossovsky MP, Darmon P, Pichard C: **Body composition changes over 9 years in healthy elderly subjects and impact of physical activity.** *Clin Nutr* 2011, **30**(4):436–442.
95. Donaldson KE, Carpenter WH, Toth MJ, Goran MI, Newhouse P, Poehlman ET: **No evidence for a higher resting metabolic rate in noninstitutionalized Alzheimer's disease patients.** *J Am Geriatr Soc* 1996, **44**:1232–1234.
96. Bove RM, Brick DJ, Healy BC, Mancuso SM, Gerweck AV, Bredella MA, Sherman JC, Miller KK: **Metabolic and endocrine correlates of cognitive function in healthy young women.** *Obesity* 2013, **21**:1343–1349.
97. Papadakis MA, Grady D, Tierney MJ, Black D, Wells L, Grunfeld C: **Insulin-like growth factor 1 and functional status in healthy older men.** *J Am Geriatr Soc* 1995, **43**:1350–1355.
98. Janssen I: **Influence of sarcopenia on the development of physical disability: the cardiovascular health study.** *J Am Geriatr Soc* 2006, **54**:56–62.
99. Masley SC, Weaver W, Peri G, Phillips SE: **Efficacy of lifestyle changes in modifying practical markers of wellness and aging.** *Altern Ther Health Med* 2008, **14**:24–29.
100. Houston DK, Toozee JA, Neiberg RH, Hausman DB, Johnson MA, Cauley JA, Bauer DC, Cawthon PM, Shea MK, Schwartz GG, Williamson JD, Tylavsky FA, Visser M, Simonsick EM, Harris TB, Kritchevsky SB: **25-hydroxyvitamin D status and change in physical performance and strength in older adults.** *Am J Epidemiol* 2012, **176**:1025–1034.
101. Middleton LE, Manini TM, Simonsick EM, Harris TB, Barnes DE, Tylavsky F, Brach JS, Everhart JE, Yaffe K: **Activity energy expenditure and incident cognitive impairment in older adults.** *Arch Intern Med* 2011, **171**:1251–1257.
102. Koster A, Visser M, Simonsick EM, Yu B, Allison DB, Newman AB, van Eijk JTM, Schwartz AV, Satterfield S, Harris TB: **Association between fitness and changes in body composition and muscle strength.** *J Am Geriatr Soc* 2010, **58**:219–226.
103. de Rekeneire N, Resnick HE, Schwartz AV, Shorr RI, Kuller LH, Simonsick EM, Vellas B, Harris TB: **Diabetes is associated with subclinical functional limitation in nondisabled older individuals: the Health, Aging, and Body Composition study.** *Diabetes Care* 2003, **26**:3257–3263.
104. de Rekeneire N, Visser M, Peila R, Nevitt MC, Cauley JA, Tylavsky FA, Simonsick EM, Harris TB: **Is a fall just a fall: correlates of falling in healthy older persons. The Health, Aging and Body Composition Study.** *J Am Geriatr Soc* 2003, **51**:841–846.
105. Watts AS, Vidoni ED, Loskutova N, Johnson DK, Burns JM: **Measuring Physical Activity in Older Adults With and Without Early Stage Alzheimer's Disease.** *Clin Gerontol* 2013, **36**:356–374.
106. Canon ME, Crimmins EM: **Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline.** *Journal of Nutrition, Health & Aging* 2011, **15**:695–698.
107. Garry PJ, Wayne SJ, Vellas B: **The New Mexico aging process study (1979–2003) a longitudinal study of nutrition, health and aging.** *Journal of Nutrition, Health and Aging* 2007, **11**(2):125–130.
108. Haren MT, Banks WA, Perry III HM, Patrick P, Malmstrom TK, Miller DK, Morley JE: **Predictors of serum testosterone and DHEAS in African-American men.** *Int J Androl* 2008, **31**:50–59.
109. Dvorak RV, Poehlman ET: **Appendicular skeletal muscle mass, physical activity, and cognitive status in patients with Alzheimer's disease.** *Neurology* 1998, **51**:1386–1390.
110. Debetto S, Markus HS: **The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis.** *BMJ* 2010, **341**:c3666.
111. Janssen I, Heymsfield SB, Wang ZM, Ross R: **Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr.** *J Appl Physiol* 2000, **89**:81–88.
112. NIH Toolbox. <http://www.nihtoolbox.org/Pages/default.aspx>.

doi:10.1186/1471-2318-14-85

**Cite this article as:** Kilgour et al.: A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse. *BMC Geriatrics* 2014 **14**:85.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)



# 11 $\beta$ -Hydroxysteroid Dehydrogenase Activity in the Brain Does Not Contribute to Systemic Interconversion of Cortisol and Cortisone in Healthy Men

Alix H.M. Kilgour, Scott Semple, Ian Marshall, Peter Andrews, Ruth Andrew, and Brian R. Walker

MRC Centre for Cognitive Aging and Cognitive Epidemiology (A.H.M.K.), Geriatric Medicine Unit, and Centre for Clinical Brain Sciences (S.S., I.M., P.A.), University of Edinburgh, Edinburgh, United Kingdom; Clinical Research Imaging Centre (S.S.) and BHF Centre for Cardiovascular Science (S.S., R.A., B.R.W.), Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom; Critical Care (P.A.), Western General Hospital, NHS Lothian University Hospitals Division, Edinburgh EH4 2XU, United Kingdom

**Context and Objective:** 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1) catalyses regeneration of cortisol in liver, adipose tissue, and skeletal muscle, making a substantial contribution to circulating cortisol as demonstrated in humans by combining stable isotope tracer infusion with arteriovenous sampling. In the brain, 11 $\beta$ HSD1 is a potential therapeutic target implicated in age-associated cognitive dysfunction. We aimed to quantify brain 11 $\beta$ HSD1 activity, both to assess its contribution to systemic cortisol/cortisone turnover and to develop a tool for measuring 11 $\beta$ HSD1 in dementia and following administration of 11 $\beta$ HSD1 inhibitors.

