Physical inactivity and sedentary time: impact on metabolic health and development of type 2 diabetes

INSTITUTE OF AGEING
AND CHRONIC DISEASE

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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March 2018

I. Abstract

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Physical inactivity is a major risk factor for global mortality; it is associated with the development of obesity, metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D) and cardiovascular disease (CVD). Despite the worldwide recommendations and health guidelines recommending an active lifestyle, low levels of physical activity (PA) and sedentary behaviour are increasingly prevalent in modern society. The primary aim of this thesis is to examine the role of sedentary time and physical inactivity with a specific emphasis on the development of multi-organ insulin resistance as precursor for the development of T2D.

The initial data within this thesis provided evidence for the importance of a physically active lifestyle irrespective of obesity. Ninety-eight individuals were recruited, screened, identified as free from disease and subsequently categorised according to obesity status and MetS (termed herein unhealthy). Seventy-three individuals were non-obese, of which 11 were unhealthy, and 25 individuals were obese, of which 13 were unhealthy. PA did not fully explain metabolic health status but sedentary time was higher in obese individuals. Secondary data in this cohort revealed an independent association between sedentary time and liver fat, which is a proxy for poor metabolic health and clinical manifestations related to T2D. Of note, moderate-vigorous physical activity, which is the cornerstone of PA recommendations, did not significantly influence metabolic health, obesity or liver fat. Research examining the consequences of a physically inactive lifestyle (i.e. sedentary behaviour) is warranted.

The major data detailed within this thesis were derived from inducing short-term physical inactivity in first-degree relatives (FDR) of patients with T2D, compared with healthy controls (CON). Forty-five habitually active participants (16 FDR, 29 CON) were assessed at baseline, after 14 days of inactivity and after 14 days of resuming normal activity. Sedentary time increased in parallel with a decrease in PA during the period of inactivity. Cardiorespiratory fitness reduced, lower limb lean mass decreased, while gynoid fat increased and significantly more android fat was accumulated in FDR. Significant increases in liver fat were accompanied by increased plasma triglycerides, which were increased to a greater extent in FDR. Whole body insulin sensitivity significantly with no difference between the groups. Resumption of normal activity reversed all observed changes. FDR seem susceptible to some of the the risks of physical inactivity.

Taken together, these data support the importance of reducing physical inactivity and sedentary time. FDR have an increased risk of developing T2D compared with CON and therefore these data must be used as a platform to develop guidelines and strategies aimed at preventing chronic physical inactivity and sedentary behaviour, particularly in these individuals. Considering linked epidemics of T2D and physical inactivity, innovative strategies must be developed to promote the importance of a habitually active lifestyle, either as a minimum or in addition to current guidelines.

II. Acknowledgements

Firstly, I would like to thank my supervisory team. Dr Daniel Cuthbertson, for providing me with the opportunity to undertake this PhD, in an area that I feel truly passionate about. Professor Graham Kemp, for facilitating my magnetic resonance scanner training and for his invaluable knowledge of this field. Dr Victoria Sprung, who has been ever present 'on deck' and provided unwavering support and encouragement in so many ways.

I wish to extend my thanks to the wider team who have been instrumental during data collection; to my colleagues from Aintree University Hospital and MR centre and students from the University of Liverpool, your contributions and support have been essential to the successful delivery of these projects. A special thanks to Juliette Norman, for sharing this unique experience with me. Many hands really do make lighter work. Also, to Dr Andrew Thompson, who became involved later in the process; for your patience and guidance I am so grateful.

I would like to thank Diabetes UK for funding the methodologies detailed within this thesis and the dissemination of findings through, national and international conference attendance.

To my participants, I sincerely thank you. Quite simply, I would never have been able to complete this work without your sacrifice and commitment. It was a hugely demanding protocol and I truly hope you will see the direct contribution to the field that has come from your generosity.

My final thanks go to my family and loved ones for their support, their patience and for ensuring a degree of work life balance. I am eternally grateful to you all. In particular, my parents, who have supported me unconditionally throughout more than two decades of education.

III. Declaration and publications

I declare that the analysis and write up of this thesis is entirely my own. Dr Victoria Sprung, Juliette Norman, Andrew Irwin, and Val Adams assisted with data collection; Dr Daniel Cuthbertson, Dr Tom Steele and Dr Sravan Thondam with tissue collection; Dr Andrew Thompson with statistical analysis; Ms Katie Mitchell with nutritional analysis and Professor Graham Kemp with MR analysis.

Publications derived from work contained within this thesis

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Thompson, A., Mitchell, K.L., Halford J.C.G., Harrold J.A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) Metabolic decompensation and altered body composition after short-term physical inactivity in first-degree relatives of patients with type 2 diabetes versus healthy controls. *Manuscript in final stages of submission to Diabetologia.*

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Thompson, A., Mitchell, K.L., Halford J.C.G., Harrold J.A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) Habitual physical activity, independent of moderate-vigorous activity, predicts amount of liver fat in obese and non-obese individuals. *Manuscript in preparation.*

Other publications completed during PhD tenure

Cuthbertson, D. J. & **Bowden Davies, K. A.**, (2017) Higher levels of cardiorespiratory fitness keep liver mitochondria happy! *J Physiol* 595: 5719-5720.

Bowden Davies, K.A., Connolly, J., Burghardt, P., Koch, L.G., Britton, S.L., & Burniston, J.G. (2105) Label-free profiling of white adipose tissue of rats exhibiting high or low levels of intrinsic exercise capacity. *Proteomics* 15: 2342-2349.

Abstract (conference) publications

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) Habitual physical activity, independent of moderate-vigorous activity, predicts amount of liver fat and metabolic health status. *Obes Facts* 1: 125.

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Halford J.C.G., Harrold J.A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) Short-term increased sedentary behaviour leads to loss of muscle mass in young individuals. *Obes Facts* 1: 144.

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Halford J.C.G., Harrold J.A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) The metabolic consequences of short-term increased sedentary behaviour. *Obes Facts* 1: 149.

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J. (2017) Implementing a step-reduction model to examine the metabolic effects of physical inactivity. *Diabetic Medicine* 34: 58.

Bowden Davies, K. A., Sprung, V. S., Norman, J. A., Wilding, J. P., Kemp, G. J., & Cuthbertson, D. J. (2016) A baseline assessment of individuals with and without a family history of type 2 diabetes: the effects of 14 day step-reduction. *Diabetic Medicine* 33: 74.

Oral presentations

British Chapter of the International Society for Magnetic Resonance in Medicine (BC-ISMRM), Liverpool (September 2017). 'Habitual physical activity, independent of moderate-vigorous activity, predicts amount of liver fat and metabolic health status'.

European Association for the Study of Diabetes (EASD): Non-alcoholic fatty liver disease (NAFLD) international study group meeting, Sweden (May 2017). Two presentations: I) 'The metabolic and skeletal muscle consequences on sedentary behaviour' and II) 'Habitual physical activity, independent of moderate-vigorous activity predicts metabolic health and liver fat'.

Musculoskeletal Biology Departmental Day, University of Liverpool (December 2016). 'Implementing a step reduction model to examine the metabolic effects of physical inactivity'.

Diabetes UK Student Networking Day, London (November 2016). 'The effects of a 14 day step reduction protocol: preliminary analysis'.

Institute of Ageing and Chronic Disease, University of Liverpool (September 2016). 'Impact and future direction of my research associated with obesity and metabolic disease'.

Faculty of Health and Life Science, University of Liverpool (June 2016). 'The effects of a 14 day step reduction protocol in individuals with and without a family history of type 2 diabetes (T2D)'.

Institute of Aging and Chronic Disease, University of Liverpool (September 2015). 'Inactivity in those with and without a family history of type 2 diabetes (T2D)'.

Lay presentations

Patient and public engagement meeting, Liverpool (May 2017). 'Feasibly and feedback on studies of physical inactivity and overfeeding'.

Diabetes UK Group meeting, St Helens (September 2016). 'Update on current Diabetes UK funded research and other relevant projects'.

KIND charity, Liverpool (August 2016). 'Obesity and health: move more'.

Meet the Scientist World Museum event, Liverpool (March 2016). 'Obesity and physical activity'.

Diabetes UK Volunteer meeting, Liverpool (February 2016). 'Obesity, type 2 diabetes and physical activity'.

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V. Abbreviations

abSAT Abdominal subcutaneous adipose tissue

ALT	Alanine transaminase
AMPK	AMP-activated protein kinase
AST	Aspartate transaminase
AUC	Area under curve
BMI	Body mass index
BMR	Basal metabolic ratw
CHD	Coronary heart disease
cIMT	Carotid intima-media thickness
CON	Control
CPET	Cardiopulmonary exercise test
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
DIT	Diet-induced thermogenesis
DNL	De novo lipogenesis
DXA	Dual-energy x-ray absorptiometry
FDR	First-degree relative
FFA	Free fatty acid
FFM	Fat free mass
FMD	Flow mediated dilation
GGT	Gamma-glutamyltransferase
GLUT4	Glucose transporter type 4
HbA1C	Glycated hemoglobin
HDL	High density lipoprotein
HIRI	Hepatic insulin resistance index
HOMA	Homeostatic model assessment
HSE	Health Survey for England
IDF	International Diabetes Federation
IHCL	Intrahepatocellular lipid
IMCL	Intramyocellular lipid
IR	Insulin resistance
LDL	Low density lipoprotein
LPL	Lipoprotein lipase

MetS	Metabolic syndrome
METS	Metabolic equivelents
MHNO	Metabolically healthy non-obese
MHO	Metabolically healthy obese
MISI	Muscle insulin sensitivity index
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MUNO	Metabolically unhealthy non-obese
MUO	Metabolically unhealthy obese
MVC	Maximum voluntary contraction
MVPA	Moderate-vigorous physical activity
NAFLD	Non-alcoholic fatty liver disease
NEAT	Non-exercise activity thermogenesis
NEFA	Non-esterified fatty acid
OGTT	Oral glucose tolerance test
PA	Physical activity
PCr	Phosphocreatine
PFT	Personal fat threshold
PHE	Public Health England
Pi	Inorganic phosphate
SAT	Subcutaneous adipose tissue
SkM	Skeletal muscle
T2D	Type 2 diabetes
TG	Triglycerides
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
$\dot{V}O_2$	Maximal oxygen consumption
WHO	World Health Organisation

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Chapter 1.

Introduction and literature review

Introduction

The global prevalence of diabetes has risen from 108 million in 1980 to 422 million in 2014 (WHO, 2016) and is projected to continue rising (Mathers and Loncar, 2006). Approximately 5 million people in England are at a high risk of developing the disease, which can lead to a number of other health problems including cardiovascular disease (CVD), stroke, nephropathy, neuropathy, limb amputation and blindness (PHE, 2016). Type 2 diabetes (T2D) accounts for the majority of people with diabetes and is largely related to excess body weight (overweight and obesity) and physical inactivity. If current trends persist, one in three people will be obese by 2034 and one in ten will develop T2D (Diabetes UK, 2016). The total financial cost (direct care and indirect costs) associated with diabetes in the UK currently stands at £23.7 billion and is predicted to rise to £39.8 billion by 2035 (Hex et al., 2012). These trends are mirrored globally and pose a serious threat to population health and economy.

Alarmingly, T2D is something that in many cases can be averted with careful lifestyle choices such as a well-balanced diet, regular physical activity and maintaining a healthy body weight. The World Health Organisation (WHO) targets for 2025 are *'halt the rise in diabetes and obesity'* and reduce the *'prevalence of insufficient physical activity'* (WHO, 2014). This is proving to be a major public health challenge as despite global recommendations, physical inactivity is rising in modern society and is recognised ahead of obesity as the fourth leading risk factor for global mortality, following smoking, hypertension and hyperglycaemia (WHO, 2010). The mechanisms by which physical inactivity causes metabolic complications has important implications for understanding the development of associated diseases. Innovative

strategies are needed to reduce the closely linked epidemics of physical inactivity, obesity and T2D.

Literature review

This literature review will begin by describing whole body integrative physiology and inter-organ cross-talk. Obesity as a public health issue is then outlined, along with its associated chronic disease outcomes. The characterising features of metabolic syndrome (MetS) are reported and the idea of a 'healthy' type of obesity will be discussed. The concept of body fat distribution as opposed to total body fat will be detailed, as well as discussion of the role fat distribution plays in the development of insulin resistance (IR), non-alcoholic fatty liver disease (NAFLD) and ultimately T2D. The link between cardiorespiratory fitness (CRF) and mortality is examined, and risk factors for T2D are identified with a specific focus on habitual physical activity (PA) and sedentary behaviour. The review concludes with suggested research priorities for understanding the consequences of physical inactivity and based on these, the aims and objectives of the thesis are stated.

1.1 Metabolic regulation

The coordinated regulation of metabolic fuel selection is crucial to energy homeostasis. This section will discuss the liver, skeletal muscle and adipose tissue as major metabolic organs with a focus on how inter-organ cross-talk between these tissues mediates whole body metabolism. The physiology of metabolic regulation will be outlined first as understanding this is central to the appreciation of the pathophysiology associated with metabolic disease. The theory discussed is underpinned by textbooks (Frayn, 2010, Baynes and Dominiczak, 2014) and detail of

how these process are altered with in metabolic disease is taken from specific studies and cited throughout.

1.1.1 Post-absorptive metabolism

In the post-absorptive state (Figure 1.1A), the liver plays a primary role in maintaining plasma glucose concentration. Glucose enters the blood from the breakdown of liver glycogen and hepatic gluconeogenesis. The stimulus for this is a decreased insulin/glucagon ratio. A large proportion of this glucose is completely oxidised by the brain, the remaining is used by red blood cells and other glycolytic tissues whereby carbon is returned to the liver as lactate to be reused in gluconeogenesis. In the absence of nutrient intake, circulating insulin from the pancreas is low therefore skeletal muscle uptake of glucose is low but liberation of fatty acids (lipolysis) from adipose tissue is high. Plasma glucose and insulin concentrations are at their lowest (of a 24 hour cycle) whereas non-esterified fatty acids are at their highest, all are somewhat stable. Non-esterified fatty acids are the preferred fuel for muscle. Fatty acids are also oxidised in the liver for gluconeogenesis and is accompanied by ketone body formation. Although not a major fuel, ketone bodies are oxidised by other tissues including the brain, skeletal muscle and adipose tissue. Furthermore, protein is broken down within skeletal muscle from muscle glycogen breakdown and a small amount of glucose uptake. Amino acids are oxidised, transferred to pyruvate to form alanine and taken up by the liver for gluconeogenesis.

1.1.2 Post-prandial metabolism

After the ingestion of a meal (Figure 1.1B), nutrients present at the portal vein and then general circulation within 15-30 minutes. The concentration of plasma glucose rises and the pancreas responds by insulin secretion. Plasma insulin concentration rises and glucagon decreases. These hormones in circulation have a direct suppressive effect

on lipolysis in adipose tissue. Non-esterified fatty acids are reduced and no longer oxidised by skeletal muscle and glucose uptake is increased. In turn, glycogen synthesis is stimulated, glycolysis increases, the output of lactate and pyruvate increases, and glucose oxidation increases. In the liver, glycogen metabolism switches from breakdown to synthesis. Some amino acids are taken up by the liver although mostly branched-chain amino acids enter skeletal muscle; these are used in the muscle as an oxidative fuel but also for protein synthesis. Hepatic gluconeogenesis is maintained through lactate which leads to glucose 6-phosphate and directed into glycogen synthesis as opposed to glucose release.

Overall, the ingestion of a meal causes a suppression of fatty acid release and promotes glucose utilisation, particularly to storage as glycogen. Muscle insulin action increases skeletal muscle glucose uptake, and adipose insulin action decreases hepatic fatty acid delivery and re-esterification of hepatic fatty acids into triglycerides. Direct hepatic insulin action will activate *de novo* lipogenesis (DNL) and conversion of excess carbohydrate substrate into triglyceride and will promote export of hepatic triglyceride to adipose tissue as very low density lipoprotein (VLDL). Towards the end of the post-prandial period, triglycerides are transported to adipose tissue as chylomicrons where lipoprotein lipase (LPL) activity has been increased by greater circulating insulin and esterification and storage of fatty acids is promoted. After 3-5 hours, glucose and insulin concentrations decline which in turn promotes lipolysis and plasma non-esterified fatty acids begin to rise.

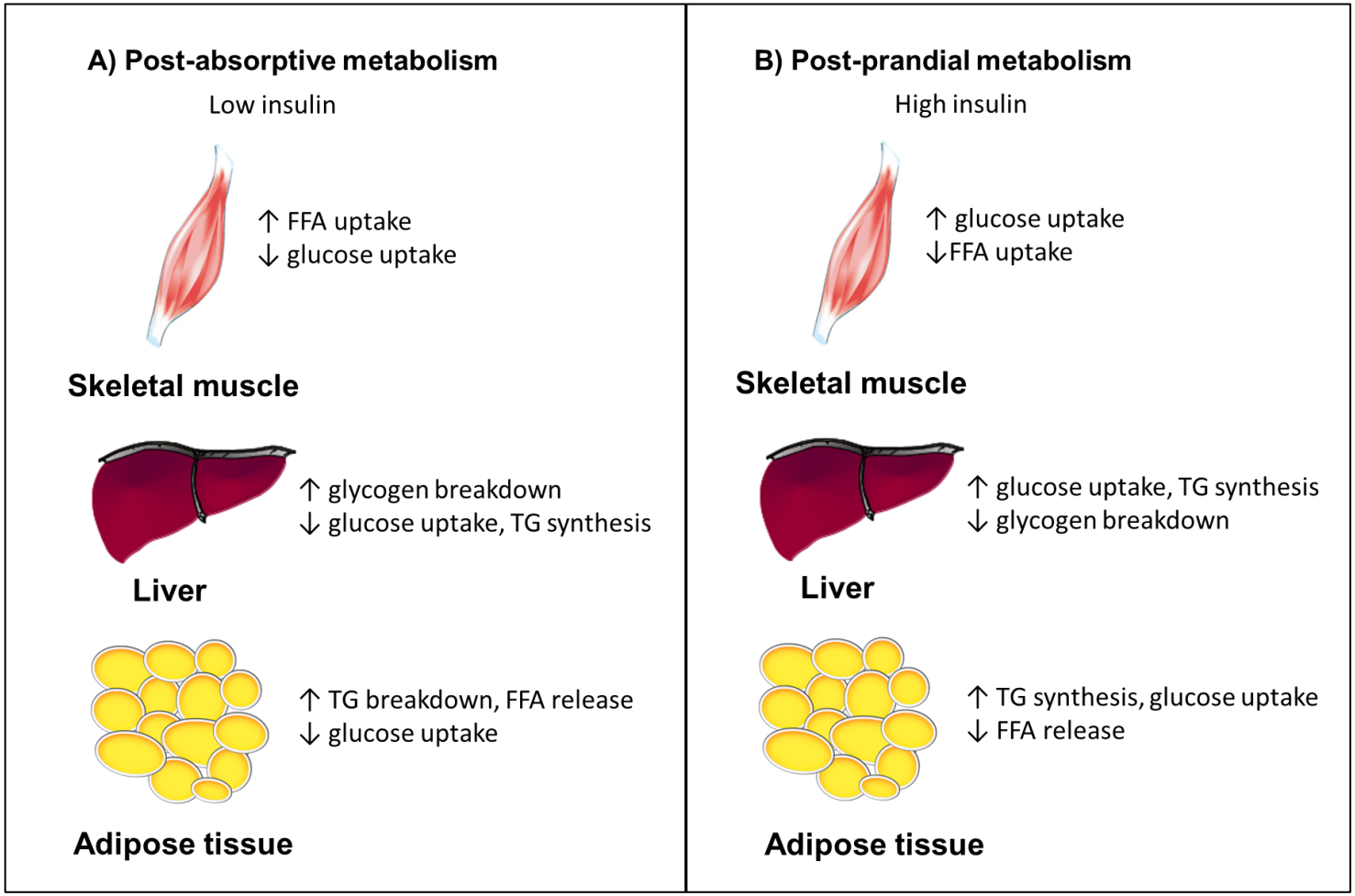


Figure 1.1 Schematic of A) post-absorptive metabolism and B) post-prandial metabolism. FFA, free fatty acids TG triglyceride.

1.1.3 Excess calorie consumption

In the context of obesity, it is important to note how subsequent meals (either in calorific excess or timed too closely) may promote energy storage. The insulin-stimulated process can become 'primed' by the previous bolus. Therefore, non-esterified fatty acids stay low, skeletal muscle and liver glycogen synthesis remains and storage of triglyceride in adipose tissue is almost continual. Over time, this will induce metabolic harm, the pathophysiology of this is discussed in detail later.

1.2 Obesity

Obesity can be defined as excessive adipose tissue, commonly known as body fat. Body mass index (BMI) is currently the most widely used method to classify weight and associated risk of co-morbidities (Table 1.1); calculated as body mass (kg) / height squared (m^2). Obesity is defined as ≥30 kg/m^2; in 2014, WHO estimated a 39% (1.9 billion adults) global prevalence of being overweight, of which 13% (600 million adults) were obese. In England, obesity prevalence increased from 15% in 1993 to 27% in 2015 (Health Survey for England, 2015). The prevalence of morbid obesity (≥40 kg/m^2) has more than tripled since 1993, and reached 2% of men and 4% of women in 2015.

The increasing prevalence of obesity is a major public health threat. Robust evidence has associated a higher BMI with a greater risk of premature all-cause mortality in 1.46 million white adults (Berrington de Gonzalez et al., 2010) and across four continents (Global et al., 2016). Obesity is also strongly linked with the incidence of numerous conditions including hypertension, dyslipidaemia, IR, MetS, non-alcoholic fatty liver disease (NAFLD), T2D, cardiovascular disease (CVD), stroke, and some cancers (Prospective Studies, 2009). In 2015/16, there were 525,000 NHS hospital admissions whereby obesity was a factor. The projected future cost of treating obesity

and obesity-related disease is staggering. It is estimated to increase by £1.9–2 billion/year in the UK and $48–66 billion/year in the USA by 2030 (Wang et al., 2011). Understanding the pathophysiology of obesity-related disease is paramount to reduce this economic burden.

Table 1.1 World Health Organisation **(**WHO) classification of body mass index (BMI) and risk of co-morbidities.

Classification	**BMI (kg/m^2)**	**Risk of co-morbidities**
Underweight	<18.5	Risks associated with being underweight
Normal weight	18.5 – 24.9	Average risk
Overweight	≥25	Increased risk
Pre-obese	25 – 29.9	Mild
Obese class I	30 – 34.9	Moderate
Obese class II	35 – 39.9	Moderate to severe
Obese class III	≥40	Severe

1.2.1 Metabolic abnormalities in obesity

Dysregulated metabolism, i.e. inefficiencies in the balancing and use of energy within the body, is thought to be the primary pathway of obesity-related disease (Frayn, 2010). Obesity arises when energy intake exceeds energy expenditure for a prolonged period of time and, resultantly, excess energy is stored in the form of adipose tissue. Excessive amounts of adipose tissue can lead to a series of metabolic abnormalities including impaired circulation of blood throughout the body as indicated by elevated blood pressure (hypertension), impaired use of lipids as indicated by unbalanced levels of cholesterol and triglycerides in the blood (dyslipidaemia) and impaired use of glucose by body tissues as indicated by abnormally high levels of glucose in the blood (hyperglycemia). Gender and ethnic specific criteria has been developed to stratify the risk of these metabolic abnormalities along with central (i.e. abdominal) adiposity. These risk factors, which are closely linked and often appear together, can form a condition known as '*metabolic syndrome*'.

1.3 Metabolic syndrome (MetS)

MetS is not a disease *per se*, but is a term that highlights a grouping of traits that increase risk of disease, approximately 3-fold for CVD and 5-fold or more for T2D (Stern et al., 2004). There are multiple definitions of MetS; three globally recognised criteria are defined (Table 1.2). The International Diabetes Federation (IDF) definition (Alberti et al., 2009), estimates around 20-25% of the world's adult population have MetS. The prevalence of MetS increases markedly with obesity (Katzmarzyk et al., 2005), whereby central adiposity is thought to play a key role and may precede the appearance of associated cardiometabolic abnormalities and chronic diseases. The presence of hypertension, dyslipidaemia, increased central adiposity (waist-hip ratio or waist circumference) and hyperglycemia in some capacity is agreed between

definitions, as are gender differences. The National Cholesterol Education Program Adult Treatment Panel (NCEP / ATP III) (Stone et al., 2005) and IDF both consider prescription medicine as a surrogate risk factor for hypertension and dyslipidaemia. Additionally, IDF propose country (ethnic origin) specific waist circumference cut-offs in order to account for population differences. Identifying individuals with MetS has potential importance within a health care setting where chronic diseases are often clinically ‘silent’ for a long time (O'Neill and O'Driscoll, 2015).

Table 1.2 World Health Organization (WHO), National Cholesterol Education Program Adult Treatment Panel (NCEP / ATP III) and International Diabetes Federation (IDF) definitions of metabolic syndrome (MetS).

	WHO (1998)	**NCEP/ ATP III (2005)**	**IDF (2009)**
Criteria	IGT, IFG, T2D plus two of the following:	Any three of the following:	Three out of the following:
Waist circumference (WC)	Men: WHR >90; Women: WHR >0.85 and/or BMI >30 kg/m^2	Men: WC ≥102 cm; Women: WC ≥88 cm	Population country specific WC*
Blood pressure	≥140/90 mmHg	≥130/85 mmHg or medicated	≥130/85 mmHg or medicated
Glucose	IGT, IFG or T2D	≥5.6 mmol/l	≥5.6 mmol/l
Triglycerides	≥1.7 mmol/l	≥1.7 mmol/l or medicated	≥1.7 mmol/l or medicated
High density lipoprotein	Men: <1.0 mmol/l Women: <1.3 mmol/l	Men: <1.0 mmol/l Women: <1.3 mmol/l or medicated	Men: <1.0 mmol/l Women: <1.3 mmol/l or medicated

*If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured; see IDF criteria for population specific values. IGT, impaired glucose tolerance; IFG, impaired fasting glucose; T2D, type 2 diabetes; WHR, waist-hip ratio; BMI, body mass index

1.3.1 Metabolically *healthy* obese and metabolically *unhealthy* non-obese

Historically, BMI has been widely used to stratify obesity status. Herein, metabolically unhealthy refers to the *presence* of metabolic syndrome (MetS+) and the term metabolically healthy refers to the *absence* of metabolic syndrome (MetS-). More recently, there is a growing recognition that not all obese individuals are unhealthy, termed *metabolically healthy obese* (MHO) and likewise, not all non-obese individuals are healthy, termed *metabolically unhealthy non-obese* (MUNO). Whilst this research is somewhat equivocal, the evidence to support these phenotypes has been recently reviewed (Phillips, 2017, Stefan et al., 2017). Many authors believe that cross-sectional observations are a transient phase; it has been questioned whether these phenotypes really exist and if so the clinical significance of these. These inconsistencies may be due a lack of a universal definition of MetS and thus a considerable variation in prevalence reports.

In a 20 year follow up study, it was found that one-half of MHO had made an adverse transition to *metabolically unhealthy obese* (MUO) and were also more likely to make this transition than MUNO (Bell et al., 2015a). Irrespective of the research disparity, these individuals are prevalent (estimated between 2-28% in Europe) within society and constitute a unique subset of characteristics. MHO display preserved insulin sensitivity, normal blood pressure, healthy lipid profiles and favourable fat distribution (Phillips, 2017). Contrary, MUNO display insulin resistance (IR), hypertension, dyslipidaemia as well as adipose tissue dysfunction and a body composition whereby the anatomical site of fat storage appears to be more important than total amount of body fat (Stefan et al., 2017). Body fat distribution is thought to be a distinguishing feature of metabolic status. It could be argued that independent of obesity, improving metabolic status is important to attenuate the development of cardiometabolic risk.

1.4 Body fat distribution

An increase in BMI, as seen with obesity, commonly represents an accumulation of fat. However, excessive total body fat content does not always lead to cardiometabolic disruption and a vast amount of evidence has revealed that body fat distribution more accurately predicts risk (Tchernof and Després, 2013). The presence of fat stores within intra-abdominal anatomical structures, termed visceral adipose tissue (VAT), has emerged as one of the most prevalent manifestations of MetS and represents an essential feature of the obesity epidemic (Després and Lemieux, 2006). VAT is considered as part of a complex phenotype including adipose tissue storage dysfunction and ectopic triglyceride accumulation in several sites including skeletal muscle (intramyocellular lipid, IMCL), liver and pancreas (Després, 2011).

A wealth of evidence has associated a greater waist-hip ratio with increased VAT and liver fat; strongly linking these and cardiometabolic disease (Després and Lemieux, 2006, Fabbrini et al., 2009). The presence of fat stores at the periphery, termed subcutaneous adipose tissue (SAT), is considered less important in the pathophysiology of chronic disease development (Ibrahim, 2010) and increased lower-body SAT is thought to be protective (Karpe and Pinnick, 2015). A metabolically healthy individual has more SAT, less VAT, and lower ectopic fat deposition in skeletal muscle and the liver than a metabolically at-risk individual (Figure 1.2) (Stefan et al., 2013). Obesity is commonly associated with IR (Petersen and Shulman 2006; Gill et al. 2005), however some non-obese individuals have IR which is associated with their ectopic fat stores (Jensen et al. 1989; Lim and Meigs 2014).

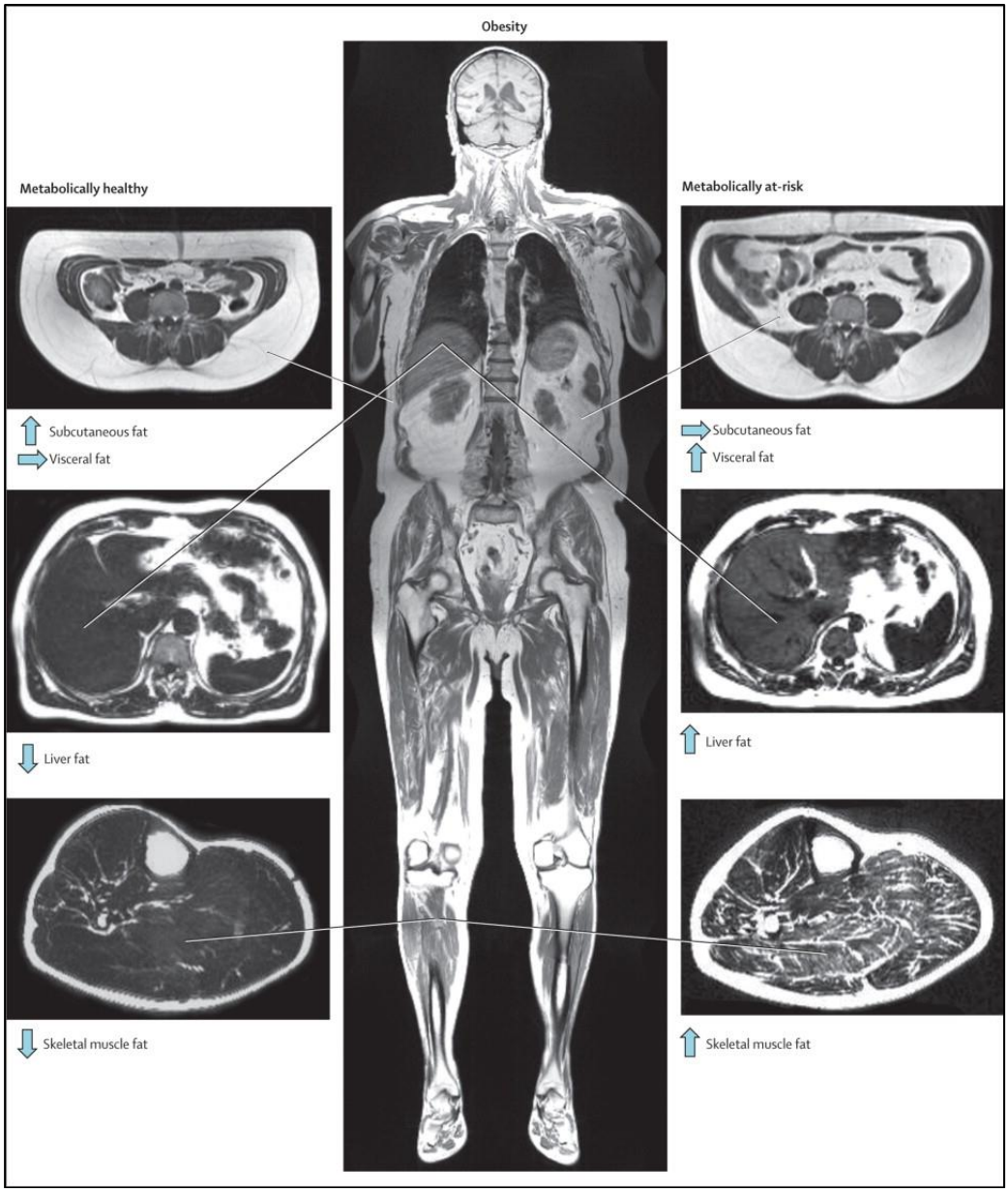


Figure 1.2 A comparison of body fat distribution in a metabolically healthy and metabolically at-risk individual (Stefan et al., 2013).

1.5 Insulin resistance (IR)

IR is a pathological condition in which high insulin concentrations fail to produce a normal response in peripheral target tissues (Benito, 2011). It is a common feature of obesity, MetS, NAFLD and T2D. *Skeletal muscle* IR disrupts insulin-mediated glucose uptake and decreases glycogen synthesis. *Hepatic* (liver) IR impairs the ability of insulin to suppress glucose production. *Adipose tissue* IR attenuates the anti-lipolytic

effect of insulin which leads to elevated free fatty acid. This metabolic setting increases the demand for pancreatic β-cells to synthesise and secrete more insulin. Eventually, the inability of pancreatic β-cells to produce enough insulin leads to elevated fasting glucose, glucose intolerance, and ultimately T2D (Petersen and Shulman 2006; Gill et al. 2005). Ectopic lipid accumulation in skeletal muscle and liver triggers pathways that impairs insulin signalling at the key insulin-responsive tissues (liver, skeletal muscle, and adipose tissue) (Samuel and Shulman, 2016).

1.6 Liver fat

The role of skeletal muscle IR in the pathogenesis of liver fat and hepatic insulin resistance has been evidenced (Petersen et al., 2007). A defect in muscle glycogen synthesis by decreased insulin-stimulated glucose transport activity due to selective ectopic IMCL accumulation and inhibition of muscle insulin signalling occurs. With post-prandial hyperinsulinemia and hyperglycemia excess energy is directed to the liver which stimulates hepatic DNL. Hepatic triglyceride synthesis is increased resulting in increased VLDL production, hypertriglyceridemia, and reductions in plasma HDL. As IR progresses towards T2D (Figure 1.3), the accumulation of liver fat (hepatic steatosis) increases and insulin signalling in the liver, skeletal muscle, and adipose tissue decreases. Rates of adipose tissue lipolysis are increased, resulting in increased fatty acid delivery to liver, which results in increased hepatic esterification of fatty acids to triglyceride. In contrast, hepatic DNL, which is dependent on hepatic insulin signalling, is reduced. The accumulation of liver fat in the development of T2D is pivotal (Taylor, 2013). To note, VAT does not appear to be pathophysiologically related (Fabbrini et al., 2009).

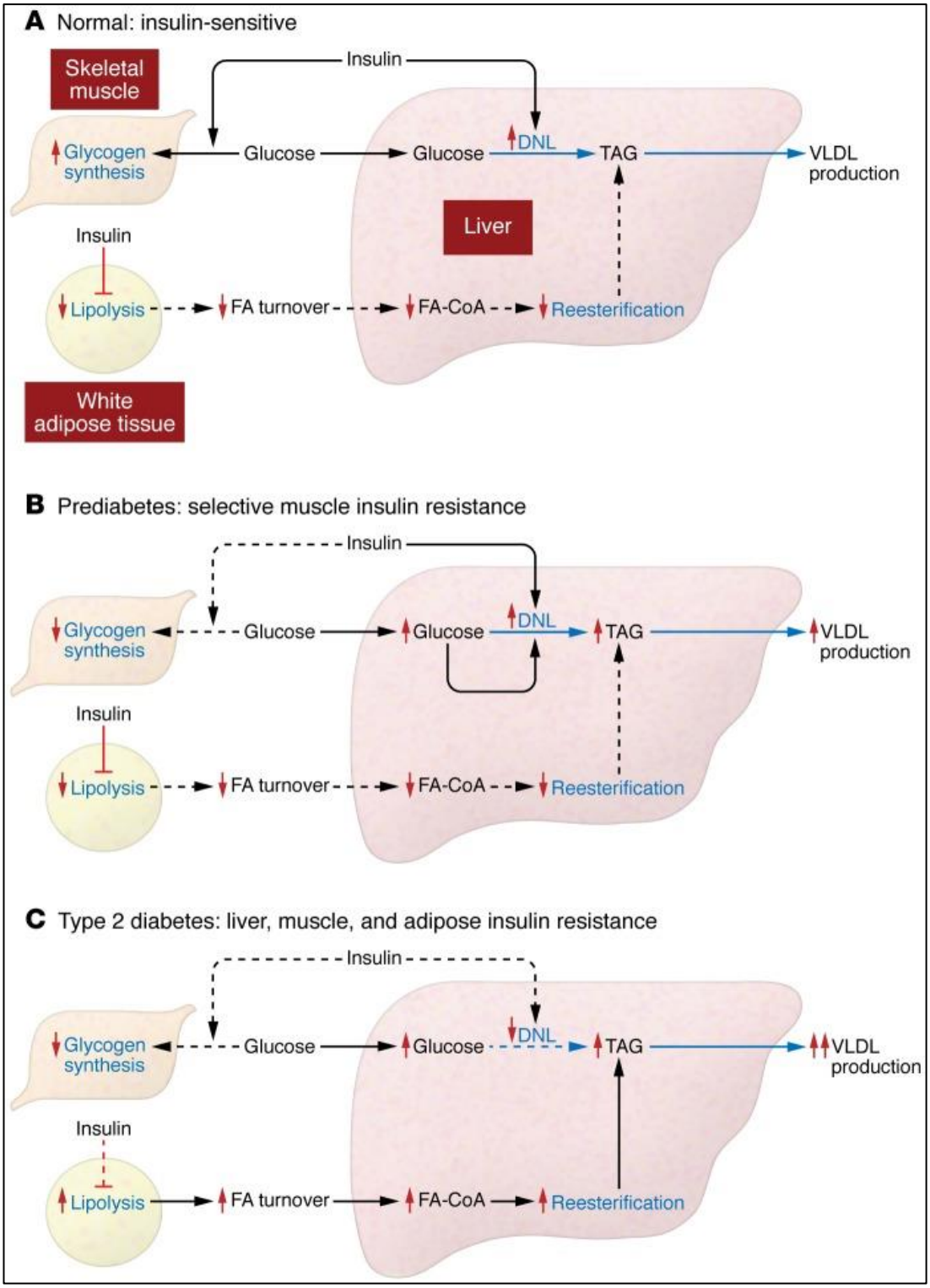


Figure 1.3 Schematic of A) normal insulin action, B) prediabetic insulin action and C) insulin action in type 2 diabetes (Samuel and Shulman, 2016).

1.7 Metabolic effects of exercise

It is well established that exercise improves insulin sensitivity (Roberts et al., 2013, Bird and Hawley, 2016). It does this primarily by contraction-induced skeletal muscle glucose uptake (Richter and Hargreaves, 2013). In addition to its mechanical role (locomotion), skeletal muscle accounts for up to 75% of blood glucose uptake in the post-prandial state. During exercise the increased contraction-stimulated glucose uptake is linked to increases in AMP-activated protein kinase (AMPK), which results in the glucose transporter type 4 (GLUT4) translocation and ultimately glucose uptake (Cartee, 2015, Sakamoto and Holman, 2008). Exercise training as been shown to improve GLUT4 concentrations in individuals with MetS and T2D (Stuart et al., 2013). Improvements appear to be tissue specific, in skeletal muscle but not hepatic IR, nor insulin-stimulated glucose uptake in adipose tissue (Malin et al., 2013, Reichkendler et al., 2013).

Exercise can also have a profound effect on the distribution and metabolic fate of an ingested meal. As such, reversal of skeletal muscle IR, with exercise, has been shown to reduce post-prandial hepatic DNL. In young, lean, but insulin resistant individuals, a single bout of exercise diverted ingested carbohydrate away from the liver and into the muscle, thereby reducing hepatic DNL and hepatic triglyceride synthesis (Rabol et al., 2011). Further, 6 weeks of exercise training has been shown to increase glucose transport-phosphorylation and muscle glycogen synthesis in insulin resistant subjects (Perseghin et al., 1996).

1.8 Mechanisms of habitual activity versus physical inactivity

A consequence of physical inactivity is a lack of AMPK activation and glucose uptake from skeletal muscle (Figure 1.4) this triggers insulin resistance and provides a substrate for DNL in adipose tissue and liver. Consequently, there is expansion of adipose tissue mass, an increase in FFA flux and serum FFA, intra-hepatic lipid accumulation and increased lipid exports as VLDL triglyceride particles and serum TG with systemic insulin resistance. On the contrary, being habitually active promotes AMPK activation and uptake of glucose in skeletal muscle; insulin sensitivity is therefore preserved and less glucose is diverted to metabolically unfavourable depots.

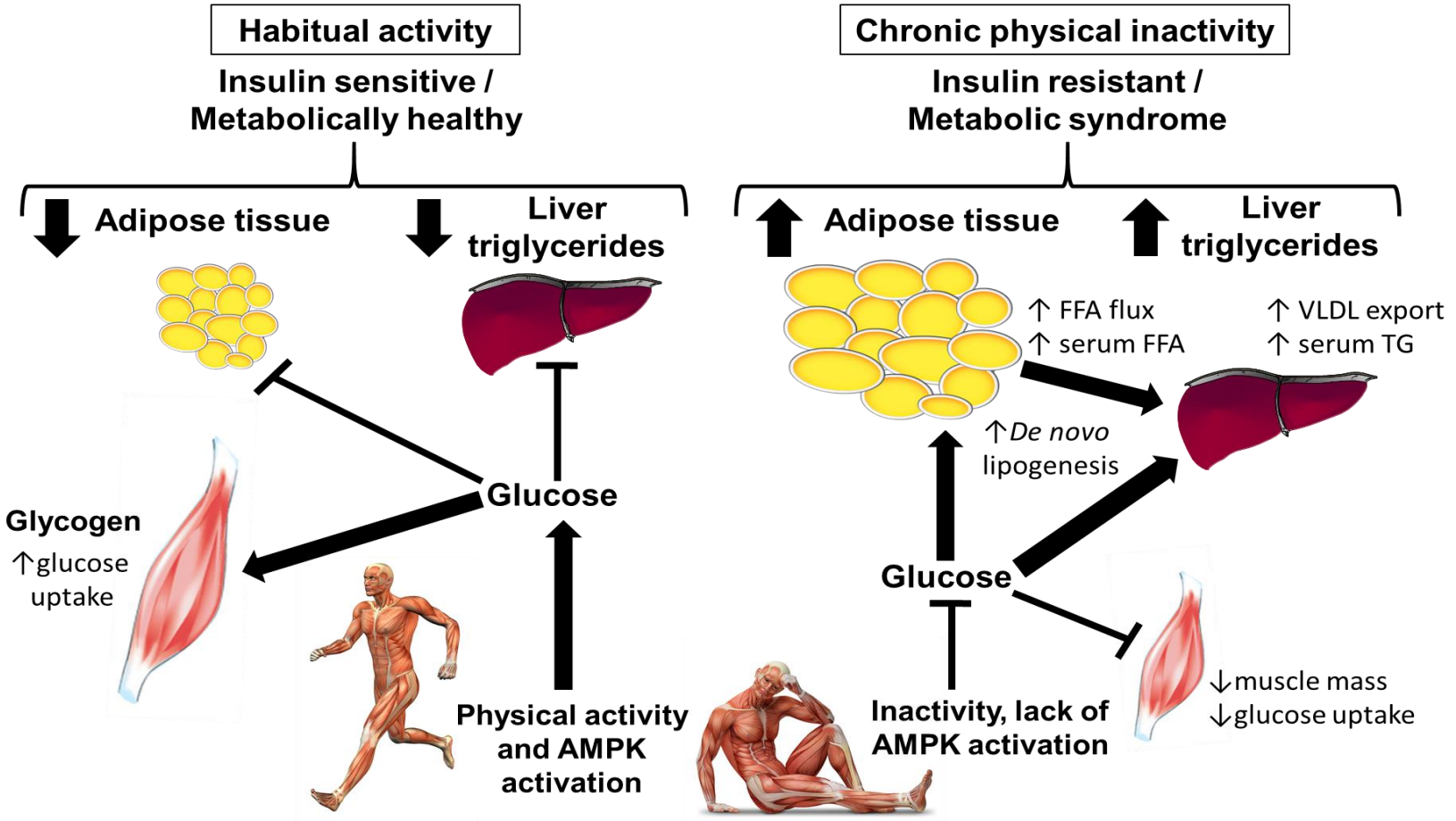


Figure 1.4 Schematic of proposed mechanisms of habitual activity versus physical inactivity. FFA, free fatty acids; VLDL, very low density lipoproteins; TG, triglycerides; AMPK, AMP-activated protein kinase.

1.9 Adipose tissue expandability hypothesis

It is important to recognise the '*adipose tissue expandability hypothesis*' to explain the accumulation of ectopic fat. It is suggested that to accommodate increased lipid supply in a state of positive energy balance, SAT must undergo expansion to avoid deposition of lipid in non-adipocyte cells (Rutkowski et al., 2015). The expansion of SAT can occur by either hypertrophy or hyperplasia but once maximal SAT expansion is reached, widespread organ specific ectopic fat deposition (steatosis) occurs (e.g. liver, pancreas) with secondary functional consequences (lipotoxicity) (e.g. hepatic insulin resistance, impaired β-cell function) (Gray and Vidal-Puig, 2007).

1.10 Personal fat threshold

A '*personal fat threshold*' (PFT) has been proposed (Taylor and Holman, 2015) which may explain the large inter-variation in ones SAT expansion capacity. The PFT is a concept of explaining the onset of T2D in non-obese individuals. All individuals have a PFT, i.e. a different weight/BMI at which SAT expansion has reached its capacity. Once further weight gain occurs, metabolic decompensation ensues. Metabolically unhealthy non-obese may have a low PFT whereas metabolically healthy obese may have a high PFT (Figure 1.5).

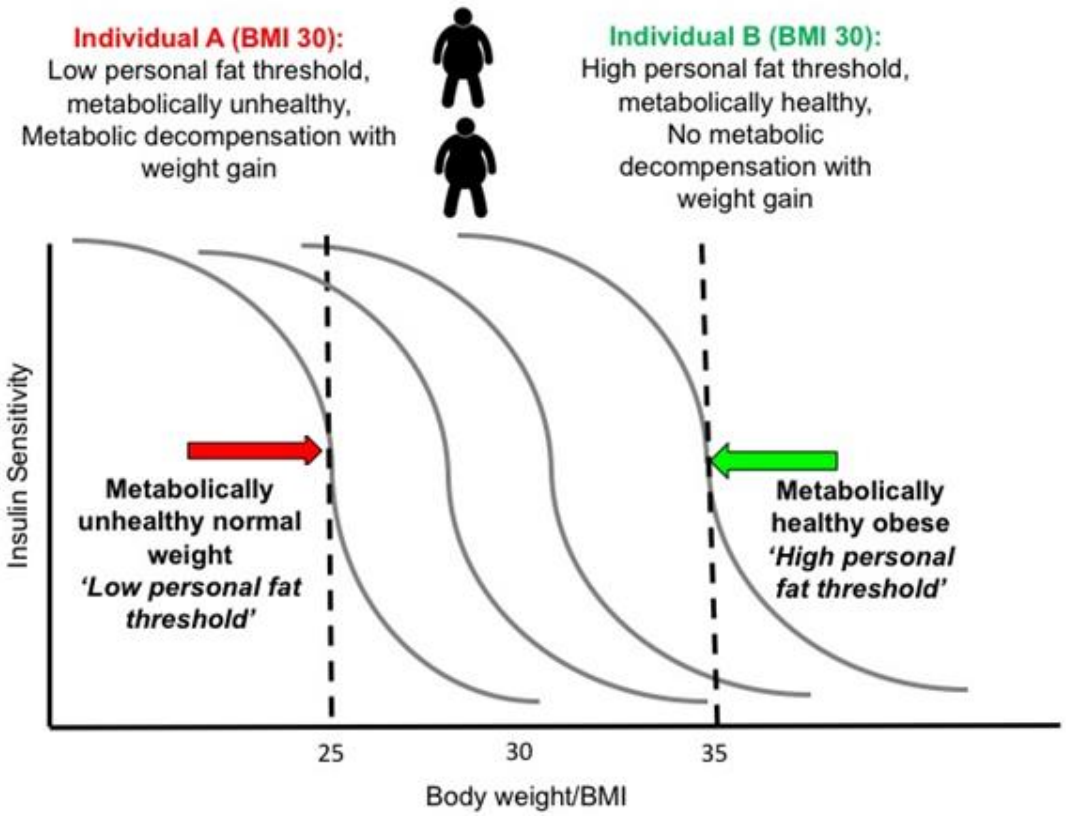


Figure 1.5 The '*personal fat threshold*' adapted to demonstrate healthy and unhealthy metabolic phenotypes (Taylor and Holman, 2015).

1.11 Non-alcoholic fatty liver disease (NAFLD)

The liver has an integral role in the regulation of glucose and lipid regulation. Consequently, dysregulated metabolism within the liver has significant implications for metabolic disease. Closely linked with the development of T2D, it is recognised that hepatic steatosis (the initial stage of NAFLD) should be targeted early to prevent its progression and associated complications. NAFLD represents a spectrum of liver disease ranging from isolated fatty liver to progressive non-alcoholic steatohepatitis (NASH), hepatic fibrosis, and cirrhosis. If cirrhosis develops ~33% of patients will develop morbid conditions or die a liver related death (Hui et al., 2003). Hepatic fat accumulation ≥5% in the absence of excess alcohol consumption (>20 g/day) is classified as NAFLD (Browning et al., 2004). It is reported that 80% of T2D patients

have NAFLD (Targher et al., 2007). NAFLD has been described as a major factor in the outcome and contribution to T2D and is considered the hepatic manifestation of MetS closely linked with obesity and IR (Rector et al., 2008). NAFLD is now the most common liver disease in both adults and children, and hepatic insulin resistance is strongly linked to NAFLD, which is a major factor responsible for the transition from normoglycemia to impaired glucose tolerance and T2D.

1.12 Type 2 diabetes (T2D)

The development of T2D is strongly linked with the aforementioned: obesity, MetS, IR and liver fat (Zaccardi et al., 2016); however non-obese individuals can also develop the disease (Vaag and Lund, 2007). T2D is a complex metabolic disorder, characterised by progressive defects in insulin secretion and action in which hyperglycemia develops (Inzucchi, 2012). The earliest indicator of T2D risk is skeletal muscle IR (Petersen et al., 2007) however this solely does not cause hyperglycemia and does not dictate pathogenesis (Taylor, 2017). T2D occurs in the presence of chronic positive energy balance in which the '*twin cycle hypothesis*' (Figure 1.6) causes a permissive environment for the development of the disease (Taylor, 2008). This hypothesis unifies the vicious cycles of liver glucose production ('*liver cycle'*) and β-cell function ('*pancreas cycle*'). β-cell dysfunction is the final and determining factor of T2D onset.

In positive energy balance, excess carbohydrate must undergo DNL, promoting the accretion of liver fat (Petersen et al., 2007). Pre-existing peripheral IR causes hyperinsulinemia which can exacerbate liver fat due to the additional requirement of insulin for DNL. The accretion of liver fat is associated with defects in suppression of glucose production (Seppala-Lindroos et al., 2002). The liver becomes less sensitive

to insulin, plasma glucose rises and basal insulin secretion is greater. With accumulation of liver fat and elevated plasma glucose, overproduction of VLDL triglyceride occurs (Adiels et al., 2006) which increases triglyceride delivery to all tissues including the pancreatic islets. Excess triglyceride in the islets will impair the acute insulin secretion in response to ingested food and cause post-prandial hyperglycemia. Eventually the inhibitory effects of triglyceride and glucose on the islets reach a level that T2D prevails.

Post-absorptive plasma glucose concentration is maintained by the liver between a narrow range in healthy individuals whereby the basal rate of glucose uptake is matched by the rate of endogenous glucose production. In T2D, hepatic insulin resistance causes poor control of glucose production which is believed to exacerbate hyperglycemia (DeFronzo et al., 1992). The post-prandial regulation of glucose depends on the suppression of endogenous glucose production and increased glucose uptake with stimulation of glucose oxidation. Hyperglycemia following ingestion of a meal is a hallmark feature of T2D (DeFronzo et al., 1982). In comparison to healthy controls, T2D patients are unable to maintain suppression of glucose production after a mixed meal (Singhal et al., 2002).

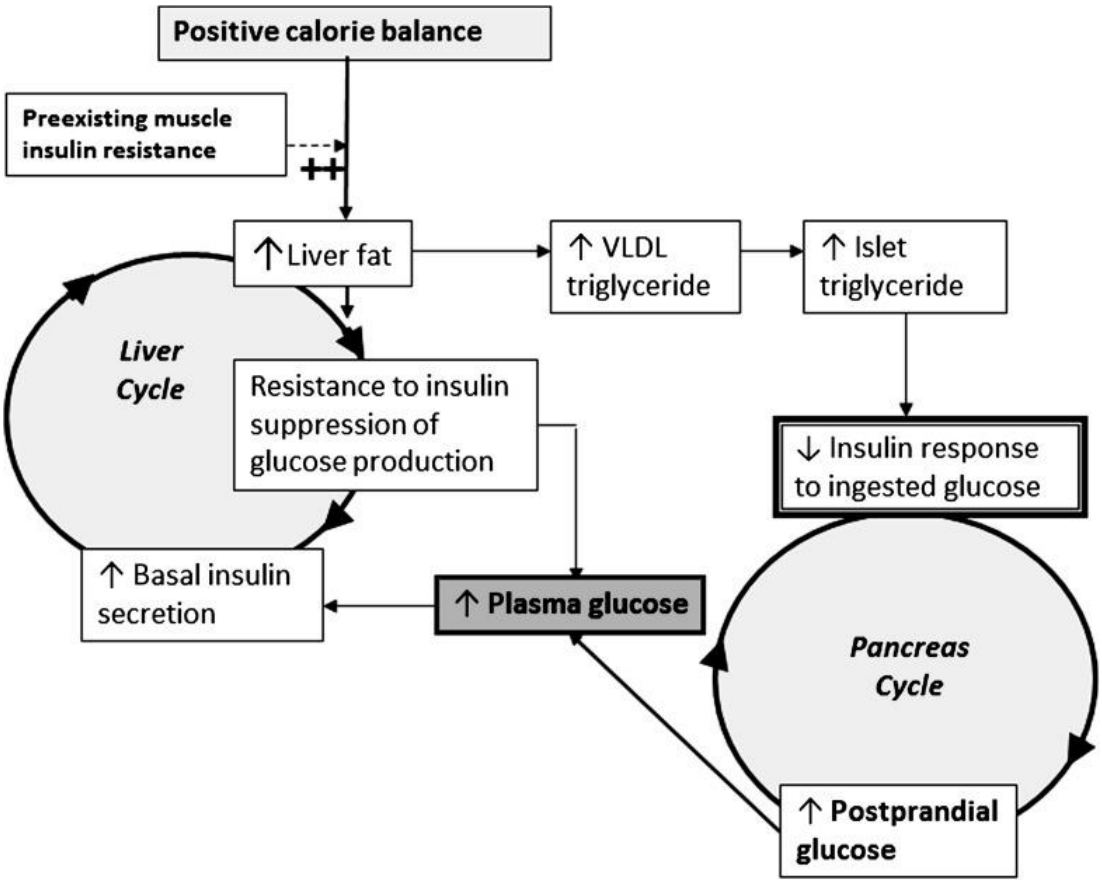


Figure 1.6 The '*twin cycle hypothesis*' of the etiology of type 2 diabetes (T2D) (Taylor, 2013).

1.12.1 Multiple pathophysiological failures that contribute to hyperglycaemia

β-cell dysfunction is the final denominator in T2D. The *'egregious eleven'* is a classification schema outlining the pathways known to mediate hyperglycemia (Schwartz et al., 2016). It is important to note that whilst this thesis focuses towards insulin resistance (liver, skeletal muscle, adipose tissue) there are an array of other mechanisms associated with the development of T2D. Other mechanisms include the brain, colon/biome, and immune dysregulation/inflammation and other β-cell dysfunctions through downstream effects, reduced insulin, decreased incretin effect, α-cell defect, stomach/small intestine via reduced amylin, and kidney (Figure 1.7).

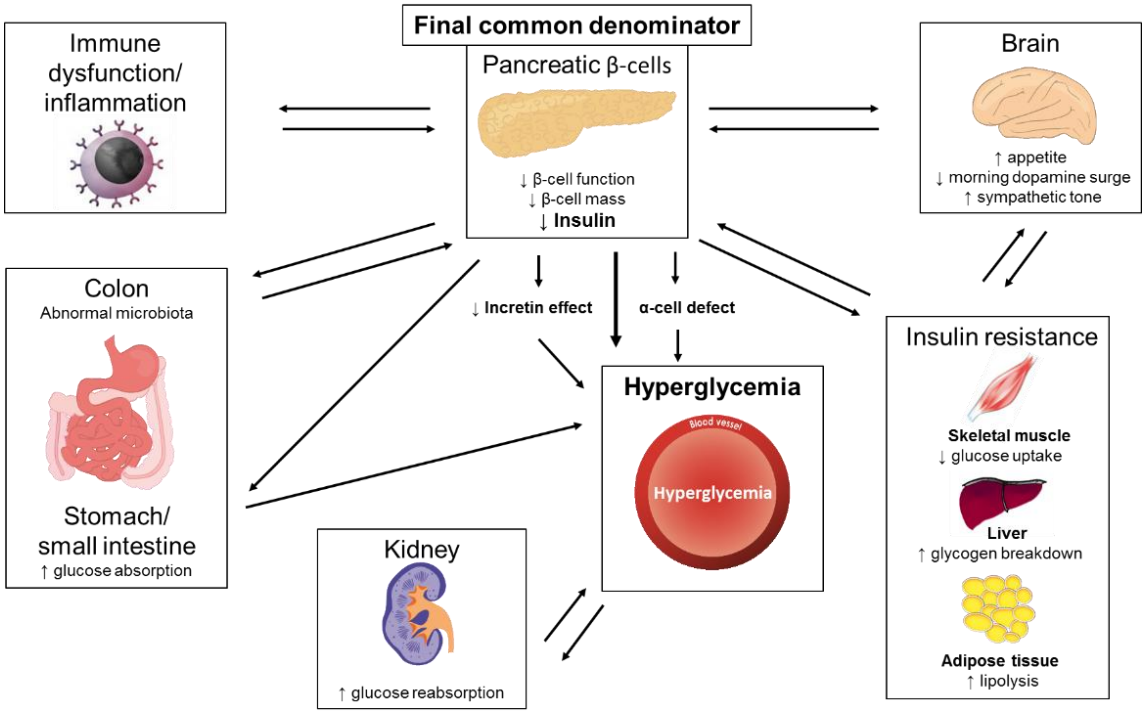


Figure 1.7 Multiple pathophysiological failures contribute to hyperglycaemia.

1.12.2 Familial predisposition for T2D

First-degree relatives (FDR) of individuals diagnosed with T2D are at a 3-fold increased risk of developing the disease themselves (Meigs et al., 2000). These individuals display IR which is compensated by β-cell hyperinsulinemia, reduced basal energy consumption and cardiometabolic abnormalities including lipid disorders and high blood pressure long before the disease onset (Groop et al., 1997). In these individuals, IR is the best predictor of the development of diabetes and plays an important part in its pathogenesis (Perseghin et al., 1996). The genetic predisposition for T2D is irrefutable (Almgren et al., 2011) but non-genetic factors also play a prominent role in whether an individual progresses to overt T2D (Poulsen et al., 1999). Non-genetic factors include pre/postnatal environment, energy intake and energy expenditure, the review herein focusses on the later.

1.13 Energy expenditure

Obesity-related disease and T2D occurs in a state of positive energy balance. Energy balance can be altered either by energy intake (nutrient consumption) and energy expenditure. Energy expenditure can be influenced in a number of ways. *Basal metabolic rate* (BMR), which is the amount of energy needed to keep the body functioning at rest, is the largest component of energy expenditure. BMR is predicted by intrinsic factors such as body composition, age, sex, and ethnicity (Weyer et al., 1999). *Dietary induced thermogenesis* (DIT), is the amount of energy needed to process food for use and storage. DIT is influenced by the energy intake, macronutrient composition, and eating pattern of the meal (Quatela et al., 2016) and constitutes to 5 to 15 % of daily energy expenditure (Westerterp, 2004). *Activity thermogenesis*, which can vary from about 15% for inactive to 50% for active individuals (Donahoo et al., 2004), is the amount of energy expended through PA. This component can be sub-divided into *exercise* activity thermogenesis and *non-exercise* activity thermogenesis (NEAT). NEAT incorporates general, everyday activity.

1.14 Cardiorespiratory fitness

The independent protective effect of high CRF against all-cause mortality has been long established (Blair et al., 1989, Ekelund et al., 1988). Obesity (discussed above) and low CRF are considered to be central mechanisms in the development of T2D. CRF is an objective marker of PA level (Garber et al., 2011). The preventive role of PA in the development of T2D (Lee et al., 2009) can be explained through epigenetic modification that may improve that may improve glucose homeostasis (Barres et al., 2012, Lindholm et al., 2014). A higher CRF at baseline is associated with lower risk of T2D during follow-up (Aune et al., 2015) and maintaining a high CRF level over time is beneficial (Momma et al., 2017). Of note, is the '*fitness versus fatness*' debate.