**Design, Setting, and Participants:** With ethical approval and informed consent, 8 healthy men aged 38.1 years (sd 16.5) underwent an ECG-gated phase-contrast magnetic resonance scan to quantify internal jugular vein blood flow and were infused with 1,2 [ $^2$ H] $_2$ -cortisone and 9,11,12,12 [ $^2$ H] $_4$ -cortisol for 3 h before samples were obtained from the internal jugular vein and an arterialized hand vein. Steroids were quantified by liquid chromatography-tandem mass spectrometry.

**Main Outcome Measures and Results:** Steady state tracer enrichments were achieved and systemic indices of cortisol/cortisone interconversion were consistent with previous studies in healthy men. However, there was no measurable release or production of cortisol, 9,12,12 [ $^2$ H] $_3$ -cortisol or cortisone into the internal jugular vein.

**Conclusions:** Although cerebral 11 $\beta$ HSD1 reductase activity may be greater in cognitively impaired patients, in healthy men any contribution of 11 $\beta$ HSD1 in the brain to systemic cortisol/cortisone turnover is negligible. The influence of 11 $\beta$ HSD1 in the brain is likely confined to subregions, notably the hippocampus. Alternative approaches are required to quantify pharmacodynamics effects of 11 $\beta$ HSD1 inhibitors in the human brain. (*J Clin Endocrinol Metab* 100: 483–489, 2015)

The 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ HSDs) are intracellular enzymes that catalyze interconversion of inactive cortisone and active cortisol; 11 $\beta$ HSD

type 1 is a predominant reductase, regenerating cortisol from cortisone, while 11 $\beta$ HSD type 2 is an exclusive dehydrogenase, inactivating cortisol to cortisone. An exten-

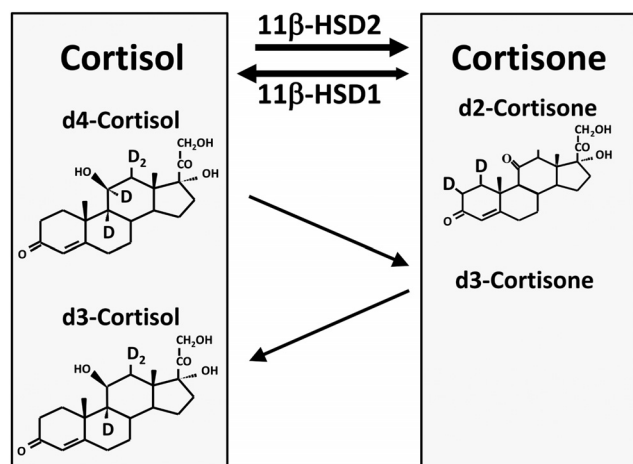
ISSN Print 0021-972X ISSN Online 1945-7197  
Printed in U.S.A.

This article has been published under the terms of the Creative Commons Attribution License (CC-BY; <https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Copyright for this article is retained by the author(s).

Received August 22, 2014. Accepted November 10, 2014.

First Published Online November 13, 2014

Abbreviations: 11 $\beta$ HSDs, 11 $\beta$ -hydroxysteroid dehydrogenases; CSF, cerebrospinal fluid; MR, magnetic resonance; Ra, rate of appearance.



**Figure 1.** Stable isotope tracers for measuring cortisol-cortisone interconversion by 11 $\beta$ -HSDs. 11 $\beta$ -HSD2 is a unidirectional enzyme catalyzing the dehydrogenase conversion of cortisol to cortisone. 11 $\beta$ -HSD1 is a potentially reversible enzyme catalyzing interconversion of cortisol and cortisone, predominantly in the reductase (cortisone to cortisol) direction. The shaded boxes on left and right represent the circulating pools of cortisol and cortisone, respectively. Production of cortisone can be measured by infusing a tracer, d2-cortisone, into the cortisone pool and measuring its dilution by cortisone. Similarly, production of cortisol can be measured by infusing a tracer, d4-cortisol, into the cortisol pool. When d4-cortisol is metabolized by 11 $\beta$ -HSD2, the deuterium in the 11 $\alpha$  position is removed, producing d3-cortisone; when this d3-cortisone is converted back to cortisol by 11 $\beta$ -HSD1 it is highly unlikely that a deuterium rather than a proton will be reincorporated, so that d3-cortisol is produced. Dilution of d4-cortisol with d3-cortisol therefore indicates 11 $\beta$ -HSD1 reductase activity.

sive literature documents the role of 11 $\beta$ HSDs in regulating intracellular glucocorticoid concentrations and hence modulating tissue-specific activation of corticosteroid receptors (reviewed in 1, 2). 11 $\beta$ HSD1 amplifies glucocorticoid action in the liver, adipose tissue, inflammatory cells, and vasculature, providing a therapeutic target for inhibition in type 2 diabetes. 11 $\beta$ HSD2 limits cortisol action, and thereby facilitates aldosterone action in the distal nephron and a few other sites, explaining the mineralocorticoid excess state which ensues when 11 $\beta$ HSD2 is inhibited by licorice.

Both 11 $\beta$ HSD isozymes also contribute to systemic turnover of cortisol. Activity of the two isozymes has been quantified in humans using stable isotope (deuterated) glucocorticoid tracers (Figure 1). Production of cortisone can be measured by the rate of dilution of 1,2- $^2\text{H}_2$ -cortisone (d2-cortisone) by cortisone, and is inhibited by licorice (3). A more complex tracer is used to measure regeneration of cortisol by 11 $\beta$ HSD1: 9,11,12,12- $^2\text{H}_4$ -cortisol (d4-cortisol) is infused and production of cortisol is measured by the rate of dilution of d4-cortisol by cortisol (an index of net cortisol production from all sources, including the adrenal gland) and by 9,12,12- $^2\text{H}_3$ -cortisol (d3-cortisol, a specific measure of cortisol regeneration

by 11 $\beta$ HSD1) (4). Using these tracers in combination with selective venous catheterization and measurement of blood flow has allowed quantification in humans of cortisol-cortisone interconversion in splanchnic (3, 5, 6), subcutaneous adipose (3, 7), and skeletal muscle (3) circulations, and exclusion of significant 11 $\beta$ HSD activity in the myocardium (8). These studies reveal rapid shuttling between cortisol and cortisone such that in healthy men at rest, remarkably, the magnitude of extra-adrenal regeneration of cortisol by 11 $\beta$ HSD1 is greater than the magnitude of adrenal cortisol secretion. They also suggest, surprisingly, that 11 $\beta$ HSD1 may catalyze both reductase and dehydrogenase activity in vivo, resulting in “recycling” between cortisol and cortisone (detected by dilution of d2-cortisone by cortisone and by simultaneous dilution of d4-cortisol by d3-cortisol) even in tissues where 11 $\beta$ HSD2 is not expressed (3).

11 $\beta$ HSDs may also play key roles in the brain (9). 11 $\beta$ HSD2 is expressed in the developing, but not the adult brain (10). 11 $\beta$ HSD1 is expressed more widely in the adult brain, and notably in the prefrontal cortex, hippocampus, and cerebellum, a distribution confirmed in humans (11). Increased local regeneration of cortisol by 11 $\beta$ HSD1 may cause glucocorticoid-dependent neurotoxicity and hence contribute to cognitive aging and dementia, while inhibition of 11 $\beta$ HSD1 has been proposed as a therapeutic strategy to treat age- and dementia-associated cognitive dysfunction. In mice, 11 $\beta$ HSD1 levels in the hippocampus and parietal cortex rise with age and correlate with impaired cognitive performance, while transgenic overexpression of 11 $\beta$ HSD1 in the forebrain accelerates age-associated cognitive decline (12). Conversely, 11 $\beta$ HSD1 knockout mice are protected from age-related learning impairment (13, 14). Moreover, selective 11 $\beta$ HSD1 inhibitors, after either systemic or intracerebroventricular administration, improve cognitive function in aged mice (14). Indeed in humans, the nonselective 11 $\beta$ HSD inhibitor carboxolone improved cognitive performance in healthy elderly and diabetic men (11). However, a recent phase II clinical trial of a selective 11 $\beta$ HSD1 inhibitor, ABT384, in patients with mild-to-moderate Alzheimer’s disease was halted prematurely because of lack of efficacy (15). It is uncertain whether this reflected selection of patients whose cognitive dysfunction is no longer responsive to reducing cortisol action, or inadequate inhibition of brain 11 $\beta$ HSD1 by ABT384.

Against this background, quantification of brain 11 $\beta$ HSD1 activity in vivo in humans would be highly desirable. Here, we aimed: (i) to determine whether, as a major organ by mass, brain 11 $\beta$ HSD1 contributes to cortisol/cortisone turnover in vivo; (ii) to establish whether 11 $\beta$ HSD1, as the only 11 $\beta$ HSD isozyme expressed in an

adult brain (11), catalyzes only regeneration of cortisol or also recycling between cortisol and cortisone; (iii) to provide a tool with which to quantify changes in human brain 11 $\beta$ HSD1 activity with dementia, and to use as a pharmacodynamics biomarker to quantify enzyme inhibition during clinical development of selective 11 $\beta$ HSD1 inhibitors for treating dementia. We therefore extended our previous studies using arteriovenous sampling with stable isotope tracer infusions in vivo to quantify 11 $\beta$ HSD activities in the human brain.

## Materials and Methods

### Participants

Participants were recruited through advertisements in the local press and around the University campus. Participants were healthy male volunteers between 18 and 70 years old. Exclusion criteria were glucocorticoid medication (by any route of administration) within the past 3 months; diabetes mellitus, cerebrovascular disease or other significant chronic illness; history of recent heavy alcohol or illegal drug use; current use of any immunosuppressive medication; abnormal screening liver, thyroid, renal or coagulation function (ie, INR > 1.5 or platelets < 50  $\times$  10<sup>9</sup>/L) or abnormal full blood count or random blood glucose; research participant in the previous 3 months; any contraindication to magnetic resonance (MR) imaging. The study complied with the Declaration of Helsinki; ethical approval was obtained from the local Research Ethics Committee (Scotland A REC, reference 12/SS/0079) and all participants gave written informed consent.