High CRF has a been demonstrated to have a stronger protective effect on diabetes in obese than in normal weight men (Holtermann et al., 2017) and a '*fit-fat index*' has been proposed as a better indication of incident risk when compared to fitness or fatness alone (Sloan et al., 2016). Recent studies have suggested that recommendations to improve CRF should move beyond weight change (i.e. emphasise PA) (Dollar et al., 2017). CRF was positively associated with increased β-cell function independent of fatness in individuals with MetS (Ramos et al., 2017). No weight loss but increased CRF and increased cardiovascular function has also been shown in patient groups (Sprung et al., 2013). There is no doubt that high CRF has therapeutic effects in the prevention the cardiometabolic complications related to T2D; and the growing body of evidence suggests this can be independent of body weight (Barry et al., 2014, Pedersen, 2007, Fogelholm, 2010).

1.15 Physical activity and health

The value of PA for health is not disputed. Research conducted by Jeremy Morris and colleagues pioneered this field (Morris et al., 1953). Their seminal study conducted in the 1950's compared the risk of coronary heart disease (CHD) in bus drivers who were physically inactive during their working day (i.e. driving) versus bus conductors who were physically active (i.e. walking up and down the bus). The investigators concluded that employees in positions that required high physical activity, i.e. the bus conductors, had lower rates of CHD. Since then, the relationship between *physical inactivity* and major non-communicable diseases has been well evidenced (Lee et al., 2012). Despite robust evidence and evolving public health messages, physical inactivity continues to emerge as a major, and indeed growing, worldwide health threat. It is estimated in the UK that 50% of women, and a third of men are damaging their health through physical inactivity. This not only has consequences for health, it also places a substantial

financial strain on health services. In 2006/07, the estimated cost of physical inactivity in the NHS was £0.9 billion (Scarborough et al., 2011).

1.16 Definitions of exercise, physical (in)activity and sedentary behaviour

Bodily movement covers a broad spectrum, from sleep, sitting down, standing up, low level physical activity to moderate and vigorous physical activity. Figure 1.8 contextualises the key terms relating to activity that will be used throughout this thesis. There have been many studies outlining the health benefits of regular *'exercise'*, focusing on high-energy expenditure (moderate-vigorous activity) and there is a well-established field of 'exercise physiology'. Exercise is deliberate movement intended to improve or maintain physical fitness, for example playing sports or going to the gymnasium. *'Physical activity'* (PA), on the other hand, is any bodily movement that requires more energy than resting, for example, getting ready for work, carrying groceries or walking to the bus stop. Performing less than 30 minutes of physical activity a week is classified as inactive. More recently, there has been emerging data on *'inactivity physiology'* with the recognition that the whole body, tissue-specific and cellular consequences of inactivity do not necessarily represent opposites to those that occur with exercise or high levels of physical activity. Studies of physical inactivity, focus on the physiological impact of a lack of human movement.

'*Sedentary behaviour*', (loosely defined as lying, reclining or sitting) is classified as ≤1.5 metabolic equivalents (METS) is a broad term were specific investigations regarding daily sitting, television viewing and screen based time have been carried out. It is important to note that sedentary behaviour is not simply a lack of physical activity, an individual can be physically active as well as sedentary especially in modern society where changes in technology, occupation, transportation and dietary availability have created a permissive environment for reduced PA and/or sedentary behaviour. Daily

sedentary time appears to exert deleterious effects on metabolism even in individuals who meet current intensity-based physical activity guidelines (Hamilton et al., 2014). The importance of considering the entire waking day, and active and inactive behaviours within it have been noted (Hamilton et al., 2007). This includes understanding how low intensity physical activity can replace large amounts of sedentary time.

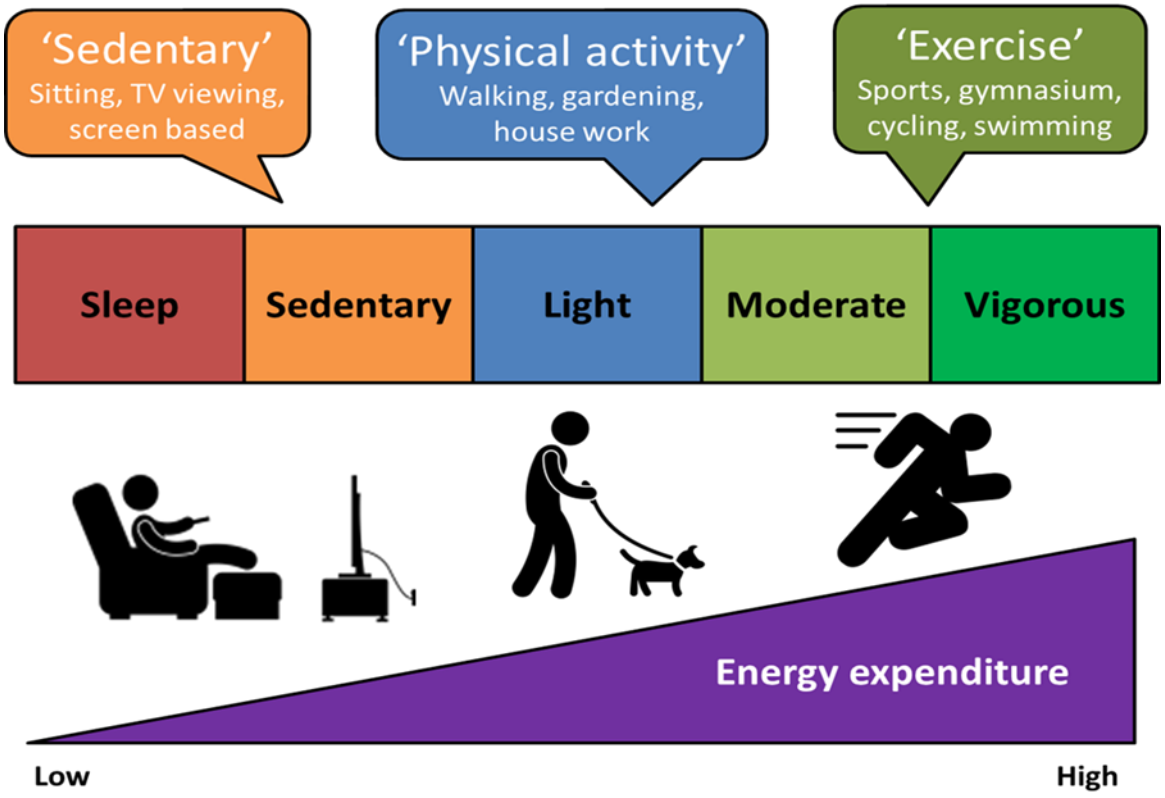


Figure 1.8 A spectrum of bodily movement to contextualise key terms; 'sedentary' behaviour at low level energy expenditure, 'physical activity' being intermediate, and 'exercise' being moderate and vigorous activity with higher energy expenditure.

1.17 Why move away from exercise research?

Regular exercise has beneficial effects on obesity, MetS, T2D, NAFLD, CVD, some cancers and overall mortality. Historically, public health activity guidelines have emphasised the importance of exercise, with 150 minutes of moderate-vigorous physical activity (MVPA) recommended per week. However, statistics reveal that a quarter of the people in the UK are failing to achieve a minimum of 30 minutes of activity per week (PHE, 2014). While the benefits of exercise are well established, low physical activity is an independent risk factor for ill-health often not considered. Relative to the substantial body of work examining the acute and chronic effects of exercise, relatively little is known about physical inactivity. Western society has changed drastically, there is evidence for a reduction in occupational energy expenditure as well as greater time spent sitting daily (Church et al., 2011, Althoff et al., 2017). The development of alternative PA strategies are challenging given that sedentary time has become a monumental part of life in the modern world. The interrelated epidemics of obesity, T2D and physical inactivity are projected to continue growing, thus, effective PA guidelines that are achievable and attainable at a population level are vital. A simple behaviour approach could be key.

1.18 Habitual physical activity

Shifting to the emphasis of PA rather than exercise, *per se,* will target the whole waking day as opposed to 30 minutes, 5 times a week. Assessment of habitual PA indicates strong links between physical inactivity and obesity in cross-sectional (Myers et al., 2016) and follow up studies (Shook et al., 2015). Importantly, even subtle changes to habitual PA can have a positive effect on health. A recent prospective cohort study evidenced an association between active commuting and incident cardiovascular disease, cancer, and mortality (Celis-Morales et al., 2017) and stair

climbing which requires no specialist equipment can improve CRF (Allison et al., 2017).

1.19 Sedentary behaviour

Sedentary time has been associated with negative health outcomes, independent of PA (Biswas et al., 2015) and MVPA (Henson et al., 2013). Further it has been associated with liver and visceral fat in individuals at a high risk of T2D (Henson et al., 2015) and normal weight healthy adults (Smith et al., 2014). Replacing sedentary time with low intensity PA can improve lipid and glucose metabolism for the prevention of T2D (Hamilton et al., 2014). Breaking up prolonged periods of sitting (e.g. standing or walking) has been shown to be beneficial in glucose metabolism in women at high risk of T2D (Henson et al., 2016) and more recently upper limb activity had positive glycaemic effects despite maintaining a seated posture (McCarthy et al., 2017).

1.20 Experimental models of physical inactivity

There is an emerging interest in studies of reduced human movement; from which drastic research has been carried out investigating the effects of bed rest (Alibegovic et al., 2010), limb immobilisation (Abadi et al., 2009) and cessation of exercise in trained volunteers (King et al., 1988). Whilst these studies are informative, they represent extreme models of physical inactivity and translations to consequences in a free-living environment are somewhat limited. More recent investigators have employed a model of reduced ambulatory activity, a step-reduction protocol whereby individuals have been transitioned from high levels of physical activity (~10,000 steps/day) to low levels of physical activity (~1,500 steps/day) for a period of 14 days. The consequences of this short-term reduced physical activity are somewhat striking, especially given the volunteers were young healthy males (Krogh-Madsen et al., 2010,

Olsen et al., 2008). Such changes have included reduced CRF, accumulation of central fat, loss of skeletal muscle mass and reductions in peripheral insulin sensitivity (Table 1.3). One of the studies also induced overfeeding (Knudsen et al., 2012) and was the only investigation to re-assess their participants after resumption of normal activity; CRF and fasting blood measures returned back to normal but changes in body composition were not fully reversed. The effects of physical inactivity has also been investigated in older adults where a reduction in skeletal muscle mass was associated with anabolic resistance (Breen et al., 2013). None of these previous studies have objectively measured sedentary behaviour.

1.21 Physical inactivity and T2D

Physical inactivity contributes to a positive energy balance and as such obesity, which are key players in the development of IR known to exacerbate the progression of T2D. One plausible paradigm suggests that physical inactivity causes a reduction in skeletal muscle insulin sensitivity, contributing to a repartitioning of energy substrates into storage, increasing central fat accumulation and ectopic storage within the liver and other organs, causing further IR (Jornayvaz et al., 2010, Rabol et al., 2011, Rector and Thyfault, 2011, Petersen et al., 2007, DeFronzo and Tripathy, 2009). As peripheral IR progresses, continued ectopic fat accumulation within the liver and pancreas precipitates development of metabolic syndrome, a progressive decline in ß-cell function and ultimately T2D (Tan and Vidal-Puig, 2008). An inactive lifestyle further increases the risk of developing T2D in FDR compared to those without (Hu et al., 1999). Bed rest studies have compared FDR and healthy controls (Alibegovic et al., 2009, Hojbjerre et al., 2011) but no previous studies have investigated the consequences of free-living short-term physical inactivity in those with a genetic susceptibility of developing T2D.

Table 1.3 Summary of human studies inducing an 'inactivity protocol' as an experimental model of reduced physical activity.

Reference	**Participant cohort**	**Inactivity protocol**	**Cardiorespiratory**	**Body composition**	**Metabolic**
(Olsen et al., 2008) Substudy 1	**Age** 27.1 ± 5.7 y **BMI** 22.9 ± 4.0 kg/m^2 **Gender** 8 males **Baseline activity** >3,500 steps/day; <2 hr exercise/week **Screening period** 7 day	**Step count** (pedometer) **Dietary records** **21 day step-reduction** Day 0: 6203 steps/day (95% CI, 5135 to 7271) Day 7: 1394 steps/day (95% CI, 1261 to 1528)	Not measured	Not measured	**3 hr oral glucose (75 g) tolerance test** ↑ insulin AUC from 757 pmol/L/3h (95% CI, 488 to 1026) at day 0 to 1352 pmol/L/3h (95% CI, 1025 to 1678) at day 21
(Olsen et al., 2008) Substudy 2	**Age** 23.8 ± 4.6 y **BMI** 22.1 ± 2.1 kg/m^2 **Gender** 10 males **Baseline activity** >3,500 steps/day; <2 hr exercise/week **Screening period** 7 day	**Step count** (pedometer) **Dietary records** **14 day step-reduction** Day 0: 10501 steps/day (95% CI, 8755 to 12247) Day 14: 1344 steps/day (95% CI, 1272 to 1416)	Not measured	**MRI** ↑ 7% intra-abdominal fat mass from 693 mL (95% CI, 485 to 902 mL) at day 0 to 740 mL (95% CI, 552 to 929 mL) at day 14 **DXA** ↔ fat mass ↓ 2 % total fat-free mass ↓ BMI	**3 hr oral glucose (75 g) tolerance test** ↑ insulin AUC from 599 pmol/L/3h (95% CI, 489 to 709) at day 0 to 942 pmol/L/3h (95% CI, 443 to 1440) at day 14 ↑ C-peptide AUC **8 hr oral fat (2 ml/kg) tolerance test** ↑ insulin AUC ↑ triglyceride AUC

(Krogh-Madsen et al., 2010)	**Age** 23.8 ± 1.5 y **BMI** 22.1± 0.7 kg/m^2 **Gender** 10 males **Baseline activity** >3,500 steps; <2 hr exercise/week, **Screening period** 7 day	**Step count** (pedometer) **Physical activity** (Actiheart) **Dietary records** **14 day step-reduction** Day 0: 10501 step/day (95% CI, 8755 to 12247) Day 14: 1344 (95% CI, 1272 to 1416) steps/day	**$\dot{V}O_2$ max (cycle ergometry)** ↓7.2% ml.min ↓6.6% ml.min.kg	**DXA** ↓ 2.8% leg lean mass ↓ 1.7% total body mass ↔ arm lean mass ↔ trunk lean mass	**Hyperinsulinaemic-euglycaemic clamp** ↓ peripheral insulin sensitivity (17% reduction in glucose infusion rate, GIR) ↔ endogenous hepatic glucose production
(Knudsen et al., 2012)	**Age** 24 ± 3.3 y **BMI** 21.6 ± 2.5 kg/m^2 **Gender** 9 males **Baseline activity** >3,500 steps; <2 hr exercise/week, **Screening period** 4 day	**Step count** (pedometer) **Physical activity** (Actiheart) **Dietary records** **14 day step-reduction** Day 0: 10278 step/day (SE 715) Day 14: 1521 steps/day (SE 131) **plus overfeeding**	**$\dot{V}O_2$ max (cycle ergometry)** ↓3.8% ml.min and ↓3.4% ml.min.kg	**MRI** ↑ 49% visceral fat **DXA** ↔ total fat-free mass ↑ total body mass, BMI**,** whole body fat, android and gynoid fat	**Hyperinsulinaemic-euglycaemic clamp** ↓ peripheral insulin sensitivity ↔ endogenous hepatic glucose production **3 hr oral glucose (75 g) tolerance test** ↑ insulin AUC ↔ glucose AUC ↓ 26% Matsuda index

1.22 Aims and objectives

From the representative literature discussed within this review, it is clear that physical inactivity and obesity can contribute to the development of IR and ultimately T2D. It is recognised that not all obese individuals are at risk of developing T2D, understanding the potential role of habitual PA in these individuals may reveal novel approaches to guidelines. Furthermore, accumulation of liver fat, which occurs only in a state of positive energy balance, is pivotal in the development of T2D; physical inactivity and sedentary behaviour may contribute significantly to this. The effects of short-term physical inactivity have been studied however the consequences in those with a genetic susceptibility (FDR) for T2D have not been investigated. This research has potential implications for the generation and implementation of PA recommendations.

1. To explore whether differences in habitual PA and sedentary behaviour explains the metabolic health status of individuals that are obese and non-obese
2. To use objective monitoring of PA and sedentary behaviour to investigate relationships between domains of physical activity and liver fat
3. To determine the effects of short-term physical inactivity in first-degree relatives of patients with T2D compared with healthy controls.

Chapter 2.

General methodology

This chapter outlines the general methods undertaken to meet the aims and objectives of this thesis. Specific methodology for each study can be found within the respective method sections for each data chapter. Unless explicitly noted, these procedures and analyses were conducted by myself (KBD).

2.1 Participants

All participants studied within this thesis were free from any disease. The cohort were from varying socio-economic backgrounds either living, working and/or studying in Merseyside and Greater Manchester (United Kingdom, UK). The demographics are somewhat representative of the adult UK population including males and females aged 18-60 years, as well variable education, nationality, religion, and ethnicity.

2.2 Recruitment

Participants were recruited following their response to local adverts (METRO and ECHO newspapers and Radio Merseyside station) as well as internal adverts (poster, email, online intranet) within the University of Liverpool and University Hospital Aintree. 'Word of mouth' also generated a significant response. Patients with hyperlipidaemia and suspected fatty liver were prospectively recruited from specialist lipid and hepatology outpatient clinics at University Hospital Aintree. First-degree relatives of type 2 diabetes mellitus (T2D) were targeted via specific meetings and clinics including; Knowsley Community Diabetes Service, University Hospital Aintree Diabetes Centre, Diabetes UK group meetings and Help BEAT Diabetes campaign.

2.3 Screening

All participants were screened by questionnaire to ensure they were non-smokers and consumed <14 units of alcohol per week, females could not be pregnant. Individuals

who had any cardiovascular, respiratory, kidney, liver and/or endocrine diseases were excluded. Safety to undergo magnetic resonance (MR) scanning was determined by experienced personnel at Liverpool Magnetic Resonance Imaging Centre (LiMRIC). MR exclusion criteria included; cardiac pace-maker, metal implants or metallic cerebral aneurysm clips, non-removable metal jewellery, body weight >140 kg and those who experience claustrophobia. Physical Activity Readiness Questionnaire (PARQ) was used to assess suitability for maximal exercise testing, those with contraindications were excluded. In studies including dual-energy x-ray absorptiometry (DXA), a 'justification of radiation exposure' was conducted by Dr Cuthbertson. DXA exclusion criteria included; body weight >200 kg, medical radiation exposure in last 7 days, non-removable metal jewellery and internal metal or plastic.

2.4 Ethics and consent

The studies included within this thesis conformed to the *Declaration of Helsinki* and were approved by the University of Liverpool Research Office, the Local Research Ethics Committee (LREC) at Liverpool Central and University Hospital Aintree (Research and Development). All participants were informed of the methods in writing and verbally before providing written informed consent.

2.5 First-degree relative (FDR) of T2D

Participants with a first-degree relative (FDR) of T2D are individuals with a parent, sibling or offspring medically diagnosed with the disease. This was verified verbally during consent.

2.6 Metabolic syndrome (MetS)

In chapter 3 individuals are categorised as 'healthy' and 'unhealthy'. Those who are unhealthy have 3 or more components of metabolic syndrome (MetS+), those who are healthy have two or less (MetS-) (Table 2.1).

Table 2.1 Criteria for metabolic syndrome (MetS).

	Metabolic syndrome Three or more of the following:
Waist circumference*	Population specific
Blood pressure	≥130/85 mmHg or medicated
Fasting glucose	≥5.6 mmol/l
Triglycerides	≥1.7 mmol/l or medicated
High density lipoprotein; HDL	M: <1.0 mmol/l, F: <1.3 mmol/l

* If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured; see IDF criteria for population specific values (Alberti et al., 2009).

2.7 Overview of assessment measures

Figure 2.1 displays an overview of assessment measures, dashed lines indicate measures not included in all studies; skeletal muscle (SkM) and subcutaneous adipose tissue (SAT) biopsies were optional. Following measures of anthropometry and 4 days of habitual assessment (which included physical activity monitoring and dietary record), participants attended two assessment visits. Assessment visit A took place at University Hospital Aintree including: fasting bloods, 2 hr oral glucose tolerance test (OGTT) and assessment of cardiorespiratory fitness ($\dot{V}O_2$). Assessment visit B took place at University of Liverpool campus including: vascular measures (carotid intima-media thickness, cIMT; flow-mediated dilation, FMD), MR scanning (magnetic resonance imaging, MRI; magnetic resonance spectroscopy, MRS) and DXA. Specific details of each measure are outlined below. Participants were instructed to fast overnight for ≥8 hours, abstain from alcohol or caffeine for 24 hours and refrain from exercise for 48 hours prior to assessment visits. All visits were standardised and took place in the morning to account for circadian variation.

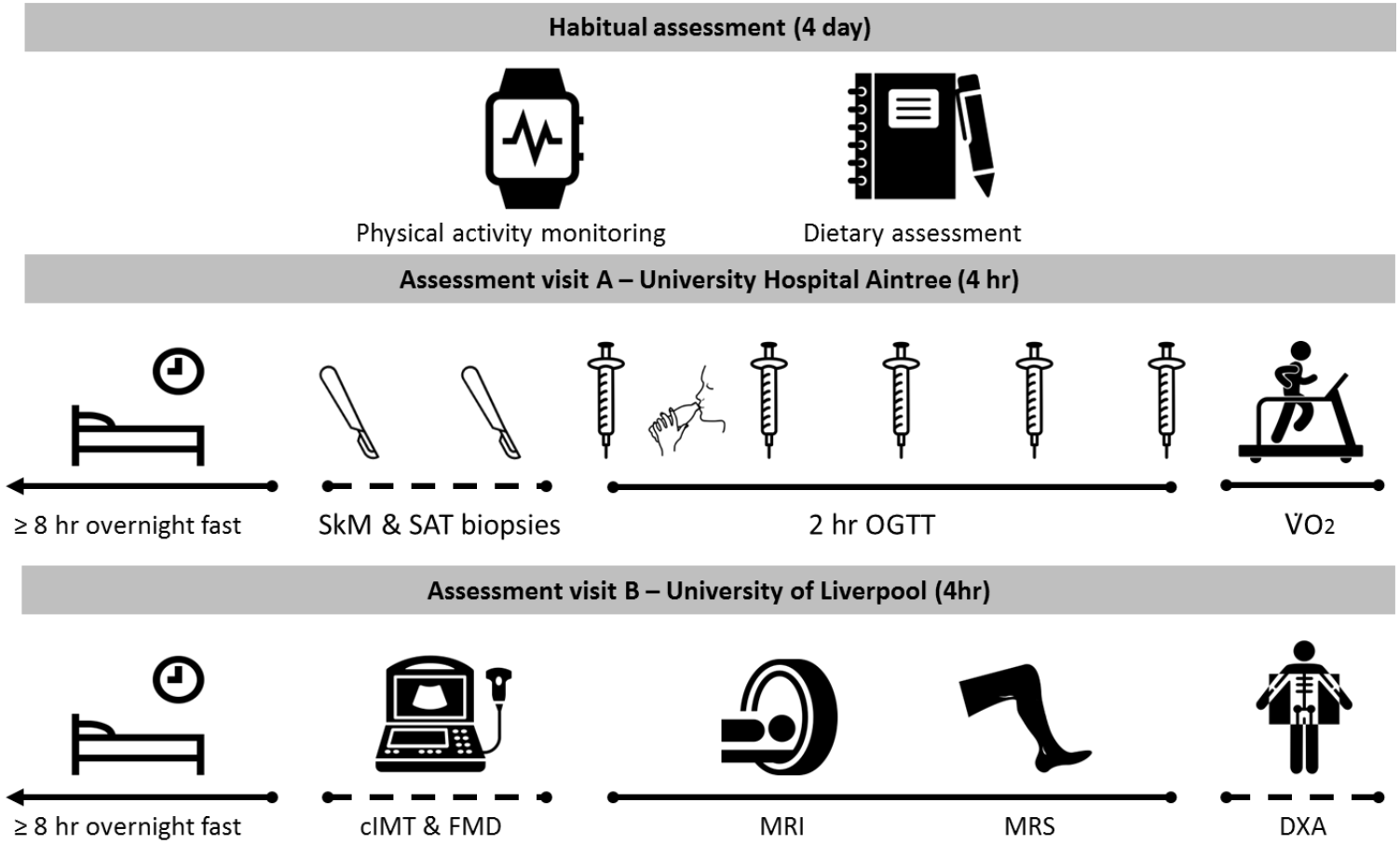


Figure 2.1 Overview of assessment measures. SkM, skeletal muscle; SAT, subcutaneous adipose tissue; OGTT, oral glucose tolerance test; $\dot{V}O_2$, cardiorespiratory assessment; cIMT, carotid intima-media thickness; FMD, flow-mediated dilation; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; DXA, dual-energy x-ray absorptiometry. Dashed lines indicate measures not included in all studies.

2.8 Anthropometric measurements

Following consent, anthropometric measurements were taken to ensure participant suitability for MR and DXA scanning. Height and weight, were also required at this stage for the configuration of the physical activity monitor; of note, gender, date of birth and handiness were required, these details were collected during screening.

Height was measured, whilst participants were standing upright (with their back and head straight so that their Frankfurt plane was horizontal), to the nearest 0.5cm using a stadiometer (Model 220, Seca, Germany). Tanita bio-impedance analysis was conducted on electronic digital scales (Model BC 420, Dolby Medical Stirling, UK); this provided total body mass, fat percentage, fat mass, fat free mass, muscle mass, total body water, basal metabolic rate, bone mass and visceral fat indicator. Body mass index (BMI; mass (kg) / height $(m)^2$) was calculated from total body mass and height. Waist circumference measurements (at the umbilicus) and hip circumference measurements (at the greater trochanter) were taken in duplicate, waist to hip ratio (WHR; waist circumference (cm) / hip circumference (cm)) was calculated from this. After a period of 5 minutes rest, blood pressure (mmHg) and resting heart rate (bpm) were determined from an average of three measures using an automated monitor (Dinamap, G & E Medical, USA).

2.9 Physical activity monitoring

Participants' habitual physical activity was assessed using a SenseWear armband (Model MF-SW, BodyMedia, UK) (Figure 2.2). Instructions were given for the armband to be worn on their left arm at all times except when showering or bathing, inclusion criteria was >90% wear time. Monitoring was conducted on 4 consecutive days from midnight to midnight on, including one weekend day, in the days preceding an assessment visit. This protocol was employed based on previous literature which suggests 3-5 days of monitoring is required to reliably estimate habitual physical activity (Trost et al., 2005). The assessment was blinded, the armband does not provide any output of information to the participant. SenseWear Professional software (version 8.0) was used to analyse the data collected. The output (Figure 2.3) included: daily average step count, total energy expenditure, active energy expenditure and time spent in domains of physical activity including: sleep, lying, sedentary (<1.5 METS), light (1.5-3 METS), moderate (3-6 METS), vigorous (6-9 METS) and very vigorous (>9 METS).

Figure 2.2 SenseWear armband worn by the participants to monitor physical activity

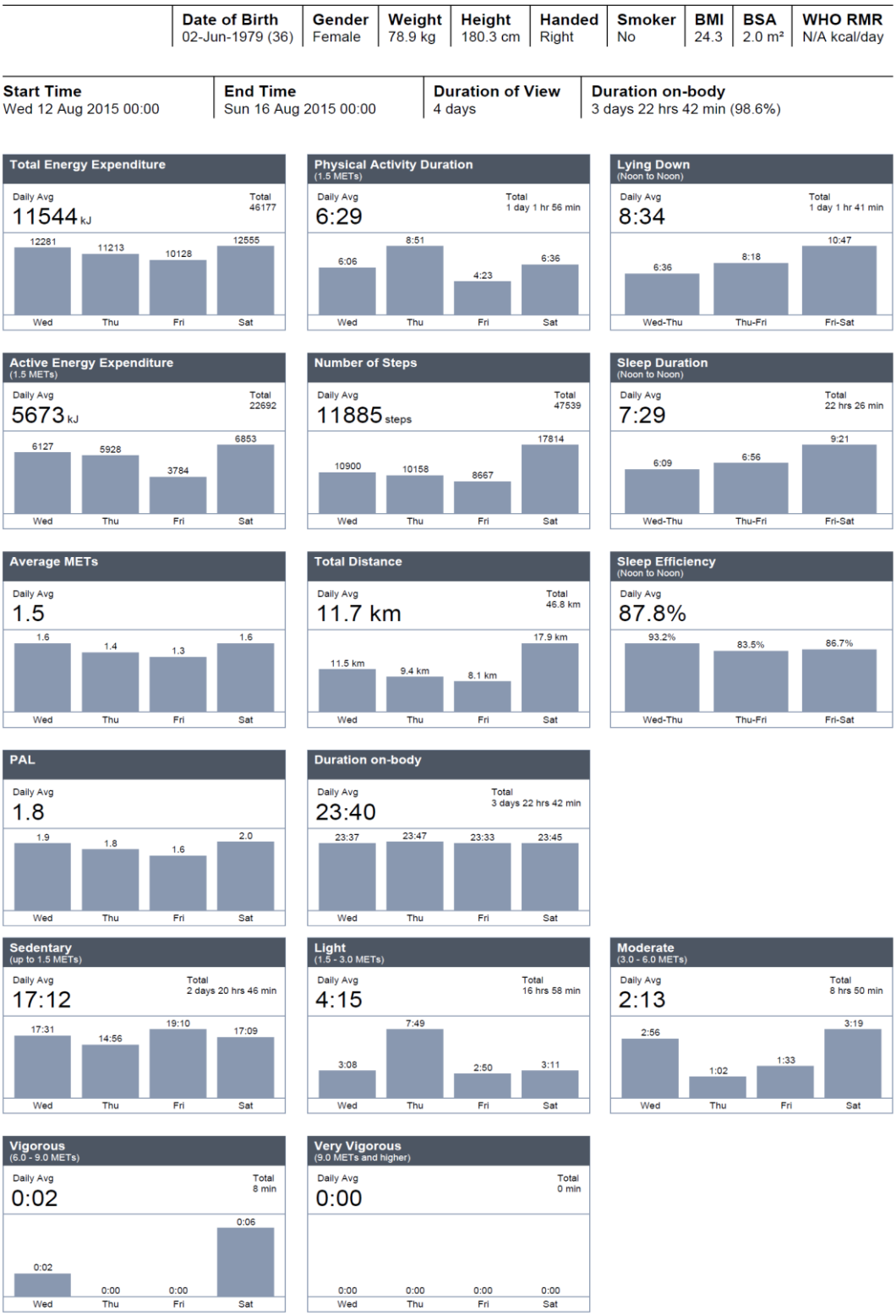


Figure 2.3 Example output derived from SenseWear armband worn by the participants for 4 consecutive days of habitual physical activity monitoring.

2.10 Dietary assessment

In paper diary format, participants recorded their entire food and fluid intake during the same 4 days of physical activity monitoring. Instructions were given to provide as much detail as possible including timing, portion size and product branding. The detail of information was verified verbally with the participant on retrieval of the diary so that any shortage of information could be completed. Total energy consumption, carbohydrate, protein and fat content was determined from dietary records by a registered nutritionist (KM) using Nutritics (Nutrition Analysis Software for Professionals).

2.11 Fasting blood profile

Participants were cannulated in the antecubital vein of one arm and blood was drawn for a baseline biochemical profile. This included: fasting glucose, fasting lipids (cholesterol: chol; triglyceride: TG; high density lipoproteins: HDL; low density lipoprotein: LDL), glycated hemoglobin (HbA1c), aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyl transferase (GTT). These samples were analysed within 24hrs by experienced technicians in the hospital laboratory at the University Hospital Aintree using an Olympus AU2700 analyzer (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, chol and HDL with cholesterol esterase/oxidase, TG with glycerol kinase, and liver enzymes with International Federation of Clinical Chemistry kinetic UV (without pyridoxal phosphate activation). LDL was calculated according to the Friedwald formula.

2.12 2 hr oral glucose tolerance test (OGTT)

Following fasting blood samples, additional baseline (0 min) samples for glucose, insulin and free fatty acids were taken. The participant then underwent an OGTT; 75g glucose solution was consumed within 5 min and subsequent blood samples were drawn (from finishing the glucose solution) at 30, 60, 90 and 120 min.

2.12.1 Glucose

The glucose profile of the OGTT was determined within 24hrs by experienced technicians in the hospital laboratory at the University Hospital Aintree by the ways aforementioned.

2.12.2 Insulin

Insulin samples were centrifuged at 35,000 rpm for 15min at 4 °C and plasma stored at -80 °C for subsequent analysis. These samples were later analysed using a commercially available radio-immunoassay (Invitrogen, UK).

2.12.3 Free fatty acids

Free fatty acid (FFA) samples were centrifuged at 35,000 rpm for 15min at 4 °C and plasma stored at -80 °C for subsequent analysis. These samples were later analysed for FFA concentration using a non-esterified fatty acids (NEFA) assay kit (Randox Daytona, UK).

2.13 Calculations of insulin sensitivity/ resistance

2.13.1 HOMA-IR

Using fasting plasma glucose and insulin concentrations, homeostatic model assessment of insulin resistance (HOMA-IR) was calculated (Matthews et al., 1985).

$$\text{HOMA-IR} = (\text{fasting insulin [}\mu\text{IU/ml]} \times \text{fasting glucose [mmol/l]}) / 22.5$$

2.13.2 Adipo-IR

Using fasting NEFA and insulin concentrations, adipose tissue insulin resistance (Adipo-IR) was calculated (Gutch et al., 2015).

$$\text{Adipo-IR} = \text{fasting NEFA [mmol/l]} \times \text{fasting insulin [pmol/l]}$$

2.13.3 Whole body insulin sensitivity (Matsuda index)

Matsuda index was calculated to estimate whole body insulin sensitivity (Matsuda and DeFronzo, 1999). An Excel template was downloaded from http://mmatsuda.diabetes-smc.jp/MIndex.html which used the following calculation:

$$\text{Matsuda index} = 1000 / \sqrt{G_0\, I_0\, G_{mean}\, I_{mean}}$$

I_0 – Fasting plasma insulin concentration [µIU/ml],

G_0 – Fasting plasma glucose concentration [mg/dl],

G_{mean} – Mean plasma glucose concentration during OGTT [mg/dl],

I_{mean} – Mean plasma insulin concentration during OGTT [µIU/ml],

10,000– Simplifying constant to get numbers from 0 to 12,

√– Correction of the nonlinear values distribution.

2.13.4 Hepatic insulin resistance index (HIRI)

Hepatic insulin resistance index (HIRI) was calculated by the product of the glucose and insulin area under curve (AUC) during the first 30 min of the OGTT (Figure 2.4) (Abdul-Ghani et al., 2007). The calculation is as follows.

$$\text{HIRI} = \sqrt{G_{0-30} \times I_{0-30}}$$

$G_{0\text{-}30}$ –Plasma glucose 0-30 min AUC concentration [mg/dl/h],

$I_{0\text{-}30}$ –Plasma insulin 0-30 min concentration [µIU/ml/h].

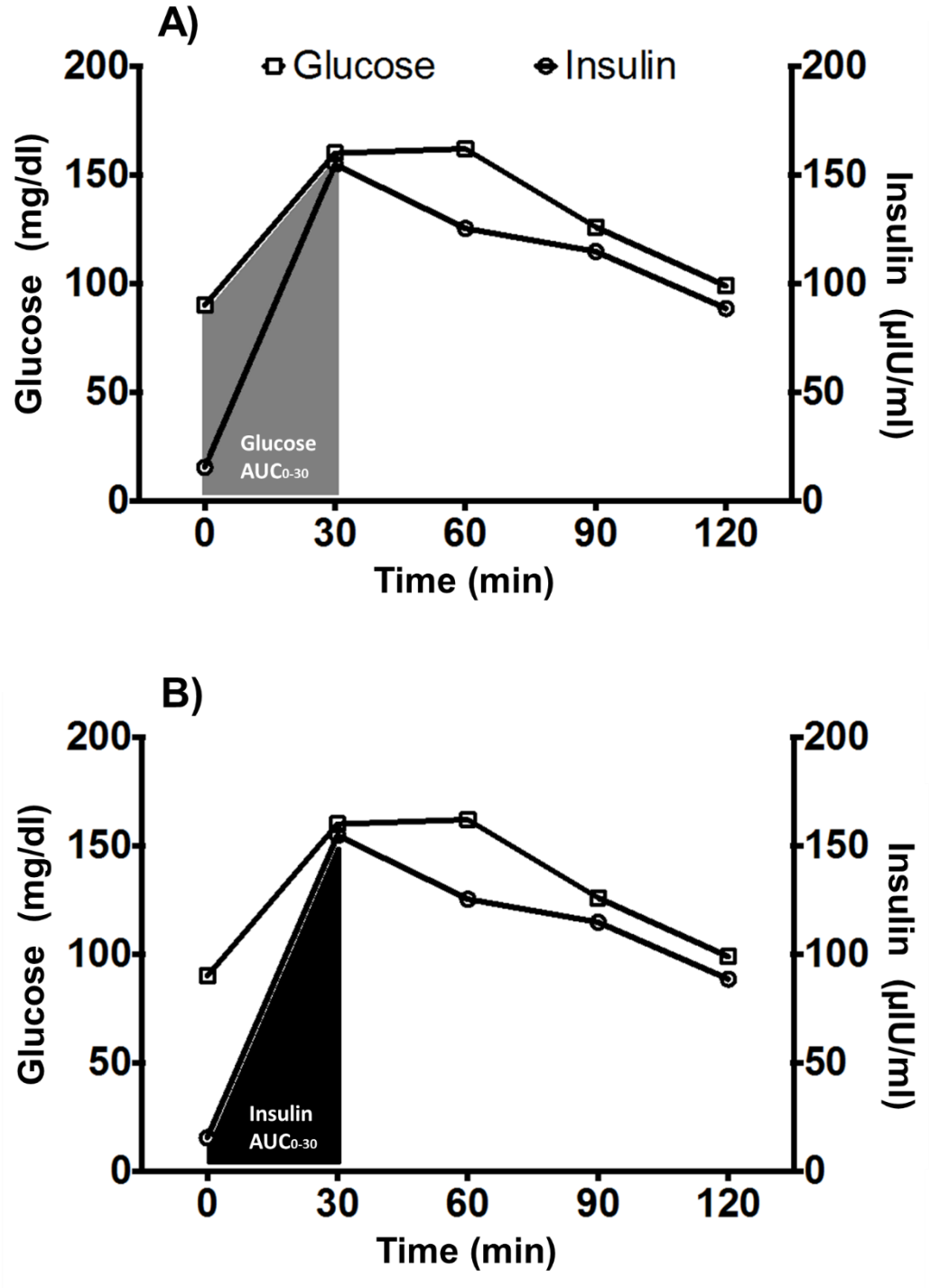


Figure 2.4 Hepatic insulin resistance index (HIRI) derived from 0 to 30 min OGTT; calculated by square root of A) glucose AUC, multiplied by B) insulin AUC, expressed in [mg/dl/h] and [mg/dl/h] respectively.

2.13.5 Muscle insulin sensitivity index (MISI)

Muscle insulin sensitivity index (MISI) was calculated from the time course of glucose and insulin during OGTT (Figure 2.5) (Abdul-Ghani et al., 2007).

$$\mathrm{MISI} = \mathrm{dG/dt} \div \mathrm{I_{mean}}$$

dG/dt [mmol/l/h] is the rate of decline in plasma glucose concentration and is calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir. It should be noted that in some cases plasma glucose concentration has rebounded after it reached its nadir. In such instances, the rebound glucose concentration was not included in the regression. I_{mean} [µIU/ml/h] represents the mean plasma insulin concentration during the OGTT.

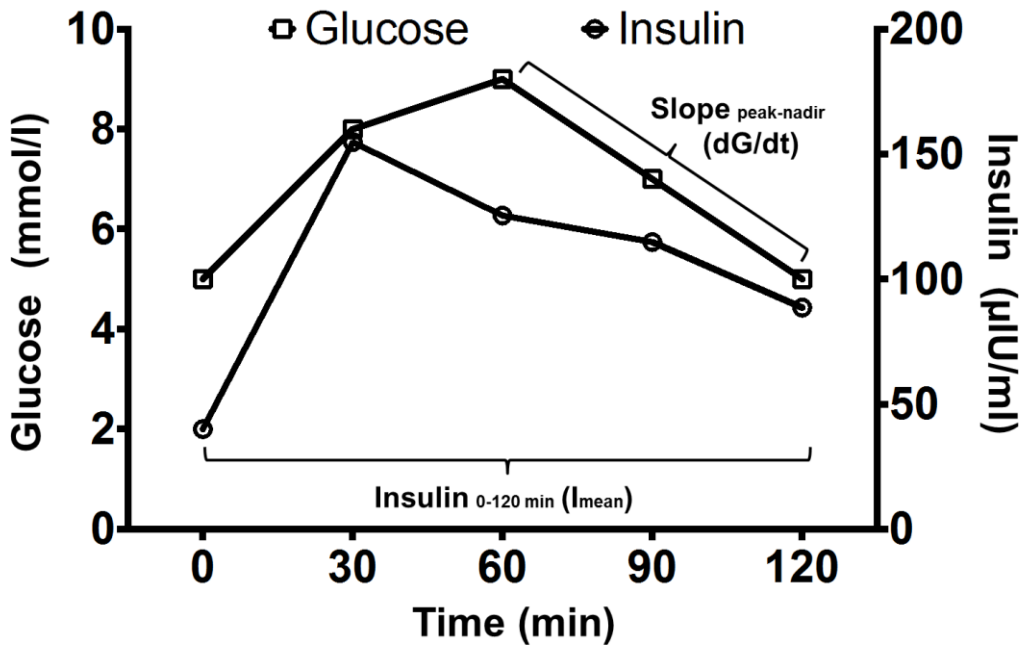


Figure 2.5 Muscle insulin sensitivity index (MISI) derived from OGTT; calculated by fitting a linear regression line through all the available points of glucose [mmol/l/h] from peak to nadir, divide absolute value of the slope by mean of all 0 to 120 min insulin [µIU/ml/h] measures.

2.14 Cardiorespiratory fitness

Physical fitness was assessed using a cardiopulmonary exercise test (CPET) which measures peak oxygen consumption ($\dot{V}O_2$ peak). The test was performed on a treadmill (Model 77OCE, RAM Medisoft Group, UK) in a temperature controlled room. Participants wore a face mask that covered their nose and mouth (Figure 2.6) and provided breath-by-breath monitoring and analysis of expiratory gases and ventilation; electrocardiographic monitoring was also conducted (Love Medical Cardiopulmonary Diagnostics, UK). Gas analysers and flow probes were calibrated prior to the test. The modified Bruce protocol was employed; after an initial 2 min warm up at 2.2 kph on a flat gradient, step-wise increments in speed and gradient were employed each minute (Bruce et al., 1973). The participants' rate of perceived exertion (RPE) (Borg, 1982) (Borg, 1982) (Borg, 1982) (Borg, 1982) was taken every minute. $\dot{V}O_2$ peak was determined as plateau in $\dot{V}O_2$ (l/min) and/or respiratory exchange ratio >1.15 combined with heart rate >90% predicted maximum (Miller et al., 1993). If volitional exhaustion occurred, the highest $\dot{V}O_2$ peak value in the minute prior was taken. An example output of the CPET is shown in figure 2.7, the blue line displaying a plateau of $\dot{V}O_2$. Breath-by-breath data was exported for calculations of absolute $\dot{V}O_2$ and calculations were derived relative to overall body mass, lean and fat free mass.

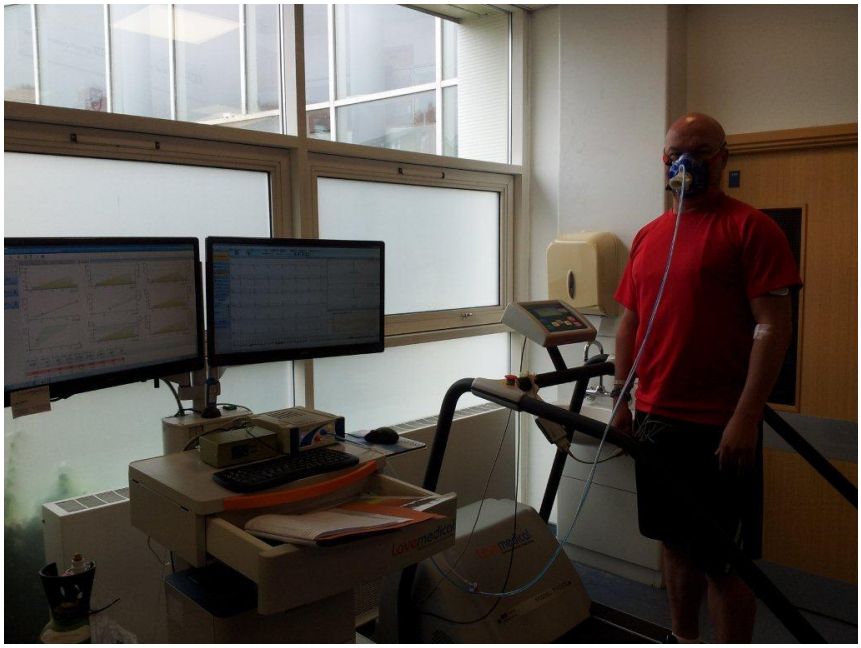


Figure 2.6 A participant set up for assessment of cardiorespiratory fitness (peak oxygen consumption, $\dot{V}O_2$ peak).

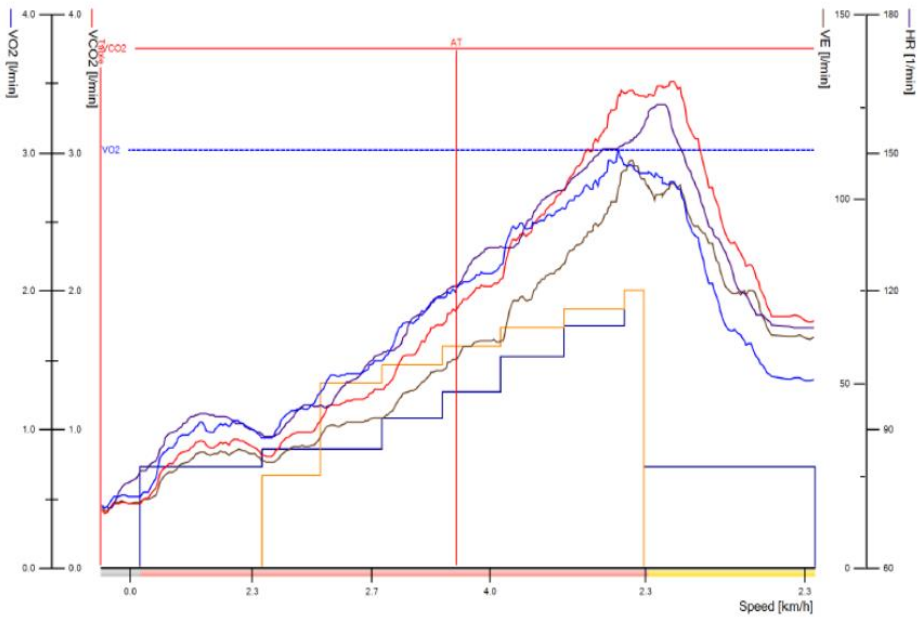
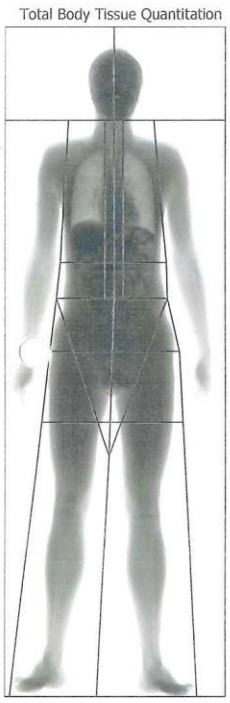
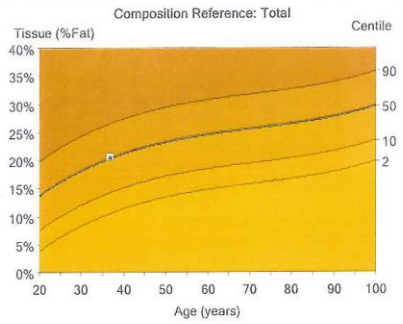


Figure 2.7 Example monitoring of cardiopulmonary exercise test (CPET) measuring peak oxygen consumption ($\dot{V}O_2$ peak). Blue line, peak oxygen consumption ($\dot{V}O_2$); red line, peak carbon dioxide consumption ($\dot{V}CO_2$), brown line, ventilatory equivalents (VE), purple line, heart rate (HR).

2.15 Dual-energy x-ray absorptiometry (DXA)

Whole body scans were performed using DXA (Lunar iDXA, GE Healthcare, UK) to determine body fat and lean body mass. All participants were placed in the standard supine scanning position in line with manufacturers guidelines (in the centre of the scanning bed with their head facing forwards, arms slightly away from the body, palms facing upwards and legs slightly apart with natural external rotation and toes facing upwards). Each scan session was preceded by a calibration routine, using multiple quality-control phantoms that simulate soft tissue and bone. Data output (Figure 2.8) was analysed using Lunar iDXA software (version 13.60.033), regional and limb specific quantifications of fat and lean tissue were given.





Region	Tissue (%Fat) [1]	Centile	Total Mass (kg) [2,3]	Fat (g) [1]	Lean (g) [1]	BMC (g)
Arms	16.3	-	8.6	1,328	6,814	487
Legs	21.0	-	26.7	5,332	20,106	1,312
Trunk	21.7	-	33.4	7,055	25,477	855
Android	23.1	-	4.9	1,108	3,692	53
Gynoid	22.8	-	12.1	2,669	9,059	341
Total	20.7	53	73.8	14,618	55,915	3,296

Figure 2.8 Example date output from dual-energy x-ray absorptiometry (DXA)

2.16 Magnetic resonance imaging and spectroscopy

MR scanning was performed at LiMRIC. The participants wore a patient gown and removed metal objects. The scans were conducted by two experienced radiographers; protocols and participant positioning were standardised.

2.16.1 Whole body imaging - adipose tissue volume and distribution

MRI was performed on 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany). Total body subcutaneous adipose tissue (SAT), abdominal SAT (abSAT) and visceral adipose tissue (VAT) were calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10mm slice thickness followed by a 10mm gap using the integral body coil). Figure 2.9 displays one trans-axial image of abdomen, SAT is represented as green and VAT red. The abdominal region was defined as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/base of the lungs. All MRI scans were analysed by Vardis Group Inc. (London, UK) using SliceOMatic (Tomovision, Montreal, Canada); data were anonymized prior to analysis ensuring blindness to all clinical details.

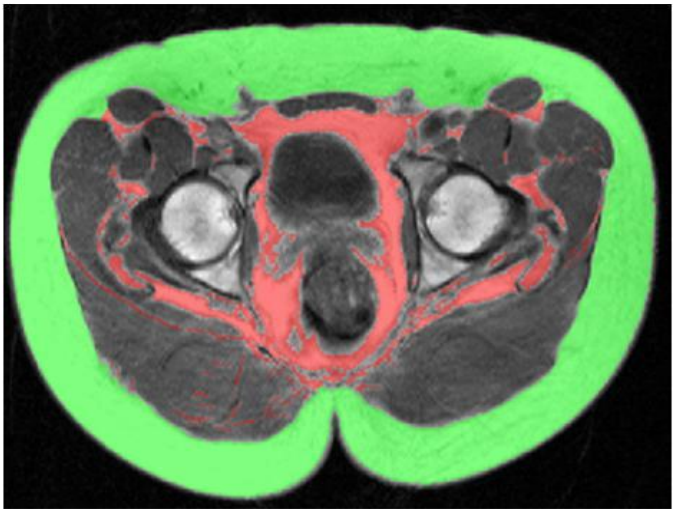


Figure 2.9 One trans-axial image of abdomen, subcutaneous adipose tissue (SAT) is represented as green and visceral adipose tissue (VAT) as red.

2.16.2 ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) - liver and skeletal muscle

^{1}H-MRS was performed on 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany). In the liver, three voxels of interest were identified at standard sites avoiding ducts and vasculature (Figure 2.10). In skeletal muscle, a single voxel was identified in each of the tibialis anterior (TA) and soleus (Sol) muscles, avoiding bone, fascia and the neurovascular bundle (Figure 2.11). Single voxel spectroscopy was conducted at each of these five sites. Voxel size was 20×20×20 mm, echo time (TE) 135 ms, repetition time (TR) 1500 ms, with 64 acquisitions. Where the musculature was too small to allow placement of a 20 mm voxel, a 15×15×20 mm voxel was placed and the number of acquisitions was increased to 200 to maintain signal-to-noise ratio. ^{1}H-MR spectra were quantified by Professor Kemp using the AMARES algorithm in the software package Java-based Magnetic Resonance User Interface (jMRUI-3.0) (Naressi et al., 2001). Intrahepatocellular lipid (IHCL) is expressed as percent of CH_2 lipid signal amplitude relative to water signal amplitude after correcting for T_1 and T_2 (Thomas et al., 2005), and intramyocellular lipid (IMCL) is expressed as CH_2 lipid amplitude relative to total creatine amplitude after correcting for T_1 and T_2 (Rico-Sanz et al., 1999).

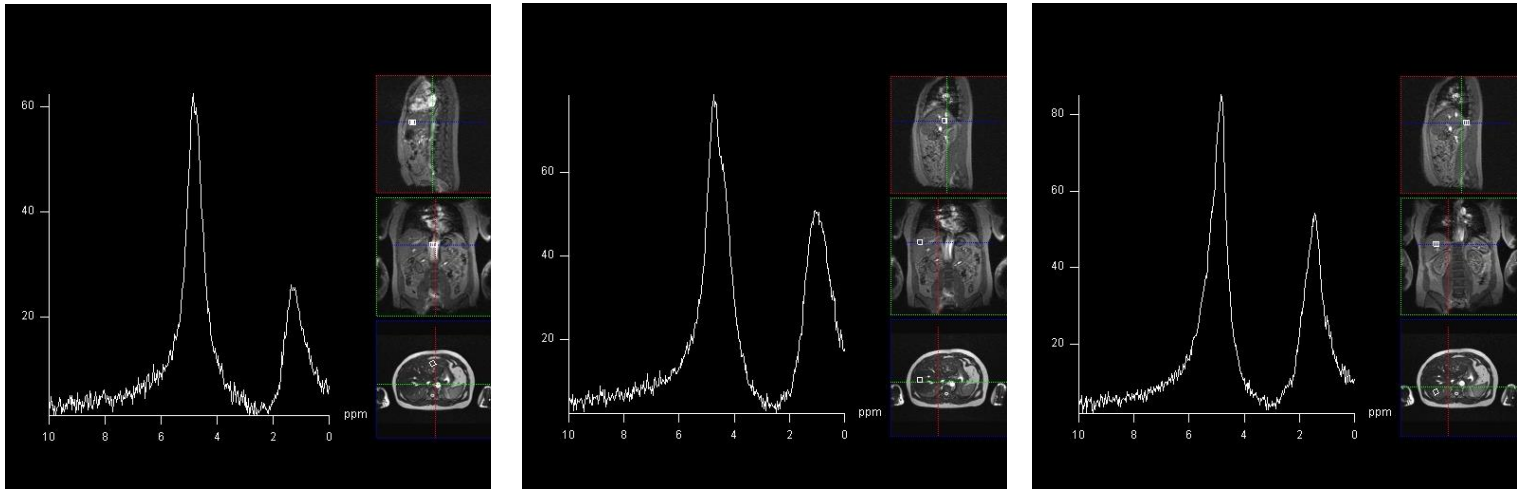


Figure 2.10 Example of a participant with high liver fat and voxel positions used during spectroscopy.

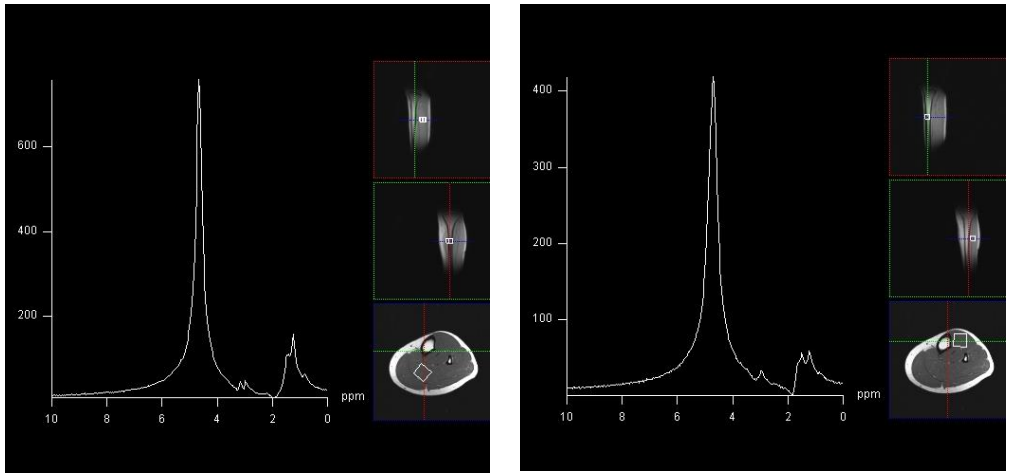


Figure 2.11 Example of the voxel positions used for soleus (left) and tibialis anterior (right) during spectroscopy.

2.16.3 ^{31}P MRS - skeletal muscle mitochondrial function

^{31}P-MRS assessments of mitochondrial function in skeletal muscle were carried out using a Siemens 3T Trio MR scanner (Siemens AG, Erlangen, Germany). An isometric knee extension exercise protocol was employed as previously described (Cuthbertson et al., 2014). In brief, participants lay supine, with the right knee flexed over a rigid foam support in a custom-built rig permitting isometric knee extension exercise against a strap across the anterior lower shin/ankle connected to an aluminium bar fitted with a strain gauge (Figure 2.12). The exercise protocol consisted of 1.1 min rest followed by 2 bouts of knee extension isometric exercise each followed by 5.2 min recovery periods. The first bout of exercise corresponded to 70% maximal voluntary contraction (MVC) and lasted 3.2 min. The second bout of exercise corresponded to 90% MVC and lasted 2 min. The knee extension exercise was paced at 0.25 Hz (2s on, 2s off) by an audible cue during each exercise. The contraction force was fed back visual via an LED display. ^{31}P-MRS data were processed using AMARES time-domain fitting algorithm in jMRUI-3.0. The chemical shift of the inorganic phosphate (Pi) peak relative to phosphocreatine (PCr) (σ parts per million) was used to determine intracellular pH. PCr recovery time courses were fitted to a monoexponential function to determine the recovery rate constant (k/min). Figure 2.13 displays an example of resting spectra (high Pi, low PCr) top right and two spectra following exercise (reduced Pi, increased PCr). For an indepth review see Kemp *et al.* (Kemp et al., 2015).

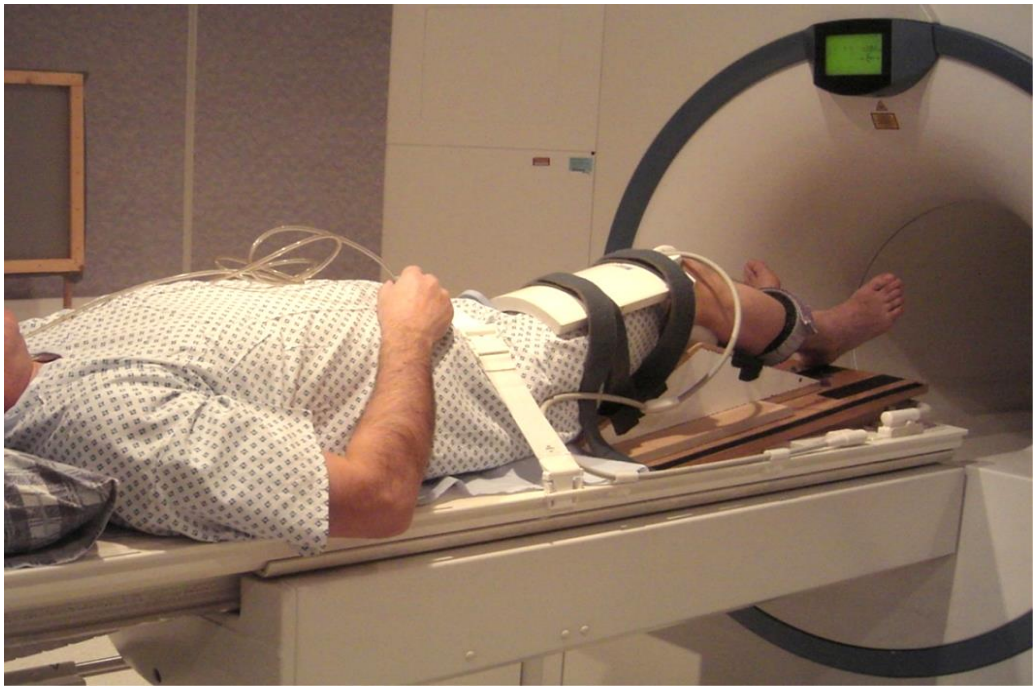


Figure 2.12 A participant set up for ^{31}P magnetic resonance spectroscopy, an isometric knee extension exercise protocol to assess skeletal muscle mitochondrial function.