### Reagents

Reagents were obtained from Sigma, Steraloids, or VWR. 1,2 [<sup>2</sup>H]<sub>2</sub>-cortisone (d2-cortisone) and 9,11,12,12 [<sup>2</sup>H]<sub>4</sub>-cortisol (d4-cortisol) were from Cambridge Isotope Laboratories. Solvents were high-performance liquid chromatography (HPLC) grade from Fisher Scientific.

### Clinical protocol

Screening tests were performed within the 2 weeks prior to the procedure. On the day of the study, subjects attended the Clinical Research Facility at around 0830 h. They were allowed to eat a light breakfast at home, but were only allowed water after their arrival at the research facility. They first underwent an ECG-gated phase contrast MR neck scan, which lasted 20–30 min. After this, a cannula was inserted into the left antecubital fossa and blood was taken to measure baseline endogenous steroids and their background isotopomers. At  $t = -5$  min a 0.7 mg loading bolus of d4-cortisol was given intravenously (IV), followed by an infusion at 0.35 mg/h (15.94 nmol/min) at  $t = 0$  min. A cannula was inserted into a vein in the dorsum of the right hand in a retrograde direction and when required the hand was placed in a hot box at 60°C to arterialize the blood. Arterialization of the blood was accepted if the oxygen saturation was >98%.

A jugular bulb cannula was inserted in the dominant internal jugular vein (assessed by MR venography) under ultrasound guidance. Placement was checked using a plain lateral C-spine x-ray: the tip of the catheter was visualized to ensure it was above

the second cervical vertebra (16). The oxygen saturation of the blood was checked to ensure it was <85%.

At  $t = 145$  min a 76.0  $\mu$ g bolus of d2-cortisone was administered IV, followed by an infusion at 105.3  $\mu$ g/h (4.88 nmol/min). From  $t = 180$  min four sets of simultaneous blood samples were taken from the arterialized and jugular bulb cannulae at 10 min intervals ( $t = 180, 190, 200,$  and  $210$  min) into Lithium heparin and plasma separated and stored at  $-80^\circ\text{C}$  until analysis.

### Magnetic resonance scanning

The MR imaging was performed with participants in the supine position on a 1.5 Tesla MR imaging unit (Signa HDxt, GE Healthcare) at the Brain Research Imaging Centre ([www.bric.ed.ac.uk](http://www.bric.ed.ac.uk)). An eight-channel neurovascular array coil was used. A noncontrast MR angiogram of the carotids from the level of the exterior auditory meatus down to the angle of the mandible was performed, followed by an MR venogram over the same area with a saturation band over the inferior aspect of the slices. The MR venogram was used to select the level for the ECG-gated phase-contrast MR scan by measuring a quarter of the way down from the jugular bulb to where the facial vein enters the internal jugular vein; axial images were taken perpendicular to the table. This level was chosen to ensure blood flow was measured in the vessel before any of the tributaries entered. The facial vein was the first, and to avoid measuring flow dynamics in the bulb itself, it may not be representative of the rest of the vessel, as there may be some pooling of blood where it first enters the bulb. Sixteen phase and magnitude images were taken at this level over one cardiac cycle. The flip angle was 25°, bandwidth 15.63 kHz, echo time (TE) 6 ms, repetition time (TR) 25 ms, and encoding velocity ( $V_{\text{enc}}$ ) 100 cm/s. Data were checked at the point of acquisition for any aliasing artifact. The field of view (FOV) was 25.6 cm  $\times$  25.6 cm and slice thickness of 5 mm. These images took approximately 8 min to acquire per patient, depending on the heart rate.

The image analysis software Medis Q flow (Medis medical imaging systems) was used to calculate jugular venous blood flow in mL/min on the side which was cannulated. The outline of the internal jugular vein was traced on each of the magnitude images from the cardiac cycle and blood flow calculated using data from the corresponding phase images. For completeness, the blood flow for the opposite internal jugular vein and both common carotid arteries were also measured.

### Laboratory analysis

Steroids (cortisol, d4-cortisol, 9,12,12 [<sup>2</sup>H]<sub>3</sub>-cortisol (d3-cortisol), cortisone and d2-cortisone) were quantified using liquid chromatography-tandem mass spectrometry (LC-MS). An internal standard solution [0.5 microg epicortisol (Steraloids), 0.25 microg 2,2,4,6,6,9,12,12 [<sup>2</sup>H]<sub>8</sub>-cortisone (d8-cortisone, Santa Cruz Biotechnology), and 0.25 microg 2,2,4,6,6,17A,21,21 [<sup>2</sup>H]<sub>8</sub>-corticosterone (d8-corticosterone, Cambridge Isotope Laboratories) with 9  $\mu$ L methanol] was added to 1.5 mL of plasma, before 15 mL of chloroform was added for extraction of the steroids. The organic phase was then reduced to dryness under oxygen free nitrogen at 60°C before being reconstituted in the mobile phase [acetonitrile: water (35:65) with 0.1% formic acid]. Samples were injected on to a Sunfire C18 column (150 mm  $\times$  4.6 mm  $\times$  5  $\mu$ m), with a column temperature of 10°C and a mobile phase flow rate of 1.5 mL/min using an Acquity Ultra Performance Liquid Chromatograph (Waters) coupled to a



Qtrap 5500 mass spectrometer (AB Sciex). Ionization was achieved in the positive electrospray mode. The following transitions (precursor $\rightarrow$ product mass-to-charge ratios) used were as follows: cortisol (363 $\rightarrow$ 121), d2-cortisol (365 $\rightarrow$ 121), d3-cortisol (366 $\rightarrow$ 121), d4-cortisol (367 $\rightarrow$ 121), cortisone (361 $\rightarrow$ 77), d3-cortisone (364 $\rightarrow$ 164), and d2-cortisone (363 $\rightarrow$ 165). Steroid concentrations and tracer/tracee ratios were calculated from calibration curves and corrected for background isotopomer enrichments as described previously (17).

### Data analysis and kinetic calculations

Whole-body rate of appearance (Ra) of cortisol, d3-cortisol, and cortisone were calculated by dividing the rate of tracer infusion by the relevant tracer/tracee ratio (d4-cortisol/cortisol, d4-cortisol/d3-cortisol and d2-cortisone/cortisone, respectively) (3, 4). Brain tissue production of cortisol was calculated using data from arterial (A) and internal jugular vein (V) samples and corrected for cerebral blood flow using Eq. (1). Brain tissue production of d3-cortisol and cortisone were also calculated with Eq. (1), substituting arterial concentrations of d3-cortisol or cortisone and the relevant arterial and venous tracer:tracee ratios, as appropriate (3). Net release or uptake of cortisol across the brain was calculated using Eq. (2). Net release or uptake of d4-cortisol or cortisone were also calculated with Eq. (2), substituting arterial and venous concentrations of d4-cortisol and cortisone, respectively.

$$\text{Tissue cortisol production} = \left( (\text{Blood flow} \times [\text{cortisol}_A]) \times \left( \frac{[\text{d4-cortisol}]_A}{[\text{d4-cortisol}]_V} \right) \right) - (\text{Blood flow} \times [\text{cortisol}_A]) \quad (1)$$

$$\text{Net cortisol release} = ([\text{cortisol}_V] - [\text{cortisol}_A]) \times (\text{Blood flow}) \quad (2)$$

### Statistical analysis

There were no prior data for cortisol or cortisone production or uptake across the brain on which to base a power calculation, therefore we used previous data from a study on cortisol and cortisone production in skeletal muscle in healthy men (3). We calculated that in order to detect the same magnitude of difference from zero for net uptake or release across the brain of cortisol, d3-cortisol or cortisone, with a similar variance to that in skeletal muscle, would require sample sizes of 3, 5, and 7, respectively (for alpha 0.05, power 80% in a two-tailed test). We therefore considered a sample size of 8 to be reasonable.

Using SPSS, the student *t*-test was used to detect a difference from zero in brain production of cortisol, d3-cortisol, and cortisone. The small *n* in the study required the use of parametric tests, despite being unable to fully check the assumptions (18). However, on visual inspection there were no obvious outliers and the data appeared to meet the assumptions of normality.

### Results

Eight healthy men were recruited with a mean age of 38.1 years (sd 16.5) and mean BMI of 24.9 kg/m<sup>2</sup> (sd 3.7). In 7 of the 8 subjects, the right internal jugular was found to be

dominant or co-dominant on the MR scan and was used for cannulation and measurement of blood flow. In one subject, the left internal jugular vein (IJV) was dominant and was cannulated instead. Mean blood flow in the selected left internal jugular vein for all subjects was 481 mL/min (sd 232).