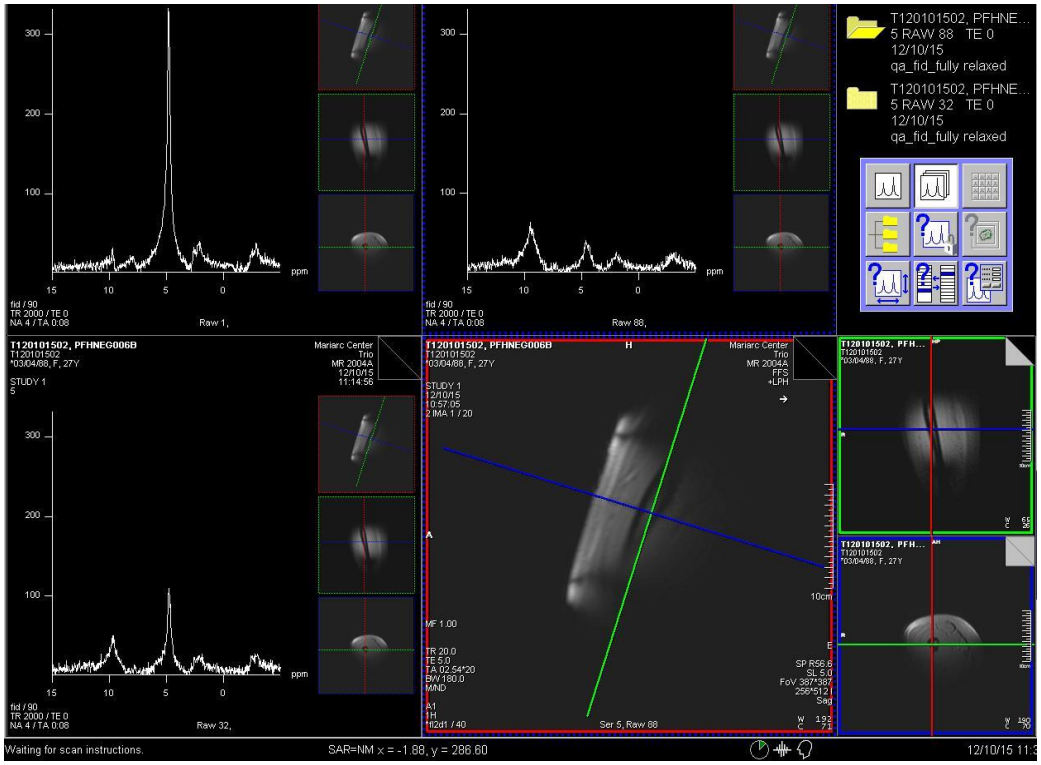


Figure 2.13 Example spectra derived from ^{31}P magnetic resonance spectroscopy. Top left, resting (high inorganic phosphate [Pi], low phosphocreatine [PCr]); top right, following 70% maximal voluntary contraction (MVC), (depleted Pi and increased PCr); bottom left, following 90% MVC (further depleted Pi and increased PCr); bottom right, spectra positioning within the quadriceps.

2.17 Tissue biopsies

Tissue biopsies were performed by experienced clinical staff in strictly sterile conditions. Samples were processed and stored by KBD. Please note, these specimen are still to be analysed and are not included in the thesis.

2.17.1 Skeletal muscle (SkM) biopsy

Skeletal Muscle samples were obtained from the right leg (vastus lateralis) using the conchotome technique. In brief, local anaesthetic (1% lidocaine) was administered before a 1cm incision was made. The samples were taken using 4.5mm jaw forceps (Tilley Henckel Punch, UK). Immediately after collection, the samples were a) snap frozen in liquid nitrogen and b) embedded on cork into Tissue Tek optimum cutting temperature (OCT) compound (Sakura Finetek, Netherlands) and immediately frozen in liquid nitrogen cooled isopentane (Sigma Aldrich, USA) (Figure 2.14). Embedded samples were initially blotted to remove excess blood and any visible collagen or fat was discarded. All muscle samples were stored at -80°C.

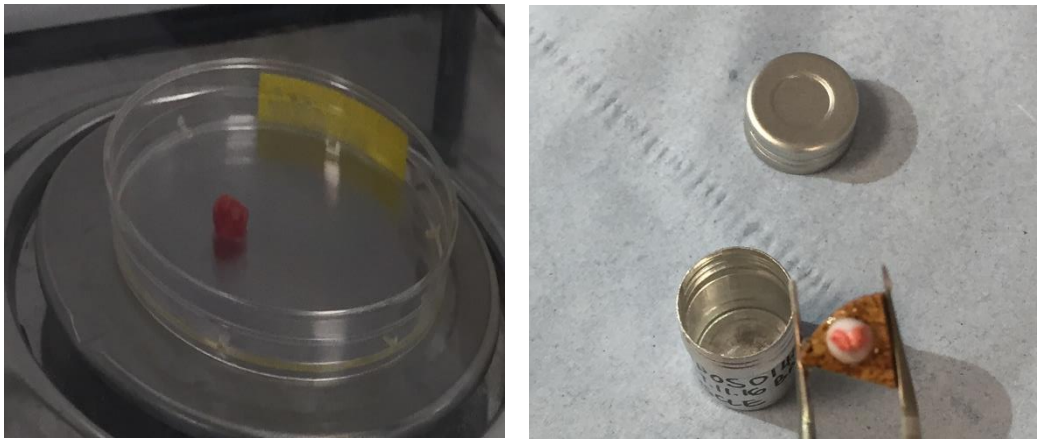


Figure 2.14 Skeletal muscle (SkM) specimen taken from vastus lateralis biopsy, before processing (right) and following embedding and freezing in liquid nitrogen cooled isopentane (left).

2.17.2 Subcutaneous adipose tissue (SAT) biopsy

Subcutaneous adipose tissue samples were obtained from the anterior abdominal wall approximately 2 cm lateral to the umbilicus. Local anaesthetic (2% lidocaine with adrenaline 1:200,000) was administered before a 1cm incision was made. The samples were taken using 15mm pincher forceps (Adsons, UK). Immediately after collection, the samples (Figure 2.15) were a) snap frozen in liquid nitrogen and stored at -80°C and b) submerged in 10% formaldehyde solution (Sigma Aldrich, USA) and stored at +4°C.

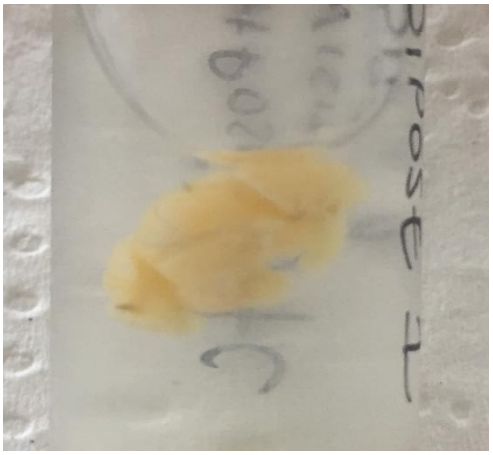


Figure 2.15 Subcutaneous adipose tissue (SAT) specimen taken from abdominal biopsy and stored in 10% formaldehyde solution.

Chapter 3.

Habitual physical activity does not explain differences in metabolic health status in obese and non-obese groups

List of authors: **Bowden Davies, K. A.**, Sprung, V. S., Norman, J. A., Irwin, A. J., Adams, V. A., Thompson, A., Mitchell, K.L., Halford J.C.G., Harrold J.A., Wilding, J. P. H., Kemp, G. J., & Cuthbertson, D. J.

KBD specific contribution to the study: Design, ethical approval and set up of the study, recruitment and participant management, coordination of testing visits including physical activity and dietary monitoring, anthropometrics, blood collection/OGTT, $\dot{V}O_2$ peak and MR/DXA scanning.

3.1 Introduction

Obesity (body mass index; BMI ≥30 kg/m^2) is strongly associated with cardiometabolic health and overall mortality (Berrington de Gonzalez et al., 2010). However, not all obese individuals are 'unhealthy' (Phillips, 2017) and likewise not all non-obese individuals are 'healthy' (Stefan et al., 2017). Meta-analysis data suggests that higher cardiorespiratory fitness (CRF), largely a product of greater physical activity (PA), can reduce mortality risk regardless of BMI (Barry et al., 2014); a higher CRF has been reported in *metabolically healthy obese* (MHO) (Ortega et al., 2013). Globally, the beneficial role of PA against cardiometabolic complications is recognised where the degree of benefit can be relative to the PA intensity (Warburton and Bredin, 2017). However, the support for PA as a determinant of health status within obese and non-obese groups is equivocal.

The stability of MHO and *metabolically unhealthy non-obese* (MUNO) is challenged and granted these phenotypes can appear as transient states. In a 20 year follow up study, it was found that one-half of MHO had made an adverse transition to *metabolically unhealthy obese* (MUO) and were also more likely to make this transition than MUNO (Bell et al., 2015a). Nevertheless, the prevalence of these 'non-conventional' phenotypes (i.e. MHO and MUNO) cannot be disregarded. The definition of metabolic health across previous studies has not been consistent however general characterisation of these groups has. MHO individuals display favourable insulin sensitivity, fat distribution (less visceral and ectopic fat), and cardiovascular function (Phillips, 2017). MUNO display less favourable features including adipose tissue dysfunction and a body composition whereby the anatomical site of fat storage appears to be more important than total amount of body fat (Stefan et al., 2017). Although MHO exhibit a cardiometabolic profile similar to *metabolically healthy non-*

obese (MHNO) their risk of CVD is greater (Caleyachetty et al., 2017). The clinical value of understanding the protective features of these phenotypes stands.

Previous research has suggested that a metabolically unhealthy individual may present as a consequence of low PA (Bell et al., 2015b, Camhi et al., 2015) whereas other research has not (Hankinson et al., 2013, Bell et al., 2014b, Phillips et al., 2013). Due to differences in methodology, cohorts and definitions these studies do not well define the PA characteristics between phenotypes. There is a growing recognition that sedentary behaviour has an independent association on metabolic health (Knaeps et al., 2016, Bell et al., 2014a). The role of sedentary behaviour has been objectively investigated within these phenotypes in older adults only (Bell et al., 2014b). However, age is an independent correlate of cardiometabolic abnormalities among non-obese and obese individuals (Wildman et al., 2008). Public health guidelines state that an individual should engage in 150 minutes of moderate-vigorous PA (MVPA) per week and historically research has focused on this. Bell and collegues found no difference between MHO and MUO in the likelihood of meeting MVPA recommendations (Bell et al., 2015b). Through objective monitoring of PA, which improves reliability compared to self-report (Dyrstad et al., 2014), would provide a comprehensive account of total daily activity which includes both sedentary behaviour and MVPA.

The aim of this cross-sectional study is to explore whether habitual PA and sedentary behaviour explains differences in metabolic health status of individuals of similar BMI. It is hypothesised that lower levels of PA and higher levels of sedentary time will be observed in unhealthy individuals irrespective of BMI.

3.2 Methods

Information regarding recruitment, screening, ethics and assessment measures be found *in Chapter 2, General methodology*.

3.2.1 Participants

Individuals with any level of physical activity were eligible. Participants were screened for and then, where appropriate, consented. Ninety-eight individuals (52 male, 46 female) with a mean age of 39 ± 13 years and BMI 27 ± 5 kg/m^2 were recruited.

3.2.2 Research design

All participants completed an assessment of habitual physical activity and dietary consumption over a period of 4 days (including one weekend day). This was followed by two assessment visits. The first at University Hospital Aintree involved anthropometry, fasting bloods, oral glucose tolerance test (OGTT) and assessment of CRF ($\dot{V}O_2$ peak). The second at University of Liverpool comprised of magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS). Due to technical issues during a period of this investigation MRI quantification of body fat was conducted on only 72 individuals. Bio-impedance data was collected in all individuals whereby $\dot{V}O_2$ peak calculations were based on both total body mass and fat free mass.

3.2.3 Individual phenotyping

Individuals were characterised into one of four groups based on their obesity (WHO classification, Table 1.1) and metabolic health status (International Diabetes Federation (IDF) criteria of metabolic syndrome (MS), Table 2.1); terms used herein i) *'healthy non-obese'*, ii) *'unhealthy non-obese'*, iii) *'healthy obese'* and iv) *'unhealthy obese'*.

3.2.4 Sample size

This study was powered on the basis of collecting tissue samples for a mechanistic study, based on previously published data this was calculated for 12 in each group. However, for the data presented here all eligible participants that had attended the University of Liverpool laboratories during data collection period were included. This explains the large *n* value of healthy non-obese, important to note is that this group was homogeneous and therefore was unlikely to introduce bias.

3.2.5 Statistical analysis

All data were explored for normality using visual inspection of frequency distributions, and transformed using $1og_{10}$ or log_{sqr}, where appropriate. Age was analysed using a one factor between-groups analysis of variance (ANOVA) whereby a significant group effect was observed ($P<0.05$). Between-group univariate general linear models were conducted for all other variables, with age as a covariate. Statistically significant interactions were explored using the least significant difference (LSD) approach to multiple pairwise comparisons. The alpha level of statistical significance was set at $P<0.05$. Data are presented as mean (95% confidence intervals [CI]), unless stated otherwise, and exact *P* values are cited (values of *P* of "0.000" provided by the statistics package are reported as "<0.0005"). Transformed data were back transformed to original units and presented as mean (95% CI). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows (Version 24.0, SPSS Inc., Chicago, IL, USA) statistic software package.

3.3 Results

3.3.1 Participant characteristics

Group characteristics are tabulated (Table 3.1). All significant differences between the groups' components of metabolic syndrome were in line with IDF classification, i.e. those who were healthy had favourable IDF-related health parameters compared to those that were unhealthy. The obese groups were well matched for age and BMI. The unhealthy non-obese were on average 15 (7, 22) years older and had a BMI of 3 (1, 5) kg/m^2 greater than healthy non-obese.

Table 3.1 Clinical, biochemical and metabolic characteristics of study participants categorised for obesity and subsequently for metabolic health.

	Non-obese			**Obese**		
	MetS- (*n*=62)	**MetS+ (*n*=11)**	***P* value**	**MetS-(*n*=12)**	**MetS+ (*n*=13)**	***P* value**
Gender	M *n*=30; F *n*=32	M *n*=9; F *n*=2	**0.042**	M *n*=5; F *n*=7	M *n*=8; F *n*=5	0.319
Age (years)	34 (31, 38)	49 (43, 55)	**<0.0005**	45 (39, 50)	46 (39, 52)	0.902
Weight (kg)	70.8 (68.1, 73.6)	80.8 (75.7, 85.9)	**0.045**	96.3 (85.2, 107.4)	99.8 (91.7, 107.9)	0.470
BMI (kg/m^2)	24.1 (23.4, 24.8)	26.9 (25.7, 28.2)	**0.018**	33.7 (30.6, 36.7)	34.1 (32.6, 35.6)	0.722
Components of metabolic syndrome [risk cut off]						
WC (cm) [M ≥94; F ≥80*]	85 (82, 87)	98 (93, 102)	**0.005**	105 (96, 115)	111 (106, 116)	0.191
SBP (mmHg) [≥130]	120 (117, 123)	144 (137, 151)	**<0.0005**	126 (117, 135)	147 (135, 158)	**<0.0005**
DBP (mmHg) [≥85]	75 (72, 77)	95 (85, 105)	**<0.0005**	77 (73, 80)	90 (82, 98)	**0.001**
Fasting glucose (mmol/l) [≥5.6]	4.9 (4.8, 5.0)	5.4 (5.1, 5.6)	0.076	5.0 (4.7, 5.2)	5.7 (5.0, 6.4)	**0.003**
Triglycerides (mmol/l) [≥1.7]	0.9 (0.8, 1.1)	1.5 (1.0, 1.9)	0.080	1.2 (0.6, 1.7)	1.8 (1.3, 2.4)	**0.016**
HDL-C (mmol/l) [M <1.0; F <1.3]	1.8 (1.7, 1.9)	1.7 (1.2, 2.1)	0.527	1.6 (1.3, 2.0)	1.3 (1.6, 1.8)	0.133
MRI derived body composition	***n*=48**	***n*=8**		***n*=8**	***n*=8**	
Total body fat (l)	21.3 (18.9, 23.7)	25.8 (20.1, 31.5)	0.164	39.6 (33.6, 45.6)	39.1 (33.2, 44.7)	0.882
Total SAT (l)	16.5 (14.2, 18.8)	18.6 (13.1, 24.1)	0.492	30.5 (24.7, 36.3)	28.2 (22.7, 33.8)	0.562
Total internal fat (l)	4.7 (4.1, 5.4)	7.3 (5.7, 8.9)	**0.006**	9.2 (7.5, 10.9)	8.5 (6.9, 10.2)	0.552
Abdominal SAT (l)	4.5 (3.5, 5.5)	5.7 (3.3, 8.1)	0.374	9.7 (7.2, 12.3)	12.1 (9.7, 14.6)	0.162
VAT (l)	2.3 (1.9, 2.8)	4.2 (3.1, 5.2)	**0.002**	5.2 (4.1, 6.2)	5.7 (4.5, 6.8)	0.490
VAT: abSAT ratio	0.6 (0.5, 0.7)	0.7 (0.5, 0.9)	0.333	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	0.793

WC, waist circumference *Europiod cut off; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; abSAT, abdominal SAT.

3.3.2 Dietary analysis

Total energy consumption, carbohydrate, protein and fat was not different between groups (P=0.926, P=0.581, P=0.342, P=0.731). Mean ±SD macronutrient percentages were 56±16% carbohydrate, 24±9% protein, and 20±7% fat.

3.3.3 Cardiorespiratory fitness

A significant group effect for cardiorespiratory fitness was evident (P<0.0005). Unhealthy obese individuals displayed significantly lower fitness than all other groups (P≤0.039; mean difference ≥5.8 ml/min/kg). When accounting for fat free mass (bio-impedance derived), unhealthy obese individuals displayed significantly lower values than both healthy groups (P≤0.012; mean difference ≥7.5 ml/min/kg) but not unhealthy non-obese (P=0.113; mean difference 5.9 ml/min/kg) (Figure 3.1A). No significant difference in either cardiorespiratory measure was observed in both non-obese groups and healthy obese (P≥0.080).

3.3.4 MRI quantification of body fat

Total body fat and total SAT was not statistically different between the two non-obese groups (P=0.164 and P=0.492, respectively) or the two obese groups (P=0.882 and P=0.562, respectively). As expected, the obese groups had significantly greater volumes of total body fat and total SAT than non-obese groups. Total internal fat was significantly lower in the healthy non-obese group than any other group (P≤0.006; mean difference ≥2.8 l), the three other groups were not significantly different (P≥0.103). Abdominal SAT values were again as expected, such that there was no significant difference between the two non-obese groups (P=0.374) or the two obese groups (P=0.162) and the obese groups had significantly greater volumes overall. VAT was significantly lower in the healthy non-obese group than any other group (P≤0.002; mean difference ≥1.9 l). The obese groups have statistically similar amounts VAT

(P=0.490). Of note, in unhealthy non-obese individuals VAT was significantly lower than unhealthy obese (P=0.047) but not healthy obese (P=0.173). VAT to abdominal SAT ratio is not significantly different between the groups (P=0.776). The demographics of those who had MRI versus those who did not was not statistically different (Appendix; Table 8.1).

3.3.5 MRS quantification liver fat and intramuscular fat

There was a significant group effect for liver fat percentage (P<0.0005) (Figure 3.1D). Healthy non-obese individuals had significantly lower liver fat % than all other groups (P≤0.050; mean difference ≥2.1 %). Unhealthy non-obese were not significantly different to either obese group (P≥0.132; mean difference ≥0.6 %); however, healthy obese was significantly lower than unhealthy obese (P=0.049; mean difference 2.6 %). There was no significant group effect for intramuscular fat % (P=0.336).

3.3.6 Glucose metabolism

A significant group effect was found for whole body insulin sensitivity quantified by Matsuda index (P=0.002) (Figure 3.1B). Healthy non-obese individuals demonstrated greater levels of insulin sensitivity than either of the other groups (P≤0.024; mean difference ≥1.6). No significant difference between Matsuda index in healthy obese and both unhealthy groups was observed (P≥0.562). There was no significant group effect of muscle insulin sensitivity index (P=0.220) or hepatic insulin resistance index (P=0.128). However when exploring the data, unhealthy obese had significantly greater hepatic insulin resistance than healthy non-obese (P=0.028; mean difference 7.7) (Figure 3.1C). HOMA-IR was significantly lower in healthy non-obese than both obese groups (P≤0.026; mean difference ≥1.7) but not unhealthy non-obese (P=0.092; mean difference 1.4). HOMA-IR was not significantly different between healthy obese and both unhealthy groups (P≥0.713).

3.3.7 Lipid metabolism

There was no significant group effect for total cholesterol and LDL cholesterol (P=0.426, P=0.408 respectively). Total cholesterol: HDL ratio was significantly different between groups. Healthy non-obese had a significantly lower ratio than both unhealthy groups ($P \leq 0.025$; mean difference ≥0.8) but not healthy obese (P=0.597; mean difference 0.2). Healthy obese had a significantly lower ratio than unhealthy obese (P=0.001) but not unhealthy non-obese (P=0.137); there was no significant difference between the two unhealthy groups (P=0.092).

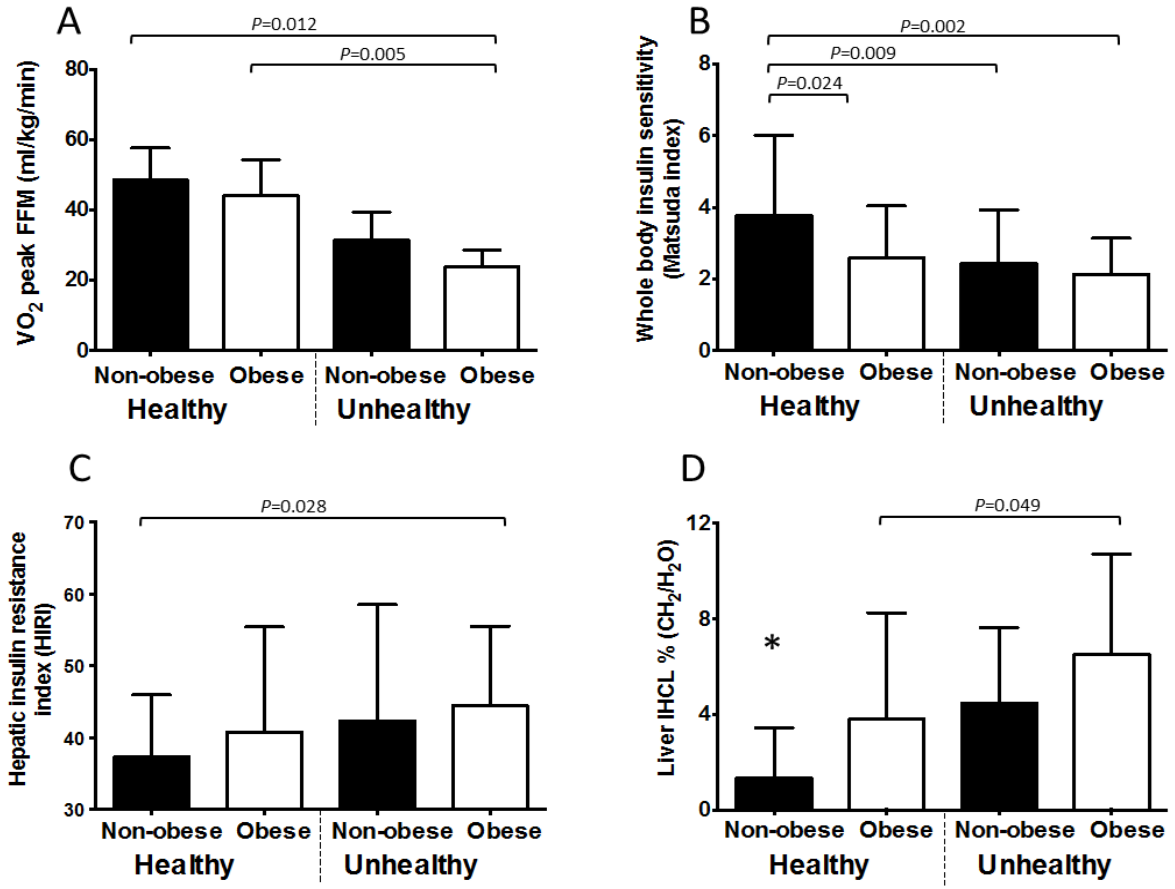


Figure 3.1 Age and BMI controlled A) $\dot{V}O_2$ peak relative to fat free mass (FFM), B) whole body insulin sensitivity, C) hepatic insulin resistance and D) liver intrahepatocellular lipid (IHCL) fat in healthy non-obese, healthy obese, unhealthy non-obese and unhealthy obese (left to right of each graph), solid bars represent non-obese individuals, open bars represent obese individuals, * denotes significantly lower liver IHCL in healthy non-obese than all other groups.

3.3.8 Physical activity

Average daily steps

A significant group effect was found for average daily steps (P=0.041) (Figure 3.2A). Healthy non-obese had significantly greater daily average steps than both unhealthy groups (P≤0.050; mean difference ≥2155 steps/day), and there was a non-significant trend for when compared with the healthy obese (P=0.092; mean difference 1840 steps/day). There was no significant difference between healthy obese and both unhealthy groups (P≥0.510).

Daily sedentary time

A significant group effect was found for daily sedentary time (P=0.002) (Figure 3.2B). Sedentary time was similar between both non-obese groups (P=0.267; 47 min/day) and both obese groups (P=0.263; 57 min/day). Unhealthy obese individuals displayed significantly greater daily sedentary time than both non-obese groups (P≤0.045; mean difference ≥107 min/day). Moreover, healthy obese individuals had significantly greater daily sedentary time than healthy non-obese (P=0.002; 97 min/day) but not unhealthy non-obese (P=0.348; 50 min/day).

Sleep duration and daily lying time

There was no significant group effect for amount of time spent lying (P=0.080) or sleeping (P=0.117). When removing sleep duration from overall daily sedentary time, a significant group effect remained (P=0.023) whereby healthy non-obese individuals displayed significantly lower values than both obese groups *(*P≤0.033) but not unhealthy non-obese (P=0.245); there was no significant difference between healthy obese and both unhealthy groups (P≥0.238). There was no significant difference

between the groups when subtracting lying time from overall daily sedentary time (P=0.084).

Daily light activity

A significant group effect was found for daily light activity (P=0.001) (Figure 3.2C). There was no significant difference in daily light activity between both non-obese groups (P=0.711; mean difference 10 min/day) and both obese groups (P=0.771; 9 min/day). However, both obese groups displayed significantly less light activity time than both non-obese groups ($P \leq 0.036$; mean difference ≥69 min/day).

Daily moderate-vigorous activity

There was no significant difference between the groups moderate-vigorous activity (P=0.322) (Figure 3.2D). When exploring moderate and vigorous activity independently, the group effect was similar for moderate activity (P=0.646) and statistically different for vigorous activity (P=0.015). Healthy non-obese individuals performed significantly greater duration of vigorous activity than both obese groups ($P \leq 0.039$; mean difference ≥16 min/day) but not unhealthy non-obese (P=0.079; mean difference 14 min/day). Vigorous activity was not significantly different between healthy obese and both unhealthy groups ($P \geq 0.440$).

Average daily METS and PA duration

Daily average METS and physical activity duration had significant group effects (P<0.0005 and P=0.020, respectively); for both measures, healthy non-obese had significantly greater values than both obese groups, but were not significantly different to unhealthy non-obese. Daily average METS in healthy non-obese were 0.3 METS greater than both obese groups (P<0.0005). The same trend was observed for PA duration, with healthy non-obese displaying significantly greater duration that both

obese groups ($P \leq 0.018$; mean difference ≥107 min/day). There was no significant difference between healthy obese and both unhealthy groups for average daily METS and PA duration ($P \geq 0.079$ and $P \geq 0.450$ respectively).

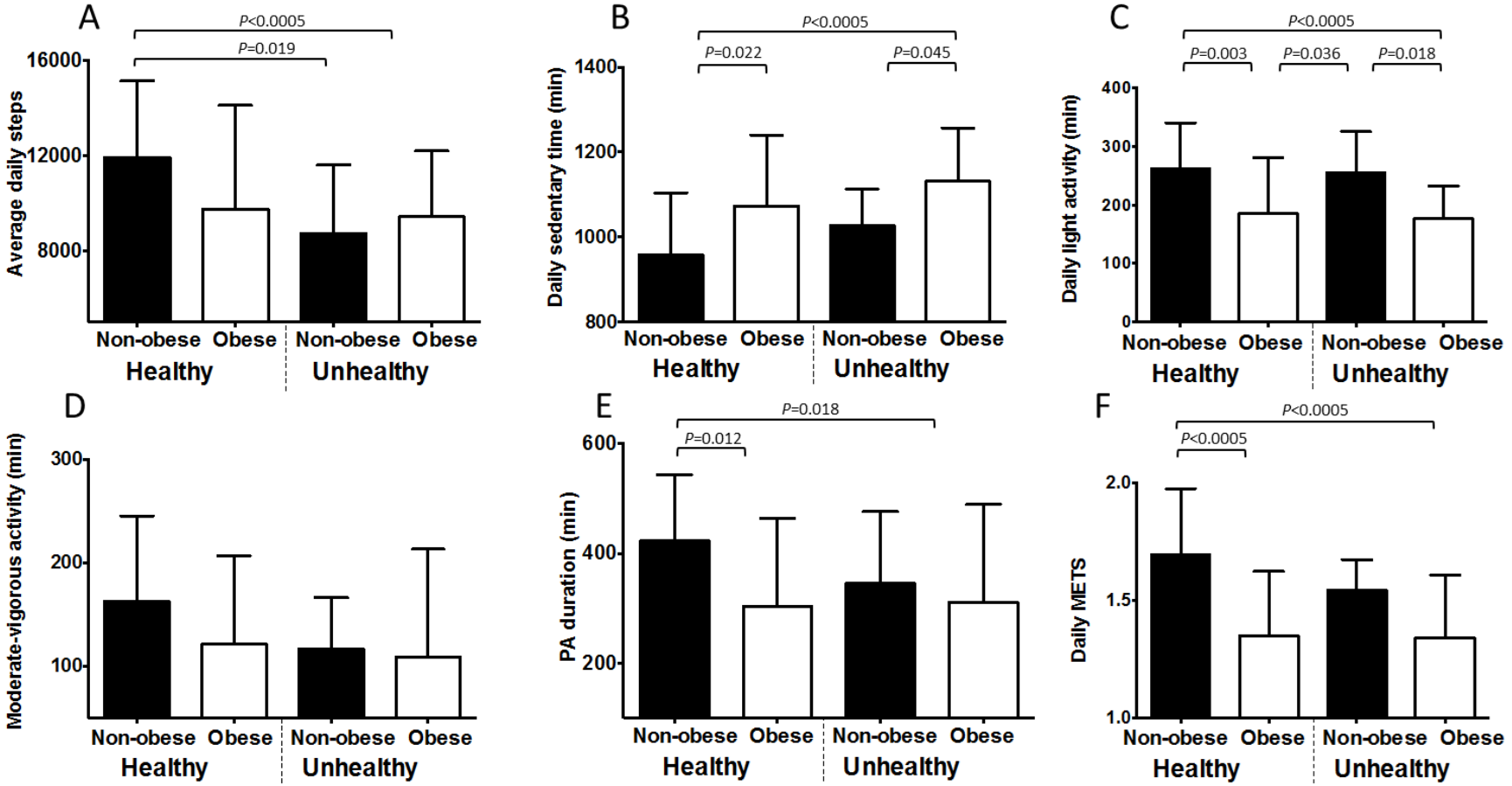


Figure 3.2 Age and BMI controlled A) average daily steps, B) sedentary time, C) light activity, D) moderate-vigorous activity, E) daily metabolic equivalents (METS) and F) physical activity (PA) duration in healthy non-obese, healthy obese, unhealthy non-obese and unhealthy obese (left to right of each graph), solid bars represent non-obese individuals, open bars represent obese individuals.