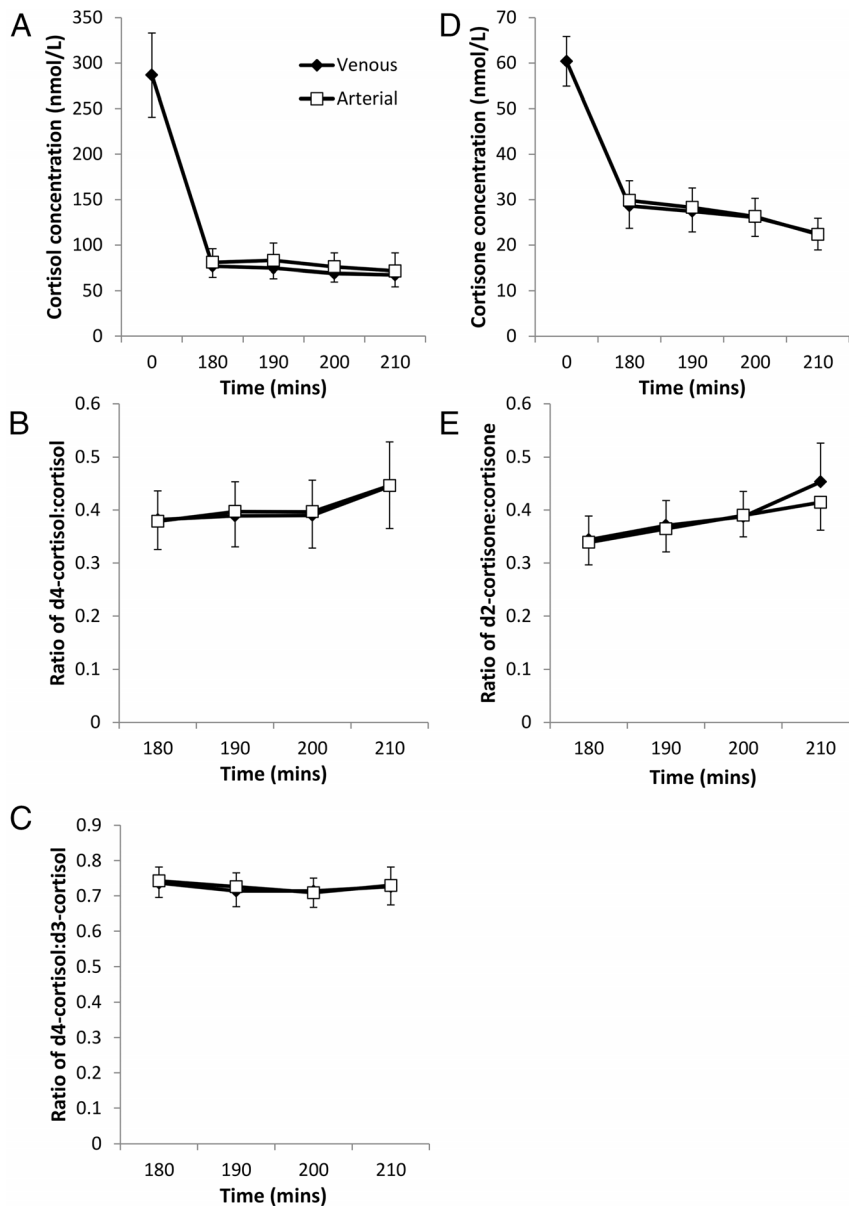
Endogenous cortisol and cortisone concentrations in both arterialized and jugular venous samples reduced from baseline to 180 min of infusion, when the first set of arteriovenous samples were obtained, which is consistent with diurnal variation (Figure 2, A and D). In both arterialized and jugular venous samples, concentrations of cortisol and cortisone and tracer/tracee ratios were similar at all four sampling time points between 180 and 210 min of infusion, consistent with the steady state being achieved (Figure 2).

Mean values in arterialized samples in the steady state were used to calculate whole body rates of appearance of cortisol, d3-cortisol, and cortisone (Table 1), all of which were readily detectable, confirming technical success of the tracer infusions. Mean steady state data from arterialized and jugular venous samples were combined with blood flow measurements to calculate net release (or uptake) of cortisol and cortisone [Eq. (2)] and to estimate cortisol, d3-cortisol, and cortisone production across the brain [Eq. (1)] (Table 1). Surprisingly, d4-cortisol concentrations were higher in the jugular vein than arterialized samples, so that there was a net release of d4-cortisol across the brain in the steady state. No other indices of brain steroid release/uptake or production were significantly different from zero.

### Discussion

We found no detectable interconversion of cortisol and cortisone across the human brain in eight healthy male volunteers, using deuterated glucocorticoid tracers and arteriovenous sampling. Previous studies using this approach have detected interconversion of cortisol plus or minus cortisone within liver, adipose tissue, and skeletal muscle (3, 5–7). Systemic cortisol/cortisone turnover values were similar in the subjects reported here to those observed in previous studies, so the approach was technically successful. The lack of statistically significant net production or release of cortisol, d3-cortisol or cortisone across the brain could not be attributed to variability in blood flow, either methodological due to the magnetic resonance method used, or biological due to asymmetry of internal jugular blood flow. Since we did not observe expected gradients in relevant steroid concentrations from arterialized to jugular venous blood, we sampled from the





**Figure 2.** Arterialized and jugular venous steroid concentrations and tracer/tracee ratios during steady state stable isotope tracer infusion. Data are mean  $\pm$  SEM for  $n = 8$ . (A) Cortisol concentration; (B) cortisone concentration; (C) d4-cortisol/cortisol ratio; (D) d4-cortisol/d3-cortisol ratio; (E) d2-cortisone/cortisone ratio. Statistical comparisons were made for the kinetic parameters derived from these "raw" data (Table 1).

dominant jugular vein, and therefore from venous drainage of a substantial proportion of brain tissue. Any contribution of  $11\beta$ HSD in the brain to whole body turnover between cortisol and cortisone is, therefore, negligible. It remains possible that, had we sampled from discreet brain subregions,  $11\beta$ HSD activity might have been measurable, but this remains a speculation. The consequences of brain  $11\beta$ HSD1 activity are likely to be confined to the subregions in which the enzyme is highly expressed (11).

In the absence of arteriovenous gradients in cortisol or cortisone concentrations, we did find a net release of d4-cortisol across the brain. It is unclear why there was

release of tracer from the brain but it may indicate overpriming and higher circulating d4-cortisol levels earlier in the infusion, with resulting re-release from the brain during steady state. However, this speculation cannot be tested in the absence of earlier samples.

One previous study has evaluated in vivo  $11\beta$ HSD1 activity in the brain, by measuring peripheral venous plasma and cerebrospinal fluid (CSF) steroid concentrations during d4-cortisol infusion (19). Unfortunately, data were presented for only two subjects without administration of a potent  $11\beta$ HSD1 inhibitor. Strangely, these subjects did not appear to reach steady state of tracer enrichment after 4 h of infusion and tracer/tracee ratios in plasma were not adjusted for d4-cortisol infusion to calculate the rate of appearance of cortisol and d3-cortisol and establish if the results were comparable with other published studies. CSF d3-cortisol concentrations were shown to be higher, relative to d4-cortisol, than plasma d3-cortisol concentrations, but only by comparing CSF data with plasma obtained 60 min earlier. This adjustment was applied on the grounds of closer correlation between plasma and CSF steroid concentrations separated by 60 min than those separated by shorter or longer intervals, but again indicates that steady state was not achieved in CSF. The authors attributed the apparent excess of d3-cortisol in CSF to brain  $11\beta$ HSD1, partly on the basis

that it was abolished in a dose-dependent fashion by administration of the  $11\beta$ HSD1 inhibitor ABT384. However, ABT384 dramatically lowered plasma d3-cortisol concentrations, consistent with potent inhibition of systemic  $11\beta$ HSD1 activity. The associated fall in CSF d3-cortisol concentrations, which fell below the limit of quantification in most samples, can be explained by the dramatic fall in plasma d3-cortisol without invoking any contribution of brain  $11\beta$ HSD1. Against this background, it has not been established whether activity of  $11\beta$ HSD1 in the brain is sufficient in magnitude to affect CSF cortisol concentrations.

**Table 1.** Calculated Steady State Kinetics for Cortisol and Cortisone for the Whole Body and Brain

|                                     | Whole Body                    | Brain                         |
|-------------------------------------|-------------------------------|-------------------------------|
| Cortisol                            |                               |                               |
| Rate of appearance of cortisol      | 47.5 (29.9–65.1) <sup>a</sup> | 0.43 (–0.27–1.12)             |
| Rate of appearance of d3-cortisol   | 22.5 (19.8–25.3) <sup>a</sup> | 0.21 (–0.20–0.62)             |
| Net brain release of cortisol       |                               | –0.54 (–3.75–2.66)            |
| Net brain release of d4-cortisol    |                               | 0.46 (0.22–0.70) <sup>b</sup> |
| Net brain release of d3-cortisol    |                               | 0.12 (–0.36–0.60)             |
| Cortisone                           |                               |                               |
| Net rate of appearance of cortisone | 14.4 (10.8–17.9) <sup>a</sup> | –0.02 (–0.35–0.30)            |
| Net brain release of cortisone      |                               | –0.23 (–0.69–0.23)            |
| Net brain release of d2-cortisone   |                               | –0.01 (–0.05–0.02)            |

Data are nmol/min shown as the mean (95% CI) for  $n = 8$ , except for net brain release of d2-cortisone, where reliable data could only be gained for  $n = 5$ . All other associations  $P > .05$ .

<sup>a</sup>  $P < .001$ .

<sup>b</sup>  $P < .005$  vs 0 using the Student one-sample  $t$ -test.

We took a different approach to measuring steroids in jugular venous blood and calculating arteriovenous differences during steady state tracer infusion. With the measurement of blood flow, this allows absolute quantification of 11 $\beta$ HSD activities for all tissue draining to the cannulated internal jugular vein. 11 $\beta$ HSD1 is known to be expressed in specific neuronal subregions of the human brain: hippocampus (in particular the dentate gyrus and the cornu ammonis), prefrontal cortex, and the area of highest expression, the granule cell layer of the cerebellum (11). The venous drainage of the human brain is complex and appears to display considerable interindividual differences. Cerebellar venous blood drains to the superior and inferior cerebellar veins and usually ultimately into the internal jugular veins. However, posture affects cerebrovenous drainage such that in the supine position the internal jugular veins drain around 95% of blood flow from the intracerebral structures, while in the erect position this can drop to as little as 25%, with the remainder draining through the vertebral venous plexus (20). Our subjects were reclining at an angle of 45° for the arteriovenous sampling, but the measure of internal jugular vein blood flow was performed supine within a magnetic resonance imaging (MRI) machine. It could be that during blood sampling more of the cerebellar venous drainage was to the vertebral venous system rather than to the in-

ternal jugular veins, and that the brain 11 $\beta$ HSD1 was underestimated as a result. Moreover, the forebrain subregions where 11 $\beta$ HSD1 is expressed represent a minority, by mass, of forebrain tissue and hence any contribution of these subregions to the plasma steroid pool may be diluted by blood from elsewhere. More selective venous cannulation might therefore detect 11 $\beta$ HSD1 activity, which was not measurable here, but this is unlikely to be feasible in healthy volunteers.

As there were no previous data on brain release or uptake of cortisol or cortisone, sample size was calculated using data from a previous study on skeletal muscle (3). With the current novel results from the brain in hand, we have performed new power calculations which show that to demonstrate that the observed mean differences are statistically significantly different from zero for Ra cortisol, Ra d3-cortisol, and net Ra cortisone, we would need sample sizes of 40, 57, and 4006, respectively (for alpha 0.05, power 90% in a two-tailed test). We do not consider it justified to undertake invasive studies in this large number of subjects in order to more precisely quantify any small amount of cortisol production in the brain, having shown its mean magnitude to be negligible relative to other tissues.