3.4 Discussion

This study is the first to objectively monitor PA and sedentary behaviour in young to middle aged adults who have been categorised for metabolic health and obesity status. The major finding of the current study was that habitual PA, including MVPA and sedentary behaviour did not explain differences in metabolic health status of individuals of similar BMI. Greater daily sedentary time and less light activity was associated with obesity but MVPA had no significant effect. CRF was markedly lower in unhealthy obese, however, when FFM was accounted the unhealthy obese and non-obese groups were similar.

The hypothesis of lower levels of PA and higher levels of sedentary time in unhealthy individuals irrespective of BMI, can be rejected in the current cohort. There are other studies that report a lack of association in these phenotypes for PA generally (Phillips et al., 2013) and for sedentary behaviour (Hankinson et al., 2013, Bell et al., 2014b). Contrary to these findings, a study in over 3,000 older adults, total PA was greater in healthy individuals across BMI groups (Bell et al., 2015b) and in a smaller study of young women healthy obese individuals demonstrated more time in light PA and less sedentary behaviour than their unhealthy counterparts (Camhi et al., 2015). Whilst prospective studies have shown that these phenotypes can be transient (Bell et al., 2015a) there is evidence to suggest that PA levels may (Moon et al., 2017) or may not (Hamer et al., 2015) play a protective role. Due to differences in methodology, cohorts and definitions it is difficult to directly compare the literature. Ethnicity can influence the cardiometabolic abnormalities in these groups (Wildman et al., 2008) therefore the IDF criteria of MetS was chosen in the current investigation which accounts for ethnic differences in waist circumference risk (Alberti et al., 2009). Despite several

investigations, robust evidence for a significant role of PA and sedentary behaviour in determining metabolic health status irrespective of BMI is lacking.

Healthy obesity remains a contentious topic, however the findings of current investigation do support the overall beneficial effect of increased PA and reduced sedentary behaviour. Those who were obese had more sedentary time and less light activity time however this association is not new (Hamer et al., 2013, Kim et al., 2013). The observed PA levels in unhealthy non-obese are worthy of discussion. Sedentary time has an independent association with metabolic health (Knaeps et al., 2016, Bell et al., 2014a); similar sedentary time in unhealthy non-obese and both obese groups supports this. When compared to healthy non-obese, daily average steps were significantly lower in both unhealthy groups and thus may explain their unfavourable health profile; the healthy groups had similar average daily steps further suggesting a protective role. MVPA was not significantly different between the groups and historically research has focused on these recommendations (i.e. exercise). This research offers an alternative approach as habitual physical activity (i.e. not exercise). CRF was markedly lower in both unhealthy groups when accounting for FFM; low CRF can increase mortality risk regardless of BMI (Barry et al., 2014). In line with these data a higher CRF has been previously reported in healthy groups (Ortega et al., 2013). Taken together with the strong association of PA with a higher CRF (Warburton and Bredin, 2017) this study outlines the overall benefits of increased PA and reduced sedentary behaviour.

When compared to both obese groups, unhealthy non-obese individuals displayed similar levels of liver fat, whole body insulin sensitivity and hepatic insulin resistance therefore BMI cannot be the sole explanation. Of note, unhealthy obese had

significantly more liver fat than their healthy counterpart. These characteristics have been previously observed (Phillips, 2017, Stefan et al., 2017). Former research has suggested that ectopic fat may be a paramount target for reduction of disease risk and perhaps more important than traditional risk factors (Arsenault et al., 2012). A more favourable fat distribution has been associated with a healthy profile in obese adults over a period of 10 years and reduces the risk of T2D and CVD (Appleton et al., 2013). Liver fat has been proposed as a better marker of metabolic health than visceral fat (Fabbrini et al., 2009) and recently as an independent risk factor for progression from a healthy to unhealthy phenotype, even in non-obese individuals (Hashimoto et al., 2017). Exploring the relationship between liver fat and habitual PA, including detailed characterisation of domains of activity (i.e. sedentary time, light activity and MVPA) may provide insight for innovative PA guidelines.

The strengths of this study include the objective monitoring of PA (more valid than self-report (Dyrstad et al., 2014) which has provided detailed phenotyping and comprehensive assessments in young to middle aged adults who have not previously been studied. The limitations include sample size, duration of PA assessment (4 days which may induce bias towards week days, we ensured that at least one weekend day was monitored) and cross-sectional design which cannot determine causality. Objective PA monitoring in a larger cohort with prospective design is required. Important to note is that clinical studies, particually observational, can introduce, by an interaction effect of the research, behaviours that are not typical of daily living (Hammond and Wellington, 2013). In this instance participants may have altered their physical activity levels knowing this was being assessed. However, this is likely to be consistent across the groups. Knowing that the cardiometabolic complications can be altered in normal-weight and overweight; future research should study these

classifications separately as opposed to non-obese in the current study. Likewise, unhealthy were categorised as 3 or more MetS components and those otherwise were healthy; recent research suggests the use of a refined healthy reference group with only 0-1 factors (Hamer et al., 2017).

In summary, habitual physical activity does not explain differences in metabolic health status in obese and non-obese groups', daily average steps may have an influence but this requires further investigation. In concordance with previous research, greater amounts of sedentary time and less daily light activity are evident in obese individuals. Overall, this research offers an alternative approach as habitual physical activity (i.e. not exercise and/or sedentary time) may have an effect that is independent of MVPA.

Chapter 4.

Sedentary behaviour but not moderate-vigorous activity is associated with liver fat in a combined cohort of obese and non-obese adults

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KBD specific contribution to the study: Design, ethical approval and set up of the study, recruitment and participant management, coordination of testing visits including physical activity and dietary monitoring, anthropometrics, blood collection/OGTT, V̇O2 peak and MR/DXA scanning.

4.1 Introduction

Strong evidence suggests that non-alcoholic fatty liver disease (NAFLD) substantially increases the risk of developing type 2 diabetes mellitus (T2D) and cardiovascular disease (CVD). A greater body mass index (BMI), body fat distribution (visceral versus subcutaneous), insulin resistance and higher circulatory lipids have all been associated with the development of NAFLD (Rinella, 2015). The role of physical activity (PA) in the prevention of liver fat in the context of non-alcoholic fatty liver disease (NAFLD) has been well studied and recently reviewed (Qiu et al., 2017, Romero-Gómez et al., 2017, Zelber-Sagi et al., 2016). However, an independent association between sedentary behaviour and NAFLD is emerging, such that PA and sedentary behaviour ought to be viewed separately.

Ryu and colleagues (Ryu et al., 2015) conducted a large cohort study which supports the importance of reducing time spent sedentary in addition to promoting PA. However, these authors utilised PA questionnaires and measured liver fat using ultrasonography. Objective PA monitors are superior to questionnaires as they reduce reporter bias (Dyrstad et al., 2014) and proton magnetic resonance spectroscopy (^{1}H-MRS) is a more sensitive and quantitative method of determining liver fat (Szczepaniak et al., 2005). Whilst smaller studies have objectively monitored PA and quantified liver fat using MRS the application of their findings are limited due to a) no liver fat measurement in control individuals (Hallsworth et al., 2015) and b) overweight and obese individuals being studied (Keating et al., 2016).

It is recognised that liver steatosis (the initial stage of NAFLD) should be targeted early to prevent complications associated with the progression of this condition. The majority of previous research has characterised those with and without NAFLD which has not well defined the PA behaviours of those that should be targeted, i.e. healthy

individuals before harmful levels of liver fat accumulate. Characterising the PA behaviours of healthy individuals provides important knowledge for changing behaviours associated with the risk of increased liver fat. One study has objectively measured PA in a community based cohort (Long et al., 2015) but again sub-optimal methods were used to determine liver fat. Objective investigation of PA and sedentary behaviour in a community based cohort utilising MRS assessment of liver fat is warranted.

The primary aim of this study was use objective monitoring of PA and sedentary behaviour to investigate relationships between domains of physical activity and MRS quantified liver fat, in healthy individuals. Knowing that insulin resistance and BMI are strongly associated with the development of fatty liver, secondary aims were to investigate their association with PA and sedentary behaviour also.

4.2 Methods

Information regarding recruitment, screening, ethics and assessment measures be found *in Chapter 2, General methodology*.

4.2.1 Participants

The individuals studied here are the cohort of *Chapter 3*. Of note, none of the individuals were clinically diagnosed with NAFLD on consent.

4.2.2 Research design

Participation included assessment of habitual physical activity and dietary consumption over a period of 4 days including one weekend day. There were two subsequent assessment visits, one at University Hospital Aintree including fasting bloods, oral glucose tolerance test (OGTT) and assessment of cardiorespiratory fitness (CRF) as well as one at University of Liverpool including proton magnetic resonance

spectroscopy (^{1}H-MRS). $\dot{V}O_2$ peak (CRF) was calculated from bio-impedance derived total body mass and fat free mass (FFM).

4.2.3 Statistical analysis

All data were explored for normality using visual inspection of frequency distributions, and transformed using $1og_{10}$ where appropriate. Univariate and multivariate linear regression were used to analyse components of physical activity and fitness associated with the outcome measure of interest. All variables reaching $P<0.05$ in univariate analysis were carried forward to a multivariate model. An *a priori* decision was made to include age and BMI in all multivariate models. The alpha level of statistical significance was set at $P<0.05$. Data are presented as mean (95% confidence intervals [CI]), unless stated otherwise and exact *P* values are cited (values of *P* of "0.000" provided by the statistics package are reported as "<0.0005"). Transformed data were back transformed to original units and presented as mean (95% CI). Pearson's correlation coefficient was performed to generate R^2 in order to determine the proportion of the variance in the dependent variable that is predictable from the independent variable. The strength of correlations are determined by Field classification (Field, 2013). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows (Version 24.0, SPSS Inc., Chicago, IL, USA) statistic software package.

4.3 Results

4.3.1 Participant characteristics

Participant characteristics are summised in Table 4.1. The number of individuals with each risk factor is summarised in Table 4.2. Overall, 74 individuals were categorised as metabolically healthy and 24 metabolically unhealthy (classified as 3 or more risk

factors for metabolic syndrome). Mean whole body insulin sensitivity (Matsuda index) of all individuals was 3.3 (2.8, 3.7). Of note, 27% of individuals had liver fat >5%

Table 4.1 Participant characteristics of healthy versus unhealthy individuals.

	Healthy	**Unhealthy**	***P* value**
Gender	M *n*=35; F *n*=39	M *n*=17; F *n*=7	0.141
Age (years)	36 (33, 39)	47 (43, 51)	**<0.0005**
Weight (kg)	74.9 (71.4, 78.5)	91.1 (84.9, 97.3)	**<0.0005**
BMI (kg/m^2)	25.6 (24.5, 26.7)	30.8 (29.0, 32.6)	**<0.0005**
WC (cm)	88 (84, 91)	105 (98, 107)	**<0.0005**
SBP (mmHg)	121 (118, 124)	145 (138, 150)	**<0.0005**
DBP (mmHg)	75 (73, 77)	88 (84, 92)	**<0.0005**
Fasting glucose (mmol/l)	4.9 (4.8, 5.0)	5.6 (5.2, 6.2)	**0.003**
Triglycerides (mmol/l)	1.0 (0.8, 1.1)	1.7 (1.1, 1.9)	**0.001**
HDL-C (mmol/l)	1.8 (1.6, 1.9)	1.5 (1.3, 1.9)	**0.046**
Liver fat % (CH_2/H_2O)	1.7 (1.0, 2.4)	5.5 (3.6, 7.4)	**<0.0005**

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol.

Table 4.2 The number of risk factors of metabolic syndrome (MetS) and liver fat in 98 individuals. Classification column is listed as healthy top and MetS bottom.

Risk factor	**Classification**	***N* (%)**
Waist circumference (cm)	≤94M/81F >94M/80F	65 (66%) 33 (34%)
Triglycerides; TG (mmol/l)	≤1.7 >1.7	83 (85%) 15 (15%)
High density lipoprotein; HDL (mmol/l)	≥1.03M/1.29F <1.03M/1.29F	91 (93%) 7 (8%)
Systolic blood pressure; SBP (mmHg)	≤130 >130	64 (65%) 34 (35%)
Diastolic blood pressure; DBP (mmHg)	≤85 >85	74 (76%) 24 (24%)
Fasting glucose (mmol/l)	≤5.6 >5.6	88 (90%) 10 (10%)

M, male classification; F, female classification

4.3.2 Physical activity and cardiorespiratory fitness

Physical activity and cardiorespiratory fitness data is summarised in Table 4.3. On average, individuals were taking 10939 steps/day (10213, 11665) and spent 1008 min/day (979, 1037) sedentary; $\dot{V}O_2$ peak average was 32.9 ml/kg/min (31.1, 34.6).

Table 4.3 Summary of physical activity and cardiorespiratory fitness data.

	Mean (95% CI)
Average daily steps (steps/day)	10939 (10213, 11665)
Daily sedentary time (min)	1008 (979, 1037)
Daily light time (min)	241 (223, 259)
Daily moderate time (min)	124 (110, 139)
Daily vigorous time (min)	19 (14, 24)
Daily lying time (min)	486 (469, 503)
Daily sleep duration (min)	403 (389, 417)
Daily metabolic equivalents (METS)	1.6 (1.5, 1.7)
$\dot{V}O_2$ peak (ml/kg/min)	32.9 (31.1, 34.6)

4.3.3 Liver fat

Univariable linear regression analysis revealed several factors associated with physical activity that were predictors of liver fat (Table 4.3). For every increase of 1,000 daily steps, liver fat decreased by 0.95% whereas for every hour increase in daily sedentary time liver fat increased by 1.09%. Greater amounts of daily vigorous activity, METS and $\dot{V}O_2$ peak were significantly associated with lower levels of liver fat. In the multivariable analysis, three of the factors associated with physical activity remained statistically significant predictors of liver fat percentage (Figure 4.1). Greater amounts of daily sedentary time is associated with greater amounts of liver fat, whilst higher overall daily METS and $\dot{V}O_2$ peak are associated with lower levels of liver fat.

Table 4.2 Univariate and multivariate regression for liver fat.

	Univariate			Multivariate		
	β coefficient	**95% CI**	***P***	**β coefficient**	**95% CI**	***P***
Age (years)	1.02	1.01, 1.06	**<0.0005**	1.00	1.00, 1.02	0.343
BMI (kg/m^2)	1.10	1.03, 1.24	**<0.0005**	1.01	0.97, 1.12	**<0.0005**
Steps (1,000)	-0.95	-0.86, -0.95	**0.023**	-0.97	-0.89, -0.97	0.103
Sedentary time (hr)	1.09	1.08, 1.29	**0.023**	1.15	1.14, 1.50	**0.036**
Vigorous activity (hr)	-0.68	-0.32, -0.68	**0.049**	-0.99	-0.95, -1.00	0.237
METS (0.1)	-0.94	-0.84, -0.95	**0.040**	-0.48	-0.13, -0.56	**0.012**
$\dot{V}O_2$ peak (ml/kg/min)	-0.97	-0.92, -0.98	**0.001**	-0.87	-0.25, -1.50	**0.007**
Light activity (hr)	-0.90	-0.72, -0.91	0.077			
Moderate activity (hr)	-0.95	-0.78, -1.04	0.478			
MVPA (hr)	-0.94	-0.79, -1.00	0.301			
Lying time (hr)	1.03	0.95, 1.20	0.596			
Sleep duration (hr)	1.03	0.91, 1.22	0.741			

Data was transformed and analysed using $1og_{10}$; data presented here is back transformed to original units. BMI, body mass index; METS, metabolic equivalents; $\dot{V}O_2$ peak, cardiorespiratory fitness; MVPA, moderate-vigorous physical activity.

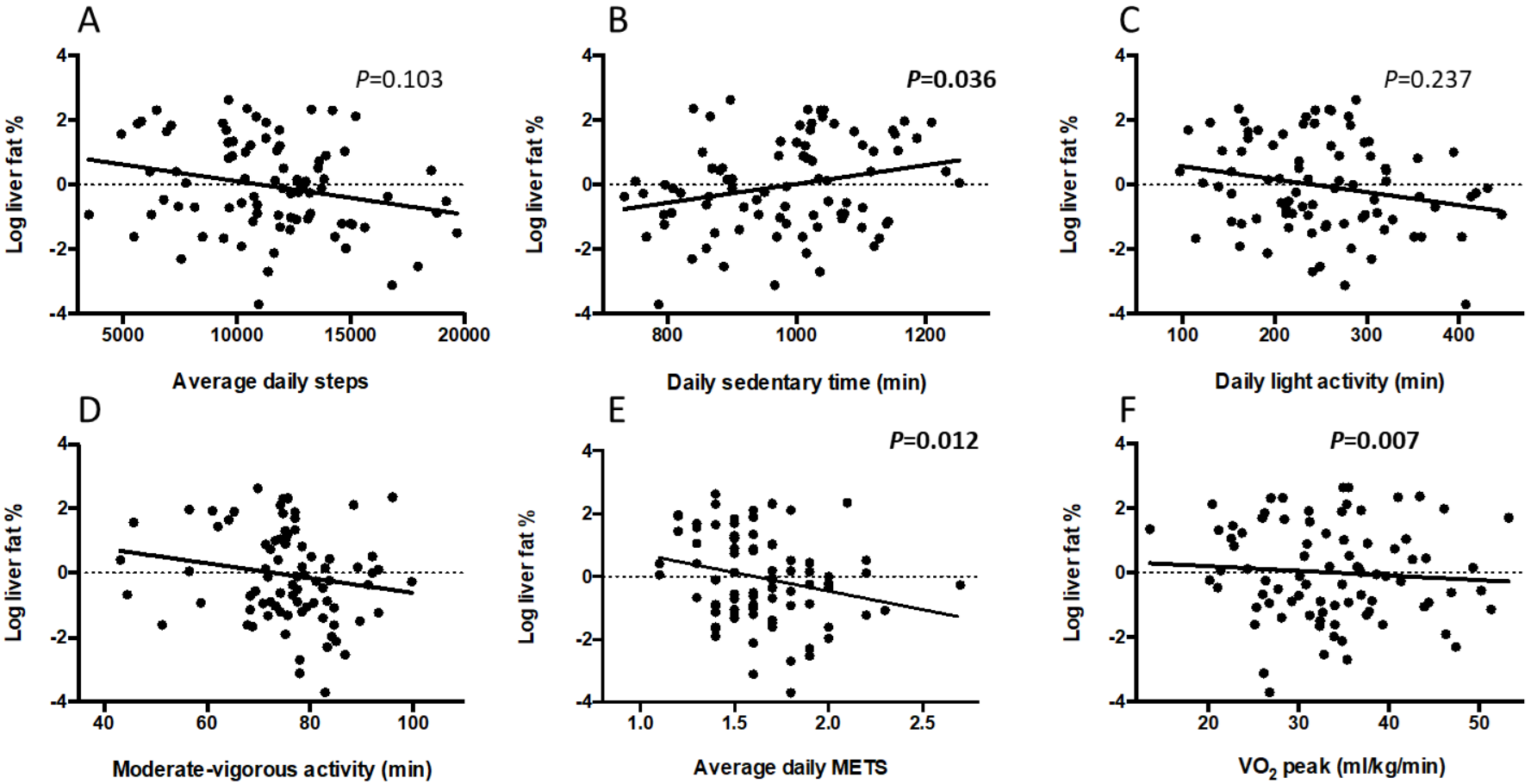

Figure 4.1 Association of liver fat (log_{10}) percentage (%) with A) daily average steps, B) sedentary time, C) light activity, D) moderate-vigorous activity, E) metabolic equivalents (METS) and F) $\dot{V}O_2$ peak; *P* value represents multivariate regression analysis.

4.3.4 Whole body insulin sensitivity

Greater daily average steps, METS and $\dot{V}O_2$ peak were significantly associated with greater whole body insulin sensitivity (Matsuda index) when exploring this data using univariable linear regression (Table 4.4). Additionally, univariable analysis also revealed that for every hour increase in daily sedentary time, Matsuda index would decrease by 0.93. However, multivariable logistic regression analysis did not reveal any independent associations between physical activity/fitness and whole body insulin sensitivity. There was a modest correlation between liver fat and Matsuda index (R^2= 0.5; P<0.005) in this cohort.

Table 4.3 Univariate and multivariate regression for whole body insulin sensitivity (Matsuda index).

	Univariate			Multivariate		
	β coefficient	**95% CI**	***P***	**β coefficient**	**95% CI**	***P***
Age (years)	1.00	0.99, 1.00	0.589	1.01	-1.00, 1.02	0.119
BMI (kg/m^2)	-0.97	-0.92, -0.98	**0.003**	-0.98	-0.94, 0.99	0.151
Steps (1,000)	1.04	1.03, 1.12	**0.013**	1.02	-1.01, 1.09	0.225
Sedentary time (hr)	-0.93	-0.83, -0.95	**0.009**	-0.94	-0.81, 0.98	0.248
METS (0.1)	1.04	1.04, 1.12	**0.045**	-0.95	-0.84, 0.99	0.237
$\dot{V}O_2$ peak (ml/kg/min)	1.02	1.01, 1.06	**0.003**	1.01	-1.01, 1.05	0.154
Light activity (hr)	1.07	-1.05, 1.25	0.111			
Moderate activity (hr)	1.09	-1.08, 1.32	0.087			
Vigorous activity (hr)	1.18	-1.06, 1.85	0.234			
MVPA (hr)	1.07	-1.05, 1.24	0.105			
Lying time (hr)	-0.94	-0.82, 0.97	0.158			
Sleep duration (hr)	-0.97	-0.85, 1.05	0.582			

Data was transformed and analysed using $1og_{10}$; data presented here is back transformed to original units. BMI, body mass index; METS, metabolic equivalents; $\dot{V}O_2$ peak, cardiorespiratory fitness; MVPA, moderate-vigorous physical activity.

4.3.5 Body mass index (BMI)

Several factors were revealed from univariable linear regression analysis as significant predictors of BMI (Table 4.5). Average daily steps, vigorous activity, METS and $\dot{V}O_2$ peak were significantly associated with BMI, such that, greater levels observed in these measures were inversely associated with BMI. For every hour increase in daily sedentary time BMI was predicted to increase by 1.0 kg/m^2; whereas for every hour increase in daily light activity BMI was predicted to decrease by 1.7 kg/m^2. Multivariable analysis revealed that sedentary time, light activity, METS and $\dot{V}O_2$ peak were all independent predictors of BMI (Figure 4.2). However, daily average steps and vigorous activity were not. In this cohort, there was a modest correlation between BMI and liver fat (R^2= 0.6; P<0.005) as well as a low negative correlation with BMI and Matsuda index (R^2= -0.3; P=0.003).

Table 4.4 Univariate and multivariate regression for body mass index (BMI).

	Univariate			Multivariate		
	β coefficient	**95% CI**	***P***	**β coefficient**	**95% CI**	***P***
Age (years)	0.14	0.07, 0.21	**<0.0005**	0.09	0.02, 0.16	**0.013**
Steps (1,000)	-0.50	-0.78, -0.22	**0.001**	-0.17	-0.45, 0.11	0.223
Sedentary time (hr)	1.03	0.63, 1.43	**<0.0005**	1.70	0.56, 2.84	**0.004**
Light activity (hr)	-1.71	-2.36, -1.07	**<0.0005**	-2.20	-3.23, -1.18	**<0.0005**
Vigorous activity (hr)	-3.64	-6.11, -1.17	**0.004**	0.54	-3.15, 4.22	0.773
METS (0.1)	-0.81	-1.11, -0.51	**<0.0005**	-1.09	-2.01, -0.17	**0.021**
$\dot{V}O_2$ peak (ml/kg/min)	-0.32	-0.44, -0.21	**<0.0005**	-0.14	-0.28, 0.00	**0.050**
Moderate activity (hr)	-0.62	-1.52, 0.27	0.172			
MVPA (hr)	-0.71	-1.43, 0.01	0.052			
Lying time (hr)	0.36	-0.38, 1.10	0.336			
Sleep duration (hr)	0.75	-0.16, 1.66	0.104			

BMI, body mass index; METS, metabolic equivalents; $\dot{V}O_2$ peak, cardiorespiratory fitness; MVPA, moderate-vigorous physical activity.

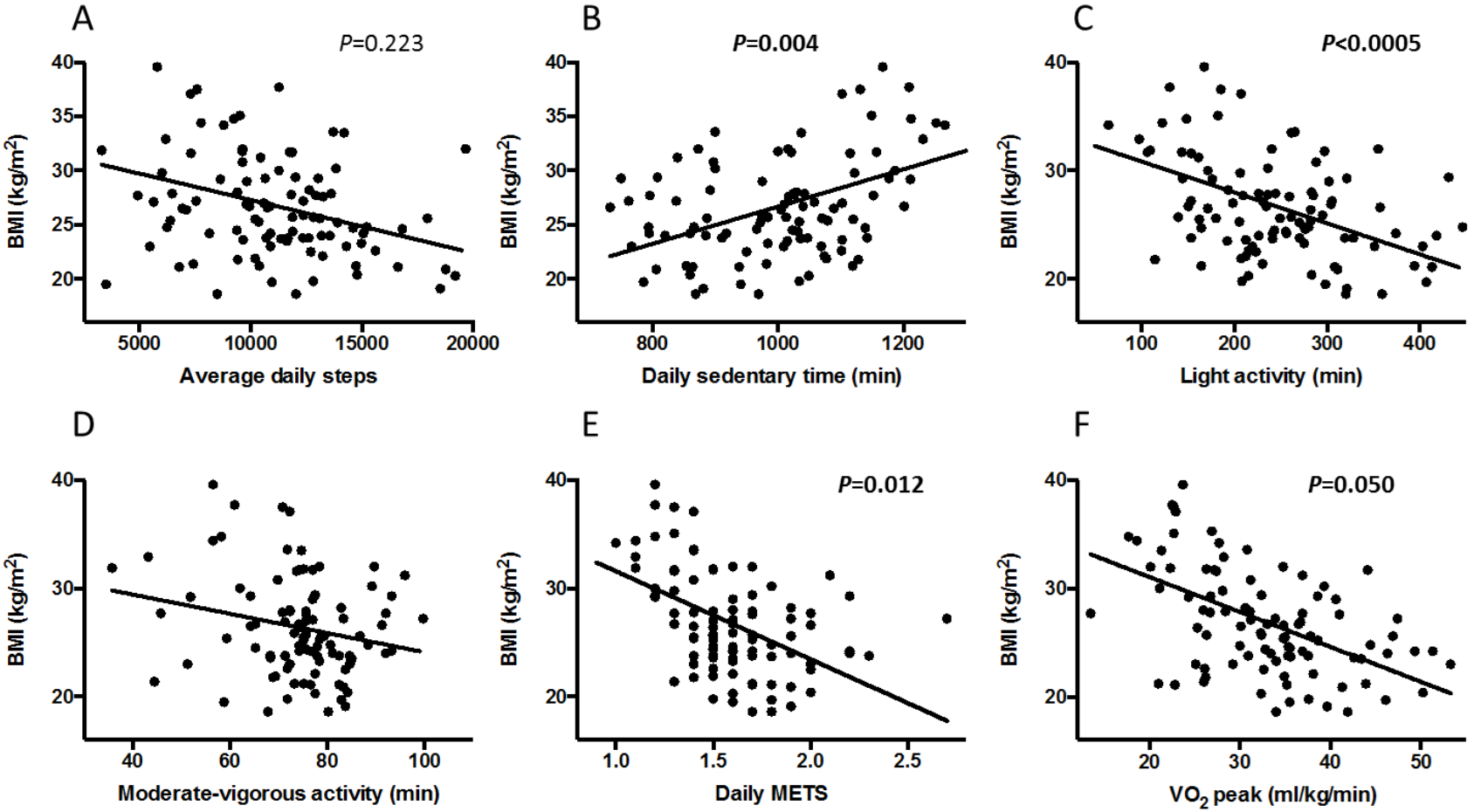

Figure 4.2 Association of body mass index (BMI) with A) daily average steps, B) sedentary time, C) light activity, D) moderate-vigorous activity, E) metabolic equivalents (METS) and F) $\dot{V}O_2$ peak; *P* value represents multivariate regression analysis.

4.4 Discussion

This study is the first to objectively monitor PA and sedentary behaviour with MRS derived quantification of liver fat in young to middle aged individuals. The data reveals that daily sedentary time is an independent predictor of liver fat percentage, such that a greater amount of daily sedentary time is associated with higher levels of liver fat. Sedentary time was also a significant predictor of BMI, as was daily light activity (1.5-3 METS). Taken together, sedentary time, overall daily METS and $\dot{V}O_2$ peak were the most predominant predictors of liver fat and BMI. MVPA was not significantly associated with either liver fat or BMI. In this cohort, whole body insulin sensitivity was not independently predicted by physical activity domains or fitness.

The strong inverse association with various types of PA and the prevalence of NAFLD has been recently reviewed (Qiu et al., 2017, Romero-Gómez et al., 2017, Zelber-Sagi et al., 2016). However, this study aimed to explore unresolved questions related to the physical behaviours associated with higher levels of liver fat. In a comparable study, Long *et al.*, found that higher levels of PA were significantly associated with liver fat however sedentary time was not (Long et al., 2015). Such contradictory findings in a similar cohort (~27% fatty liver, 28 kg/m^2 BMI) could be explained by mean sedentary time which was almost 3 hours greater than that of the current study. Moreover, liver fat was quantified by computed tomography (CT) therefore direct comparison is invalid. The majority of previous studies have examined the PA behaviours of those with and without NAFLD. This research approach does not determine whether because of low PA individuals have NAFLD or *vice versa* because of NAFLD individuals have low PA. Some authors have shown no association between liver fat and both PA and sedentary time (Keating et al., 2016) whereas others have concluded that PA and

sedentary time are independently associated with the prevalence of NAFLD (Hallsworth et al., 2015, Ryu et al., 2015).

Historically, chronic disease prevention strategies have largely focused on MVPA (i.e. exercise). Interestingly, no association was observed between MVPA and the measured health outcomes (liver fat, BMI and insulin sensitivity) in the current study. Compliance with national PA guidelines (150 min of moderate activity per week) has been associated with the lowest odds of fatty liver previously (Long et al., 2015). Although the current study did not selectively recruit for active participants, the majority were meeting PA guidelines which may explain the lack of association. That being said, strong evidence shows greater levels of MVPA are positively associated with metabolic equivalents (METS) and cardiorespiratory fitness ($\dot{V}O_2$ peak) (Garber et al., 2011). The results here reveal METS and $\dot{V}O_2$ peak as independent predictors of liver fat and BMI. The importance of overall physical activity and its influence on these two health parameters cannot be disregarded. Recent research in a population-based sample of adults shown that $\dot{V}O_2$ peak is strongly, inversely and independently related to the risk of liver fat (PÄLve et al., 2017). Importantly, and in agreement with the data presented here, the associations between $\dot{V}O_2$ peak and liver fat remained after adjustment for BMI; not all previous studies have similar findings (Minder et al., 2014, Church et al., 2006).

BMI was investigated in the current study given its potential role in the development of liver fat and other cardiometabolic complications. The results demonstrate that less daily sedentary time and greater light activity, METS and $\dot{V}O_2$ peak was associated with reduced BMI. These data further reiterate a public health message that places emphasis on day to day activity. Irrespective of BMI, many studies have strongly indicated the importance of regional body fat distribution in the prevalence of NAFLD

(Kim et al., 2016, Park et al., 2008, van der Poorten et al., 2008). It is proposed that larger areas of visceral adipose tissue (VAT) are associated with increased risk of NAFLD whereas larger areas of subcutaneous adipose tissue (SAT) are associated with a reduced risk (Kim et al., 2016). Whilst our study design included the measurement of these fat depots, technical issues during a period of these investigations meant that VAT and SAT quantification was only available for 72 individuals (Appendix 1 and 2 respectively) and as such, could not be included in overall analysis of liver fat due to differences in sample size. The abdominal location of VAT and its greater metabolic activity has been shown to have more adverse effects than SAT. Albeit controversial, Després has questioned whether increased VAT is a culprit of liver fat or merely a marker (Despres, 2012). Notably, liver fat, but not total body fat or VAT, has been identified as an independent predictor of insulin resistance (Linder et al., 2014).

Insulin resistance plays a key role in the development of liver fat via several mechanisms including reduced skeletal muscle glucose uptake, impaired suppression of hepatic glucose production and increased circulating free fatty acids from adipose tissue. Although whole body insulin sensitivity was not predicted by physical activity domains or fitness in the current cohort, certain trends deserve discussion and potential further investigation. In a recent review (Bird and Hawley, 2016), studies involving PA confirm its efficacy in improving insulin sensitivity. A lifestyle that complies with the PA guidelines is associated with optimal insulin sensitivity. The failure here to observe an association in this cohort could be attributed again to the majority of participants being ‘recreationally active’ or further due to relatively ‘good’ insulin sensitivity. Future investigation in a larger cohort with a greater span of physical activity levels and/or glycaemic control may be beneficial.

It is recognised that breaks within sedentary time are also independently associated with metabolic risk (Healy et al., 2008). Whilst the results of the current study demonstrate that overall sedentary time needs to be considered independently of physical activity, future research should investigate sedentary behaviour patterns. A low sample size is a limitation of this research, as is the cross-sectional approach which removes the ability to assign causality. It is also important to note that the assocations observed with PA domains and liver fat were very week, absolute data is shown in Appendix, Table 8.1; a larger cohort of study is required. Recent prospective studies confirm the relationship between PA and NAFLD (Gerage et al., 2017, Kwak et al., 2017). However, prospective research must also employ such objective measures of PA to provide robust evidence. It is worth noting that even in adults meeting PA guidelines, a large amount of sedentary time increases the risk of becoming overweight or developing metabolic disorders (Levine et al., 2005). Targeting a change in sedentary behaviour can be considered as either an additional to physical activity guidelines or as a minimal requirement to improve health. In a quest to develop prevention strategies that are adoptable in a population reluctant to move more, advancing the knowledge within this field is paramount.

In conclusion, a greater amount of sedentary time independently predicted a greater amount of liver fat. MVPA did not significantly influence liver fat, BMI and insulin sensitivity in this recreationally active cohort. Light activity and sedentary behaviour were associated with BMI with overall daily METS and $\dot{V}O_2$ peak associated with both liver fat and BMI. An increasing amount of evidence suggests a role of sedentary time in the development of, or predisposition towards NAFLD, independent of physical activity/exercise. Targeting sedentary time in addition to physical activity and exercise recommendations seems vital. Public health messages need to emphasise the

independent importance of reduced sedentary time in even healthy, and somewhat physically active individuals. As the epidemic of obesity, T2D and NAFLD continues to grow, 'behavioural change' strategies at the population-level are needed.

Chapter 5.

Metabolic decompensation and altered body composition after short-term physical inactivity in first-degree relatives of patients with type 2 diabetes versus healthy controls

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KBD specific contribution to the study: Design, ethical approval and set up of the study, recruitment and participant management, coordination of testing visits including physical activity and dietary monitoring, anthropometrics, blood collection/OGTT, $\dot{V}O_2$ peak and MR/DXA scanning.

5.1 Introduction

Epidemiological evidence indicates that physical inactivity and sedentary behaviour are major causal factors in the development of obesity, insulin resistance and type 2 diabetes (T2D) (Lee et al., 2012, Henson et al., 2013). However, such data provides no mechanistic insight into these pathophysiological changes. One plausible paradigm suggests that physical inactivity causes a reduction in skeletal muscle insulin sensitivity, contributing to a repartitioning of energy substrates into storage, increasing central fat accumulation and ectopic storage within the liver and other organs, causing further insulin resistance (Jornayvaz et al., 2010, Rabol et al., 2011, Rector and Thyfault, 2011, Petersen et al., 2007, DeFronzo and Tripathy, 2009). As peripheral insulin resistance progresses, continued ectopic fat accumulation within the liver and pancreas precipitates development of metabolic syndrome, a progressive decline in ß-cell function and ultimately T2D (Tan and Vidal-Puig, 2008).

To date, mechanistic human studies pertaining to inactivity have used extreme experimental models including bed rest (Alibegovic et al., 2010), limb immobilisation (Abadi et al., 2009) and cessation of exercise in trained volunteers (King et al., 1988). These models are not physiologically representative of habitual activity levels in free-living individuals, and should be interpreted cautiously. More recently, an alternative experimental model has been developed in which active (~10,000 steps/day), healthy volunteers, who do not participate in regular exercise, transition to an inactive lifestyle (reducing up to ~1,500 steps/day) for brief periods (~14 days). This approach reflects societal changes in physical activity levels (i.e. reduced physical activity and more sedentary time) (Church et al., 2011, Althoff et al., 2017). Step-reduction models have demonstrated that physical inactivity results in detrimental physiological changes including reduced cardiorespiratory fitness, accumulation of central fat, loss of skeletal

muscle mass with associated anabolic resistance, and reductions in peripheral insulin sensitivity (Krogh-Madsen et al., 2010, Olsen et al., 2008, Knudsen et al., 2012, Breen et al., 2013). Importantly, such research provides a mechanistic basis for the consequences of increased sedentary behaviour and aids understanding of the development of metabolic disease.

First-degree relatives (FDR) of individuals with T2D have a 3-fold increased risk of developing the disease themselves compared to those without a family history (Meigs et al., 2000); this risk can further be increased with low physical activity levels (Hu et al., 1999). No previous studies have employed a step-reduction protocol to examine whether individuals genetically predisposed to T2D are more susceptible to the adverse metabolic consequences of an inactive lifestyle, compared to those who are not. Given that group-specific physical activity guidelines have been proposed for other high-risk groups (e.g. South Asians) and that the exercise dose-response curve clearly differs between populations (Celis-Morales et al., 2013), this is a very pertinent research question. Furthermore, only one other study has investigated whether the detrimental effects of step-reduction are reversed when habitual physical activity is resumed (Knudsen et al., 2012).

The primary aim of this study is to investigate the consequences of short-term physical inactivity (>80% step-reduction) in FDR of T2D versus healthy controls (CON), hypothesising that FDR will have greater adverse changes as a direct result of reduced activity. The secondary aim of this study is to investigate the recovery responses when activity levels are resumed, hypothesising an impaired recovery in those with FDR.

5.2 Methods

5.2.1 Screening and eligibility

A two tiered screening process was employed. As summarised in Figure 5.1, tier one *screened for eligibility* and tier two *screened for intervention.* In the first instance, individuals who expressed their interest in the study were screened for eligibility by questionnaire over the telephone or email. This process required individuals to detail their activity levels which excluded 64 individuals who were doing >2 hours of structured exercise per week. The feasibility of step-reduction and commitment time required for assessment visits were discussed in depth with each person individually to ensure full understanding and compliance to protocol, 18 individuals declared they were 'unable to commit' due to occupation or caring commitments. Medical exemptions included unsuitability for magnetic resonance (MR) and prescribed medications, excluding 9 individuals.

Of those that were screened for intervention, which included physical activity monitoring (outlined in detail below), 13 individuals over estimated their daily step count and were therefore excluded (<10,000 steps/day). Following commencement of the study, there was a 10% drop out rate i.e. *withdrawals* which were due to participant circumstance, namely occupational commitments. The term c*ompleted intervention* refers to attendance to all assessment visits, complete adherence to step-reduction and monitoring of physical activity throughout, analysis was per protocol. One FDR

participant was excluded for insufficient wear time of the activity monitor and one CON participant for absence of significant step-reduction.

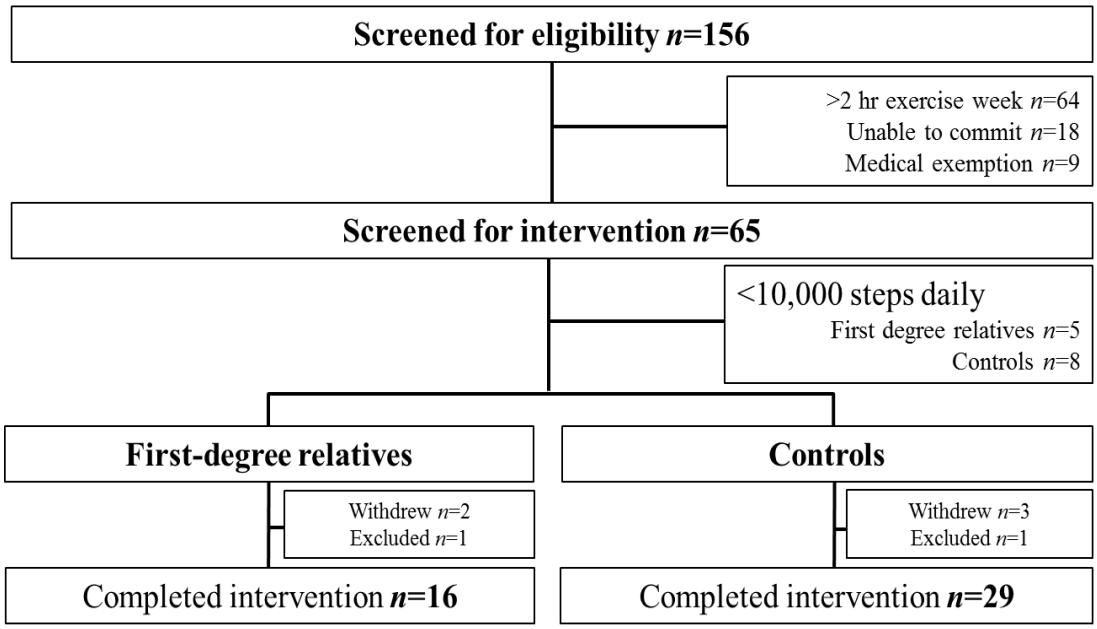

Figure 5.1 Screening, recruitment, retention and completion study numbers.

Embedded in the screening process was instruction of how to feasibly achieve ~1,500 steps per day. This had to be well thought out, discussed and planned prior to the commencement of the step-reduction period and dates meticulously planned around the participants work and social life. Bespoke advice was given to each participant including suggestions such as; public transport, car sharing, taxis, taking the elevator instead of stairs, online shopping, working from home as well as spending more time doing sedentary hobbies (e.g. television viewing, creative writing, crafts).

5.2.2 Participants

All participants were habitually physically active with no history of regular structured exercise or highly physical employment. Individuals who had any cardiovascular, respiratory, kidney, liver and/or endocrine complications were excluded as were smokers and those who consumed >14 units of alcohol per week were excluded. A FDR is classed as a parent, sibling or child of somebody who has received a clinical diagnosis of T2D. Control participants (CON) confirmed no FDR with T2D.

5.2.3 Physical activity screening and habitual dietary assessments

Physical activity (PA) screening was conducted in a free-living environment and consisted of monitoring from midnight to midnight on 4 consecutive days, including one weekend day. Eligibility requirements were >10,000 steps per day. During this period physical activity was monitored using a SenseWear armband (Model MF-SW, BodyMedia, UK). The participants were instructed to wear the activity monitor at all possible times, inclusion criteria was >90% wear time which was monitored using SenseWear Professional software (version 8.0). The data collected included average daily step count, total energy expenditure and time spent in domains of activity including: sleep, sedentary (<1.5 METS), light (1.5-3 METS), moderate (3-6 METS), vigorous (6-9 METS) and very vigorous (>9 METS) activity. Dietary records were collected during the same 4 days screening period. Participants were instructed to maintain their usual habitual behaviours throughout the screening period.

5.2.4 Research design

Following PA screening, participants underwent their initial assessment visits before being instructed to reduce their activity to ~1500 steps for a 14 day period after which the assessments were repeated (Figure 5.2). Normal habitual activity was then resumed for 14 days before participants underwent their final assessment visit. Screening and resumption of normal activity were blinded to the participant as the armband does not have a display or provide any output of information. If participants successfully met the inclusion criteria they were issued with a display device which was synchronised with the physical activity monitor armband to allow participants to adequately monitor and reduce their steps, during step-reduction. Dietary records were obtained 4 days prior to an assessment visit.

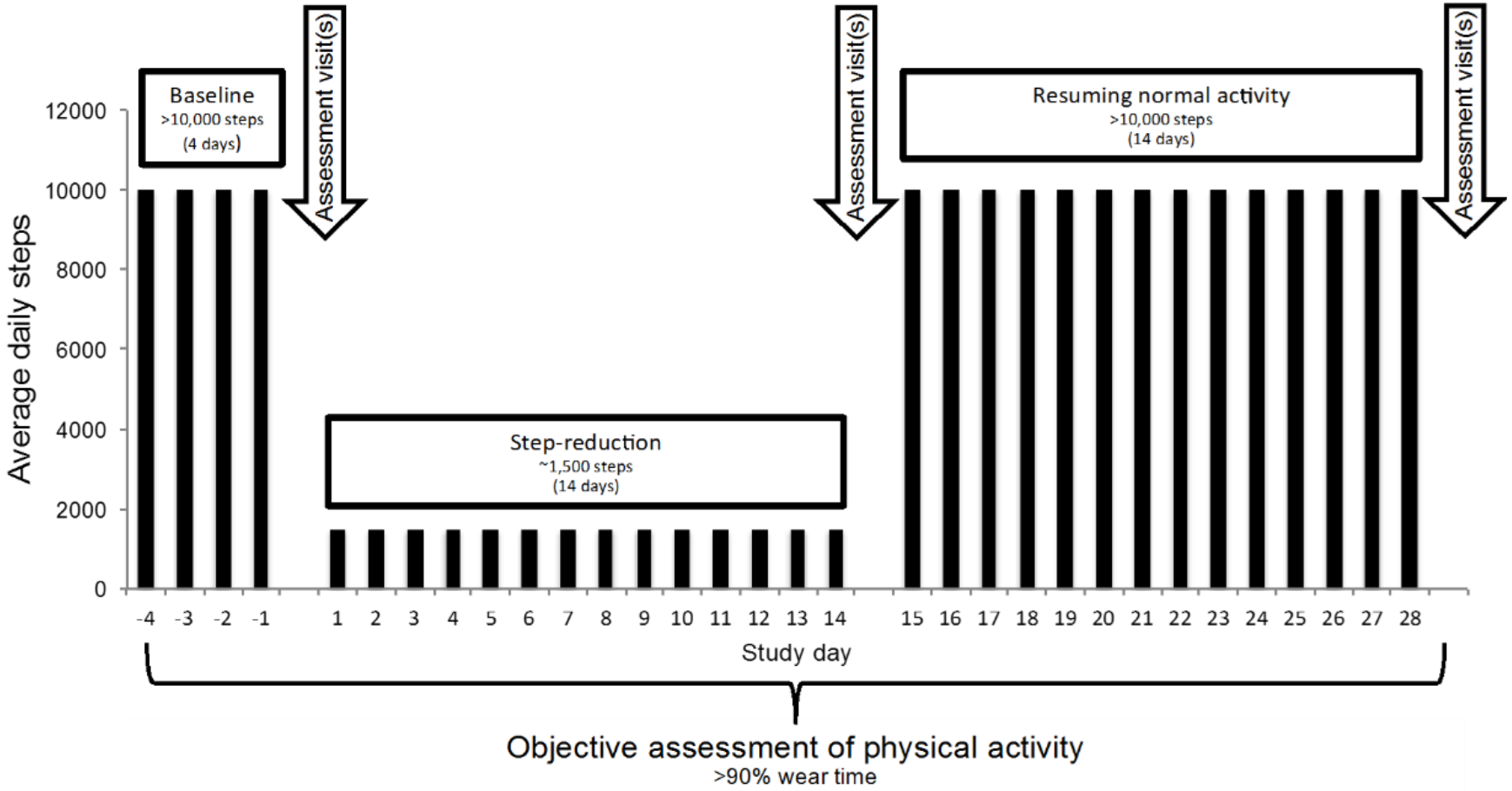

Figure 5.2 Schematic of study design for step-reduction intervention. Complete objective assessment of physical activity throughout screening (baseline), step-reduction and resumption of normal activity. Y axis represents daily average step and X axis study day.

5.2.5 Assessment measures

Assessment visits were conducted on 3 separate occasions, this included baseline (following 4 day screening), step-reduction (following 14 day reduced physical activity) and resuming activity (following 14 day resumption of normal activity) (Figure 5.2). At each time point there were two visits. Visit one included body composition measures at LiMRIC and visit two metabolic measures at University Hospital Aintree. For details of the experimental procedures please refer to *Chapter 2, General Methods*. Participants were required to fast overnight for >8 hours, abstain from alcohol and caffeine 24 hours and refrain from exercise 48 hours prior to experimental appointments. All participants were studied at the same time of day to account for circadian variation (Jones et al., 2010).

5.2.6 Sample size calculation

The primary outcome variable for this study was the change in insulin sensitivity between the two groups. Based upon previous data (Knudsen et al., 2012) and using MINITAB 16; a sample size of 50, SD=1.1 (25 in each group) was calculated using an interpendant *t*-test to have ≥80% power to detect a standardized mean difference of 0.89 for insulin sensitivity using Matsuda index with 5% significance level, assuming a 20% drop out rate (20 completed participants per group).

5.2.7 Statistical analysis

All data were explored for distribution using visual inspection of frequency distribution, and logarithmically transformed using $1og_{10}$ or log_{sqr}, where appropriate. Baseline analysis was performed on clinical characteristics of first-degree relatives (FDR) compared with control (CON) individuals using independent *t*-tests. The coefficient of determination (R^2) was calculated from Pearson correlation coefficients

to evaluate collinearity between variables. Data were then analysed whilst controlling for baseline differences as model covariates. The groups' responses to the intervention were compared by calculating delta (Δ) change and analysed using a two factor between-groups (group x time) analysis of covariance (ANCOVA) with respective baseline data entered as a covariate. The term *intervention* refers to step-reduction and resumption of normal activity. Statistically significant interactions were assessed using the least significant difference (LSD) approach to multiple pairwise comparisons. Paired sample *t*-tests were used to assess for any differences between baseline and resumption of activity. The alpha level of statistical significance was set at $P<0.05$. Data are presented as mean (95% confidence intervals [CI]), unless stated otherwise and exact *P* values are cited (values of *P* of "0.000" provided by the statistics package are reported as "<0.0005"). Logarithmically transformed data were back transformed to original units and presented in the text as mean (95% CI). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows (Version 24.0, SPSS Inc., Chicago, IL, USA) statistic software package.

5.3 Results

5.3.1 Participant demographics

A total of forty-five participants were included in this study, 28 females and 17 males. FDR included 10 female, and 6 male, CON included 18 female, and 11 male; percentage splits were 62% female and 38% male for each. Mean age of all the participants was 36 ± 14 years; FDR were on average 7 (3, 14) years older than CON (Table 5.1). An average daily step count >10,000 was required to be included in the study. FDR demonstrated a mean daily step count of 12524 steps/day (11214, 13835), CON had a mean daily step count of 13036 steps/day (12035, 14037); there was no significant difference between groups ($P=0.391$).

5.3.2 Baseline characteristics

The baseline characteristics of FDR and CON groups are listed in Table 5.1; FDR had significantly greater values for BMI, overall body mass, waist circumference and hip circumference (P<0.05). Fasting insulin and HOMA-IR were significantly higher in CON. All other variables were not statistically significant. BMI had evidence of collinearity with body mass (R^2=0.645), waist circumference (R^2=0.703) and hip circumference (R^2=0.719). As a representative measure of these differences within body composition, BMI was selected as a valid covariate within the ANCOVA model. Fasting insulin and HOMA-IR had high evidence of collinearity (R^2 =0.943), HOMA-IR was selected as a valid covariate and fasting insulin was removed from the analyses.

Table 5.1 First-degree relatives (FDR, *n*=16) and control (CON, *n*=29) subject characteristics at baseline, displaying mean (95% confidence interval) and statistical significance.

	FDR	CON	*P* value
Clinical characteristics			
Age (years)	40 (31, 48)	33 (28, 39)	0.166
Body mass (kg)	79.3 (72.2, 86.4)	68.9 (65.1, 72.6)	**0.011**
BMI (kg/m^2)	27 (24, 29)	24 (23, 25)	**0.018**
Waist circumference (cm) ¥	93 (86, 100)	84 (80, 88)	**0.029**
Hip circumference (cm) ¥	103 (97, 108)	95 (92, 98)	**0.015**
Waist: hip ratio	0.91 (0.86, 0.95)	0.89 (0.86, 0.92)	0.052
SBP (mmHg)	122 (114, 129)	121 (116, 127)	0.932
DBP (mmHg)	74 (70, 78)	76 (72, 80)	0.438
Physical fitness and activity			
$\dot{V}O_2$ (l/min)	2.5 (2.2, 2.8)	2.4 (2.2, 2.6)	0.676
$\dot{V}O_2$ peak (ml/kg/min)	32.1 (28.5, 35.8)	35.3 (32.9, 37.9)	0.136
$\dot{V}O_2$ peak lean (ml/kg/min)	50.1 (47.1, 53.1)	52.6 (50.0, 55.3)	0.189
Energy expenditure (kJ/day)	12251 (11019, 13483)	11383 (10189, 12577)	0.408
Average steps (steps/day)	12524 (11214, 13835)	13036 (12035, 14037)	0.391
Daily sedentary time (min)	1021 (948, 1093)	989 (940, 1038)	0.498
Daily light activity (min)	231 (184, 280)	240 (210, 270)	0.650
Daily moderate activity (min)	130 (102, 154)	139 (114, 164)	0.306
Daily vigorous activity (min)	21 (3, 38)	24 (12, 37)	0.550
Lipid profile			
Cholesterol (mmol/l)	5.1 (4.5, 5.7)	4.9 (4.6, 5.2)	0.681
Triglycerides (mmol/l)	1.0 (0.7, 1.2)	1.0 (0.8, 1.2)	0.524
HDL (mmol/l)	1.8 (1.5, 2.2)	1.8 (1.6, 2.0)	0.907
LDL (mmol/l)	2.8 (2.2, 3.4)	2.6 (2.3, 2.9)	0.475
Cholesterol: HDL ratio	3 (2, 4)	3 (2, 3)	0.400

Glucose regulation			
Fasting glucose (mmol/l)	5.0 (4.6, 5.4)	5.0 (4.7, 5.2)	0.994
Fasting insulin (µlU/ml) ¥	12 (9, 15)	20 (15, 24)	**0.002**
HOMA-IR ¥	2.8 (2.2, 3.4)	4.3 (3.3, 5.2)	**0.003**
Glucose AUC (mmol/l)	780 (652, 947)	799 (729, 870)	0.928
Insulin AUC (µlU/ml)	8261 (5669, 10652)	9192 (7911, 10473)	0.468
Whole body IS (Matsuda index)	4.1 (3.0, 5.3)	3.2 (2.3, 4.0)	0.155
Hepatic-IR index (HIRI)	33.4 (27.5, 39.1)	40.5 (36.3, 44.7)	0.058
Muscle-IS index (MISI)	0.07 (0.03, 0.10)	0.06 (0.05, 0.08)	0.749
Body composition			
Total body fat (%)	33 (26, 40)	30 (27, 33)	0.279
Android fat (%)	36 (27, 45)	30 (26, 35)	0.134
Gynoid fat (%)	35 (27, 43)	34 (30, 37)	0.581
Total lean mass (kg)	49.5 (44.0, 55.0)	46.0 (42.5, 49.6)	0.150
Liver IHCL % (CH_2/H_2O) ¥	3.0 (0.6, 5.4)	0.7 (0.4 1.1)	0.064
Muscle IMCL % (CH_2/creatine)	9.1 (5.8, 12.5)	6.4 (5.4, 7.4)	0.109

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; $\dot{V}O_2$, maximal oxygen uptake; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; IHCL, intrahepatocellular lipid; IMCL, intramyocellular lipid. ¥ variables analysed following logarithmic transformation.

5.3.3 Physical activity

The intervention induced significant changes in all activity measures (Figure 5.3). During the step-reduction period, average daily step count decreased by 10435 steps/day (10810, 10060; $P<0.0005$), a reduction of 81%. In parallel, sedentary time increased by an average of 130 min/day (92, 168; $P<0.0005$) and total energy expenditure (TEE) decreased by an average of 2697 kJ/day (3008, 2385; $P<0.0005$). All activity >1.5 METS (i.e. light, moderate and vigorous) decreased during the step-reduction period (all $P<0.0005$). There were no statistical differences between the groups in average daily step count, TEE, sedentary, light and moderate activity at any of the time points ($P>0.05$). Vigorous activity was significantly higher in CON ($P=0.001$); when explored, the group difference was not present at step-reduction ($P=0.988$) but rather at resuming activity ($P=0.006$). CON increased their vigorous activity during resumption of activity to a greater extent than FDR (Figure 5.3F). There were no significant differences in any of the physical activity measures between baseline and following resumption of activity ($P>0.05$).

5.3.4 Dietary analysis

Total energy consumption did not change throughout ($P=0.330$) and there was no difference between groups ($P=0.372$). Mean ±SD macronutrient percentages were 56±15% carbohydrate, 24±10% protein, and 20±9% fat, these did not change throughout ($P=0.235$, $P=0.268$, $P=0.924$ respectively) and there was no difference between groups ($P=0.660$, $P=0.179$, $P=0.177$ respectively).

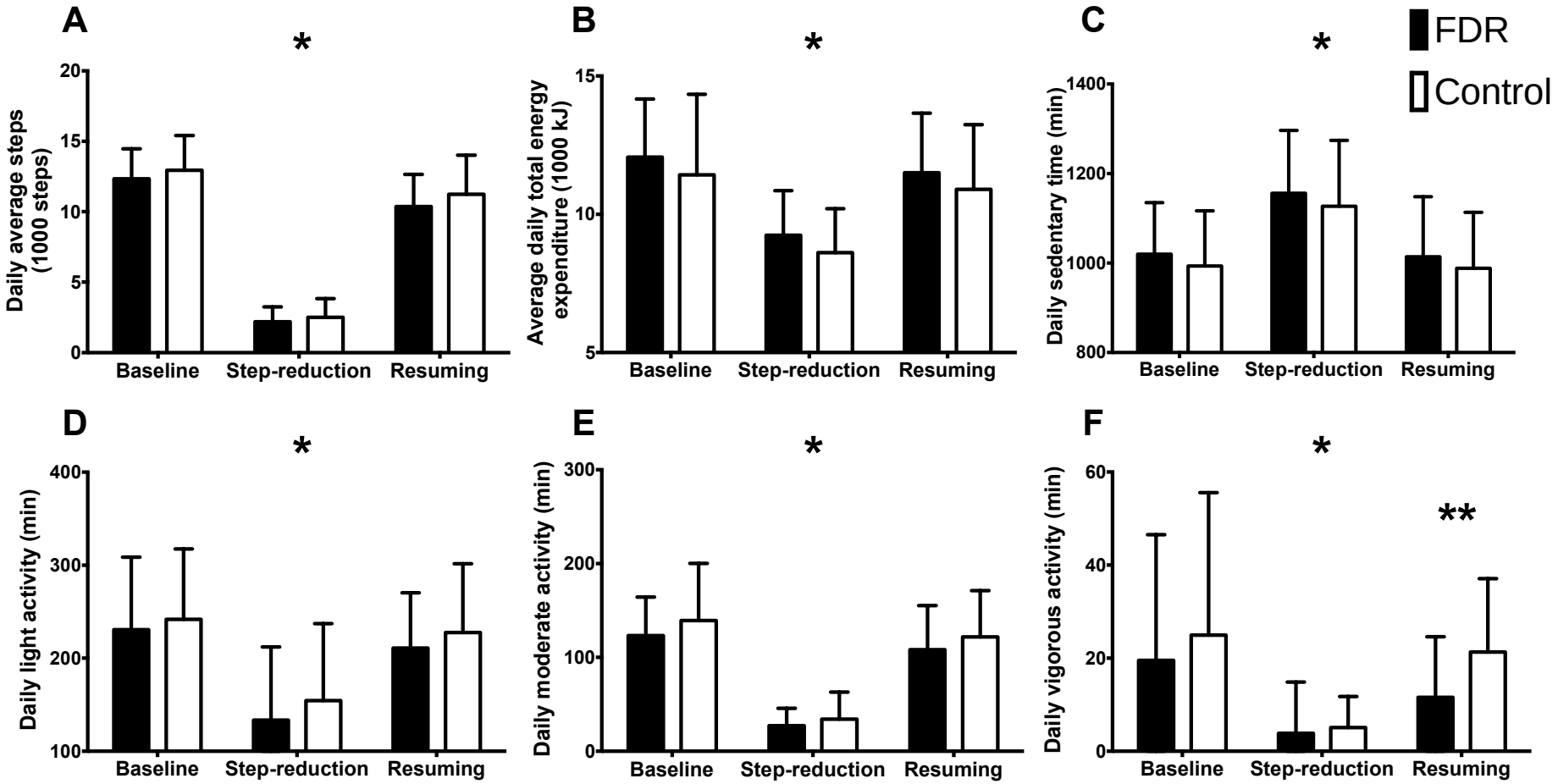

Figure 5.3 Physical activity data first-degree relatives (FDR) and controls (CON) at baseline, following step-reduction and resuming activity including A) daily average step count, B) total energy expenditure, C) daily sedentary time (<1.5 METS), D) daily light activity (1.5-3 METS), E) daily moderate activity (3-6 METS) and F) daily vigorous activity (>6 METS); mean (SD), **P*<0.05 main effect of time; ***P*<0.05 statistical difference between groups.

In the most part, the results presented herein are those observed in the entire sample (pooled FDR and CON groups). Where statistical differences occur between the groups or there is a significant interaction effect, it is explicitly noted.

5.3.5 Anthropometrics and blood pressure

Body mass, BMI and waist to hip ratio did not change in either group (P=0.611, P=0.553 and P=0.385 respectively). Pooled waist and hip circumference measures increased by 0.7 cm (0.4, 1.0) and 0.4 cm (0.1, 0.7) respectively (P<0.0005) after step-reduction. Systolic blood pressure (SBP) increased by 4 mmHg (0, 8; P=0.037) following step-reduction, diastolic blood pressure (DBP) remained unchanged (P=0.982). There were no between group differences in any of the clinical characteristic changes.