If 11 $\beta$ HSD activity in the brain had been detectable with the approach used here, this could have provided a useful pharmacodynamic tool to quantify brain enzyme inhibition by selective 11 $\beta$ HSD1 inhibitors in development for treatment of dementia (14, 19). As things stand, neither this approach nor the CSF approach attempted by Katz et al (19) appear well-suited for this purpose in healthy volunteers. Given that 11 $\beta$ HSD1 expression increases with age in mice and is predictive of cognitive decline (12), it remains possible that arteriovenous sampling could be used to detect cortisol regeneration by 11 $\beta$ HSD1 in the brains of patients with dementia or with risk factors for cognitive decline (eg, diabetes, older age).

## Acknowledgments

We thank the staff of the Imaging, Nursing and Mass Spectrometry Cores of the Wellcome Trust Clinical Research Facility, Edinburgh for assistance in conducting the study. In particular, we thank Mr Sanjaykumar Kothiya for performing the liquid chromatography-tandem mass spectrometry.

Address all correspondence and requests for reprints to: Dr Alixe HM Kilgour, Geriatric Medicine, University of Edinburgh, Room S1642, Royal Infirmary Edinburgh, 51 Little France Crescent, Edinburgh EH16 5SA, UK. E-mail: a.kilgour@ed.ac.uk.

We acknowledge support of the Wellcome Trust and British Heart Foundation. A.H.M.K. and J.M.S. are members of The University of Edinburgh Centre for Cognitive Ageing and Cog-

nitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative. Funding from the BBSRC, EPSRC, ESRC, and MRC is gratefully acknowledged.

Disclosure Summary: B.R.W. is an inventor on relevant patents owned and licensed by the University of Edinburgh, and has consulted for several companies developing selective 11 $\beta$ HSD1 inhibitors. A.H.M.K., S.S., I.M., P.A., R.A. have no conflicts of interest relevant to this article to declare.

## References

1. Seckl JR, Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology*. 2001;142:1371–1376.
2. Stewart PM, Krozowski ZS. 11 beta-Hydroxysteroid dehydrogenase. *Vitam Horm*. 1999;57:249–324.
3. Hughes KA, Manolopoulos KN, Iqbal J, et al. Recycling between cortisol and cortisone in human splanchnic, subcutaneous adipose, and skeletal muscle tissues in vivo. *Diabetes*. 2012;61:1357–1364.
4. Andrew R, Smith K, Jones GC, Walker BR. Distinguishing the activities of 11beta-hydroxysteroid dehydrogenases in vivo using isotopically labeled cortisol. *J Clin Endocrinol Metab*. 2002;87:277–285.
5. Basu R, Singh RJ, Basu A, et al. Splanchnic cortisol production occurs in humans: evidence for conversion of cortisone to cortisol via the 11-beta hydroxysteroid dehydrogenase (11beta-hsd) type 1 pathway. *Diabetes*. 2004;53:2051–2059.
6. Andrew R, Westerbacka J, Wahren J, Yki-Järvinen H, Walker BR. The contribution of visceral adipose tissue to splanchnic cortisol production in healthy humans. *Diabetes*. 2005;54:1364–1370.
7. Stimson RH, Andersson J, Andrew R, et al. Cortisol release from adipose tissue by 11beta-hydroxysteroid dehydrogenase type 1 in humans. *Diabetes*. 2009;58:46–53.
8. Iqbal J, Andrew R, Cruden NL, et al. Displacement of cortisol from human heart by acute administration of a mineralocorticoid receptor antagonist. *J Clin Endocrinol Metab*. 2014;99:915–922.
9. Yau JLW, Seckl JR. Local amplification of glucocorticoids in the aging brain and impaired spatial memory. *Front Aging Neurosci*. 2012;4:24.
10. Brown RW, Diaz R, Robson AC, et al. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology*. 1996;137:794–797.
11. Sandeep TC, Yau JL, MacLulich AM, et al. 11Beta-hydroxysteroid dehydrogenase inhibition improves cognitive function in healthy elderly men and type 2 diabetics. *Proc Natl Acad Sci U S A*. 2004;101:6734–6739.
12. Holmes MC, Carter RN, Noble J, et al. 11beta-hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus and parietal cortex and causes memory impairments. *J Neurosci*. 2010;30:6916–6920.
13. Yau JL, Noble J, Kenyon CJ, et al. Lack of tissue glucocorticoid reactivation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. *Proc Natl Acad Sci*. 2001;98:4716–4721.
14. Sooy K, Webster SP, Noble J, et al. Partial deficiency or short-term inhibition of 11beta-hydroxysteroid dehydrogenase type 1 improves cognitive function in aging mice. *J Neurosci*. 2010;30:13867–13872.
15. Marek GJ, Katz DA, Meier A, et al. Efficacy and safety evaluation of HSD-1 inhibitor ABT-384 in Alzheimer's disease. *Alzheimers Dement*. 2014;10:S364–373.
16. Shaaban Ali M, Harmer M, Latto I. Jugular bulb oximetry during cardiac surgery. *Anaesthesia*. 2001;56:24–37.
17. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med*. 2013;368:1477–1488.
18. Bland JM, Altman DG. Analysis of continuous data from small samples. *BMJ*. 2009;338:a3166.
19. Katz DA, Liu W, Locke C, et al. Peripheral and central nervous system inhibition of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in man by the novel inhibitor ABT-384. *Transl Psychiatry*. 2013;3:e295.
20. Valdeza JM, von Münster T, Hoffman O, Schreiber S, Einhäupl KM. Postural dependency of the cerebral venous outflow. *Lancet*. 2000;355:200–201.