5.3.6 Biochemical measures

Fasting glucose and insulin

Fasting glucose was not significantly altered by the intervention, however, fasting insulin increased following step-reduction and decreased following resumption of habitual activity but to a similar extent in both groups (P=0.016). For fasting insulin changes, FDR showed augmented changes (P=0.046) while CON was not statistically different (P=0.278) (Table 5.2).

Table 5.2 Data of first-degree relatives (FDR) and controls (CON) following step-reduction and resuming activity; delta (Δ) change values ± SD; between-group difference (95% CI); *p* value for main group difference; [*p* value for pooled effect of time].

	FDR		**CON**		**Between-group difference**		***p* value** Group [Time]
	Δ Step-reduction	**Δ Resuming activity**	**Δ Step-reduction**	**Δ Resuming activity**	**Step-reduction**	**Resuming**	
Clinical characteristics							
Body mass (kg)	-0.1 ± 1.0	-0.1 ± 1.2	0.1 ± 1.0	0.3 ± 1.2	-0.2 (-0.8, 0.5)	-0.4 (-1.2, 0.4)	0.241 [0.611]
BMI (kg/m^2)	0 ± 0.3	0 ± 0.4	0 ± 0.3	0 ± 0.4	0 (-0.3, 0.2)	0 (-0.4, 0.2)	0.369 [0.553]
WC (cm) ¥	0.6 ± 1.0	-0.3 ± 0.9	0.7 ± 1.0	-0.4 ± 0.8	-0.1 (-0.8, 0.6)	0.1 (-0.5, 0.7)	0.934 **[<0.005]**
HC (cm) ¥	0.3 ± 0.5	-0.3 ± 0.6	0.1 ± 0.5	-0.1 ± 0.6	0.2 (-0.2, 0.5)	-0.2 (-0.7, 0.2)	0.670 **[<0.005]**
SBP (mmHg)	-1 ± 9	-3 ± 8	2 ± 8	-4 ± 8	-2 (-8, 4)	1 (-4, 7)	0.654 **[0.037]**
DBP (mmHg)	-1 ± 8	-2 ± 9	-1 ± 8	-1 ± 8	1 (-5, 6)	-1 (-7, 5)	0.846 [0.982]
Glucose metabolism							
Fasting glucose (mmol/l)	-0.1 ± 0.5	0.1 ± 0.4	-0.1 ± 0.5	-0.1 ± 0.4	0.1 (-0.3, 0.3)	0.2 (-0.1, 0.5)	0.171 [0.294]
Fasting insulin (pmol/l) ¥	34 ± 9	-35 ± 11	17 ± 8	-11 ± 11	18 (-24, 59)	-25 (-79, 30)	0.669 **[0.016]**
HOMA-IR ¥	1.1 ± 2.1	-1.1 ± 2.6	0.4 ± 2.1	-0.4 ± 2.6	0.7 (-0.7, 2.1)	-0.7 (-2.5, 1.0)	0.911 **[0.027]**
Whole body IS (Matsuda)	-0.7 ± 1.2	0.3 ± 2.3	-0.9 ± 1.2	1.3 ± 2.2	0.1 (-0.7, 1.0)	-1.1 (-2.7, 0.5)	0.254 **[<0.005]**

Lipid profile							
Cholesterol (mmol/l)	0.2 ± 0.8	-0.2 ± 0.9	0.2 ± 0.8	-0.3 ± 0.8	-0.1 (-0.6, 0.4)	0.9 (-0.5, 0.7)	0.962 **[0.041]**
HDL (mmol/l)	-0.1 ± 0.3	0 ± 0.4	0 ± 0.3	0 ± 0.4	0 (-0.2, 0.2)	0 (-0.3, 0.2)	0.495 [0.641]
LDL (mmol/l)	0.8 ± 0.5	-0.1 ± 0.6	1.2 ± 0.5	-0.2 ± 0.5	-0.2 (-0.5, 0.2)	0.2 (-0.2, 0.5)	0.930 **[0.013]**
Body composition							
Total lean mass (kg)	-0.6 ± 0.9	0.4 ± 1.2	-0.1 ± 0.9	0.4 ± 1.1	-0.4 (-1.0, 0.2)	0.2 (-0.6, 1.0)	0.598 **[0.005]**
Leg lean mass (kg)	-0.1 ± 0.4	0.2 ± 0.5	-0.2 ± 0.4	0.2 ± 0.5	0.2 (-0.1, 0.4)	-0.1 (-0.4, 0.3)	0.589 **[0.004]**
Arm lean mass (kg)	0.1 ± 0.2	0.1 ± 0.2	-0.1 ± 0.2	0.1 ± 0.2	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.2)	0.607 [0.502]
Cardio-respiratory fitness							
$\dot{V}O_2$ (l/min)	-0.2 ± 0.3	0.3 ± 0.4	-0.1 ± 0.3	0.1 ± 0.4	-0.1 (-0.3, 0,1)	0.2 (-0.1, 0.5)	0.376 **[0.002]**
$\dot{V}O_2$ peak (ml.min^{-1}.kg^{-1})	-3.0 ± 4.8	3.6 ± 5.6	-1.4 ± 4.6	0.9 ± 5.5	-1.6 (-4.8, 1.6)	2.7 (-1.1, 6.7)	0.370 **[0.002]**
$\dot{V}O_2$ lean (ml.min^{-1}.kg^{-1})	-4.0 ± 7.3	4.9 ± 8.5	-1.9 ± 6.9	1.1 ± 8.1	-2.2 (-7.1, 2.7)	3.9 (-1.8, 9.6)	0.394 **[0.006]**

BMI, body mass index; WC, waist circumference; HC hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; $\dot{V}O_2$, maximal oxygen uptake; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance. ¥ Variables analysed following logarithmic transformation.

NB. Absolute values of each variable are presented to the nearest accuracy of measurement. Where Δ change values do not demonstrate a difference, data are presented with one decimal place (dp), if by laws of rounding one dp does not suffice *that value only* is increased to 2 dp.

Lipid profile

Total cholesterol significantly increased by 0.5 mmol/l (0.1, 0.9; p=0.041) following step reduction: HDL cholesterol remained unchanged (*P*=0.641) while LDL significantly increased by 0.3 mmol/l (0.1, 0.6; *P*=0.013). Triacylglycerol pooled outcomes also increased following step-reduction by 0.5 mmol/l (0.2, 0.7; *P*=0.002) but a between-group difference was present whereby FDR had a 0.3 mmol/l greater change (0.1, 0.6; *P*=0.044) (Figure 5.4).

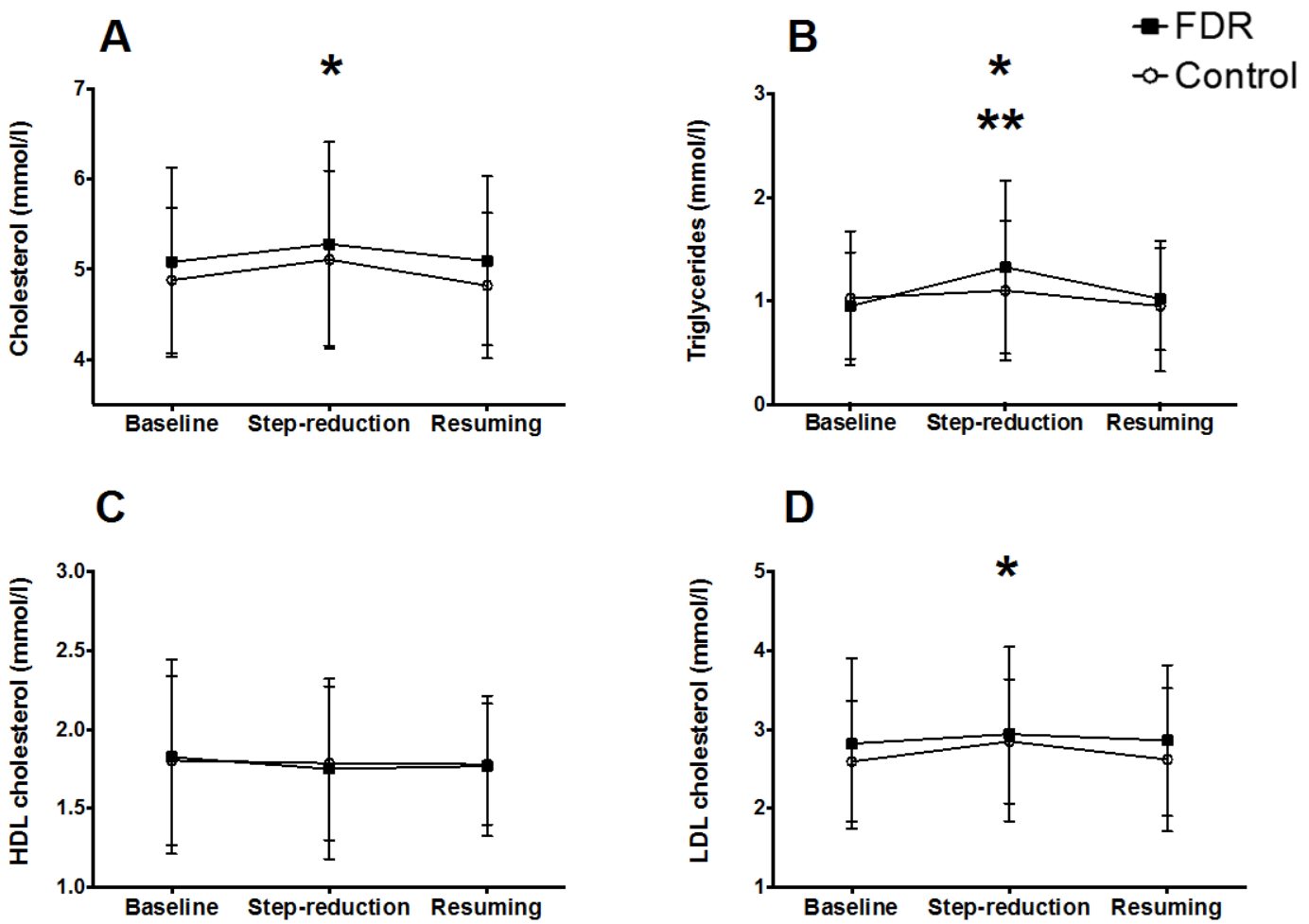

Figure 5.4 Lipid blood data first-degree relatives (FDR) and controls (CON) at baseline, following step-reduction and resuming activity including A) cholesterol, B) triglycerides, C) high density lipoprotein (HDL) cholesterol D) low density lipoprotein (LDL) cholesterol; mean (SD), *P<0.05 main effect of time; **P<0.05 statistical difference between groups.

5.3.7 Cardiorespiratory fitness

The period of reduced physical activity significantly lowered cardio-respiratory fitness across the study population, whichever way this was expressed (absolute, relative to body weight or lean body mass): $\dot{V}O_2$ by 0.3 l/min (0.5, 1.0; *P*=0.002), $\dot{V}O_2$ peak per kg of body mass by 2.2 ml/kg/min (0.9, 3.6; *P*=0.002) and $\dot{V}O_2$ peak per kg of lean body mass by 2.9 ml/kg/min (0.9, 5.0; *P*=0.006) (Figure 5.5A) with no between-group differences for any of these measures.

5.3.8 Body composition

Lean body mass

Total lean mass decreased by 0.3 kg (0.1, 0.6; *P*=0.005), as did total leg lean mass by 0.2 kg (0.1, 0.3*; P*=0.004), but there was no significant change in arm lean mass (*P*=0.502).

Regional fat mass

Step-reduction induced similar changes in both groups total body fat which increased by 0.9% (0.6, 1.3; *P*<0.0005) and android fat which increased by 1.7% (1.1, 2.3; *P*<0.0005). In the two groups combined, gynoid fat increased by 0.6% (0.3, 0.8; *P*=0.001). However, a between-group difference was found whereby FDR accumulated 1.5% more android fat (0.4, 2.6; *P*=0.008) following step-reduction (Figure 5.6B).

Liver fat

IHCL increased following the period of reduced activity by 0.7 % CH_2/H_2O (0.2, 1.2; *P*=0.001). There were no statistically significant differences between groups in liver IHCL responses to the intervention, although a main effect for group approached statistical significance (*P*=0.066), FDR showing a trend for greater changes in IHCL compared to CON.

Skeletal muscle fat

Muscle IMCL increased 1.0 % CH_2/creatine (-0.3, 2.3) after the step-reduction phase but this did not reach statistical significance (P=0.094).

Within-group comparisons Several within-group responses were different in terms of reaching statistical significance. All measures of cardio-respiratory fitness (absolute, relative to body weight and relative to lean body) were significant in FDR but not CON. As was fasting insulin and free-fatty acid AUC.

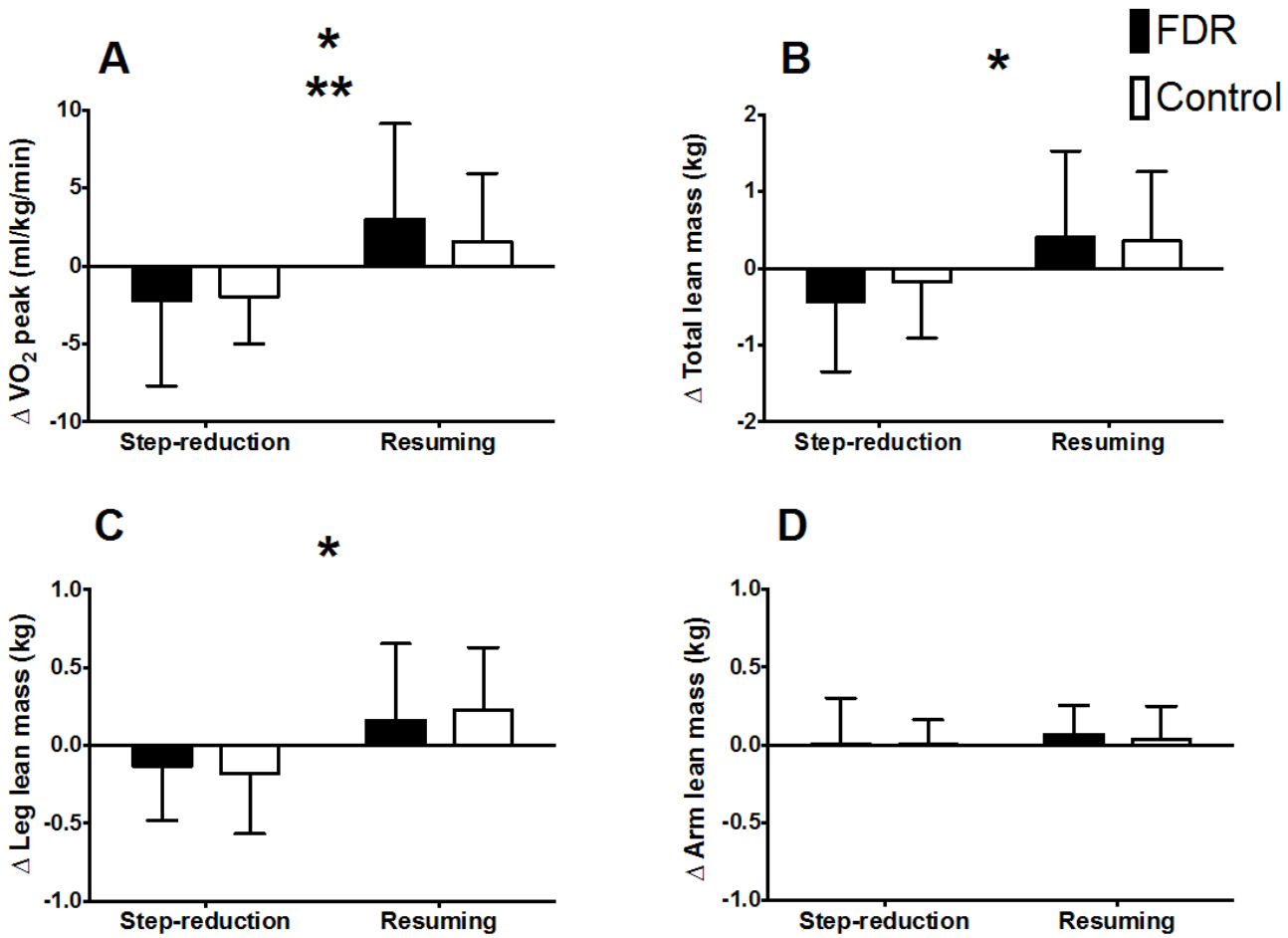

Figure 5.5 Cardiorespiratory fitness and regional lean mass delta change (Δ) in first-degree relatives (FDR) and controls (CON) following step-reduction and resuming activity; A) maximal oxygen uptake ($\dot{V}O_2$ peak), B) total lean mass, C) leg lean mass and D) arm lean mass; mean (SD), **P*<0.05 main effect of time; ***P*<0.05 statistical difference between groups.

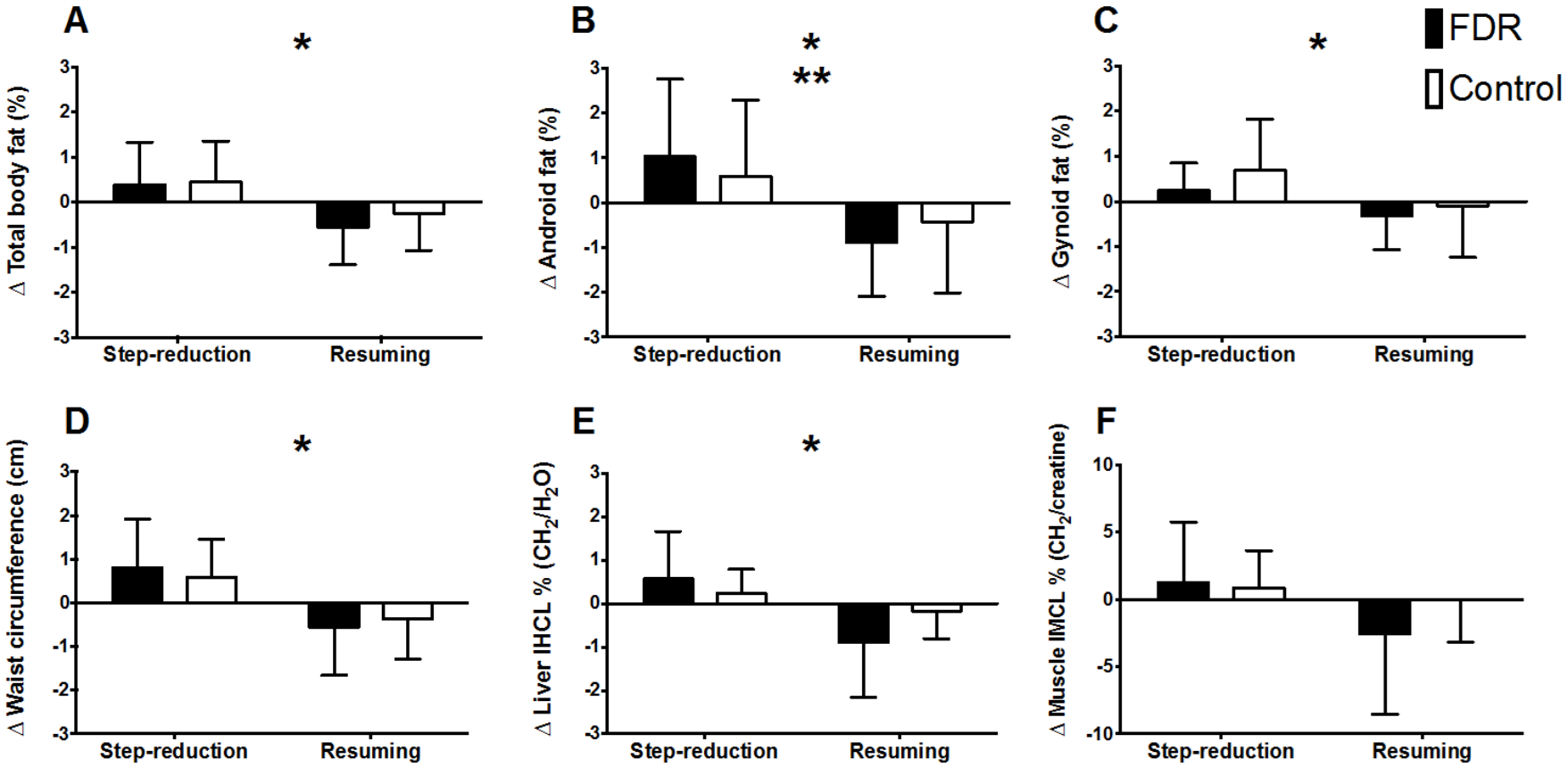

Figure 5.6 Regional and liver fat delta change (Δ) responses of first-degree relatives (FDR) and controls (CON) following step-reduction and resuming activity; A) total body fat, B) android fat, C) gynoid fat, D) waist circumference, E) liver intrahepatocellular lipid (IHCL) and F) muscle intramyocellular lipid (IMCL); mean (SD), *P<0.05 main effect of time; **P<0.05 statistical difference between groups.

5.3.9 Multi-organ insulin sensitivity

Whole body insulin sensitivity

In both groups, the primary outcome measure of whole body insulin sensitivity (Matsuda index) significantly declined following step-reduction (P<0.0005) which was accompanied by a significant increase in glucose AUC (P=0.025) and insulin AUC (P<0.0005). However, no between-group differences in these measures were observed (Figure 5.7D).

Skeletal muscle insulin sensitivity

Muscle insulin sensitivity was significantly reduced following step-reduction (P<0.0005). Overall, there was a significant between-group difference of 0.015 (0.006, 0.023; P=0.001), FDR displaying lower levels of muscle insulin sensitivity. Following resumption of activity there was a significant difference of 0.023 (0.003, 0.042; P=0.023) between the two groups, FDR displaying lower muscle insulin sensitivity (Figure 5.7D).

Hepatic insulin resistance

The intervention had no significant effect on hepatic-IR in either group (P=0.060) but HIRI was on average 3.8 greater in FDR across the intervention (1.7, 6.4; P=0.007) (Figure 5.7F)

Adipose tissue insulin resistance

The intervention had an effect on free fatty acid AUC which was borderline significant (P=0.050) (Figure 5.7C).

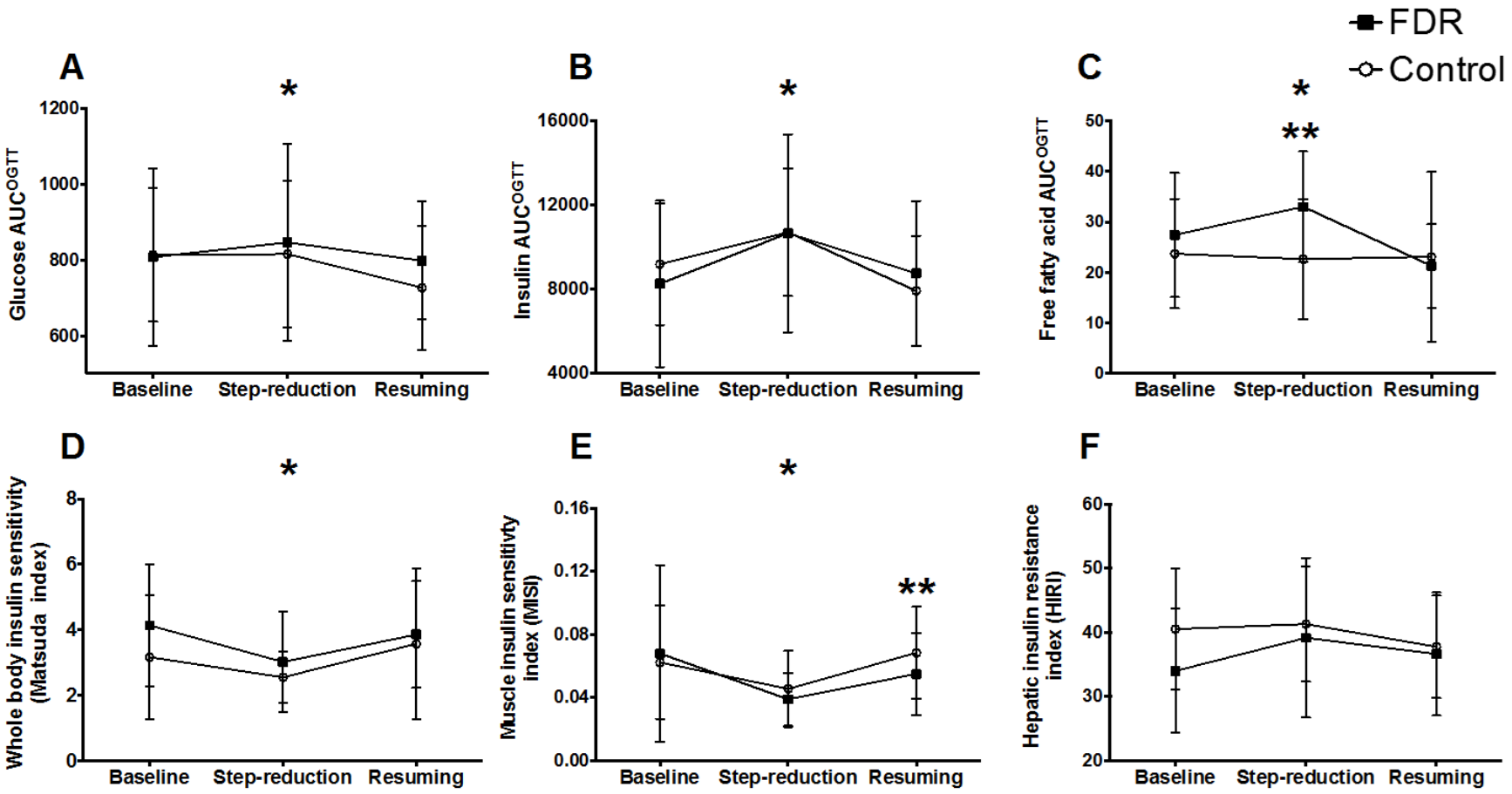

Figure 5.7 Metabolic responses of first-degree relatives (FDR) and controls (CON) at baseline, following step-reduction and resuming activity. Derived from oral glucose tolerance test (OGTT) A) glucose AUC, B) insulin AUC, C) free fatty acid AUC, D) whole body insulin sensitivity (Matsuda index) E) muscle insulin sensitivity index (MISI) and F) hepatic insulin resistance index (HIRI); mean (SD), *P<0.05 main effect of time; **P<0.05 statistical difference between groups.

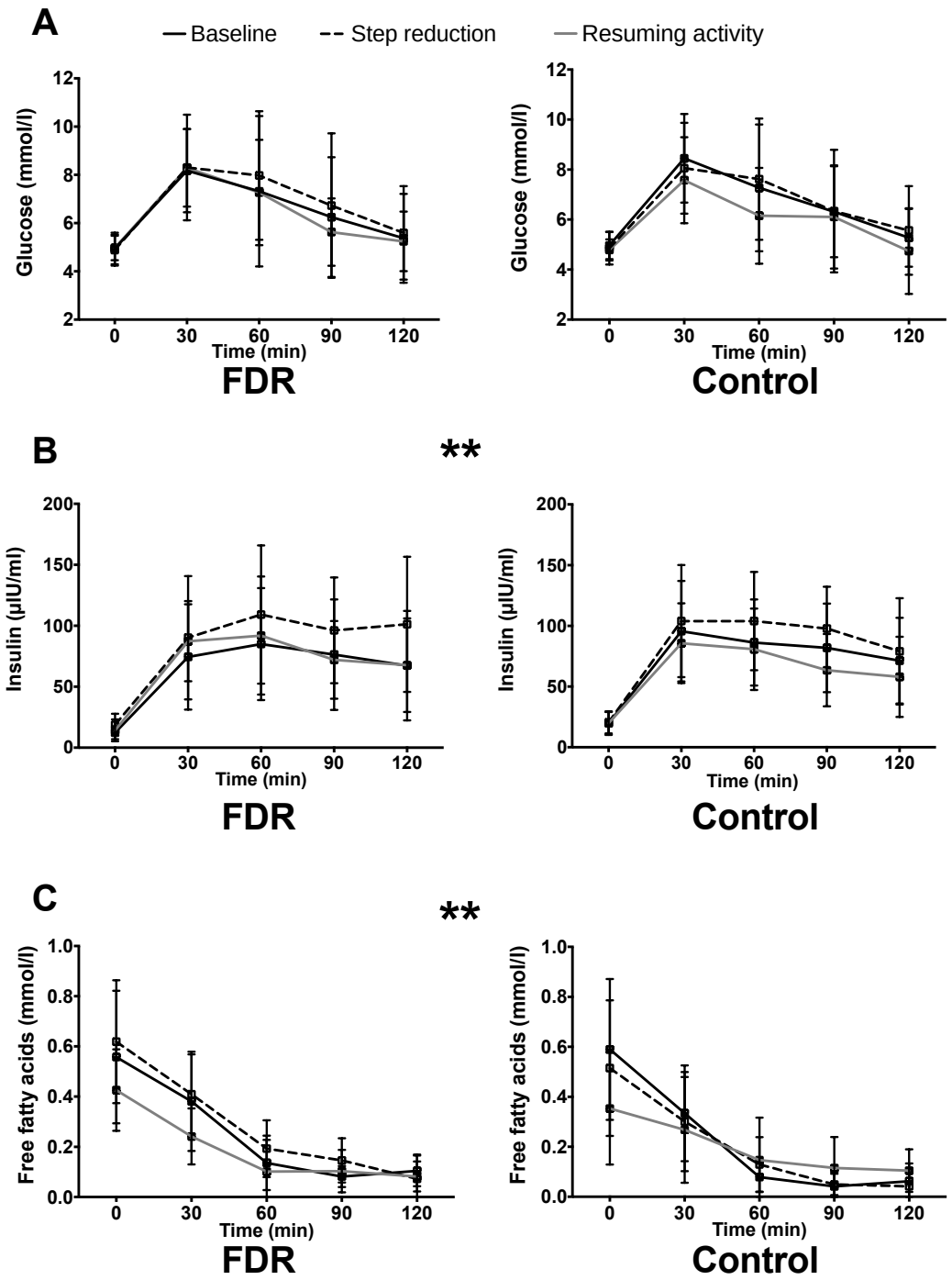

Figure 5.8 Time course data of oral glucose tolerance test (OGTT) in first-degree relatives (FDR) [left panels] and controls (CON) [right panels]; A) glucose, B) insulin, C) free fatty acids. Baseline represented as a solid black line (-), step-reduction as a dashed black line (--), resuming activity solid grey line (-). ***P*<0.05 statistical difference between groups.

5.3.10 Summary of significant changes induced by step-reduction

Table 5.3 Summary of significant changes in response to changes in physical activity, detailed for increase or decrease in both groups combined and group differences between FDR and control.

	Δ Step-reduction	Δ Resuming activity	Group difference
Physical activity			
Average daily steps	↓	↑	-
Daily sedentary time	↑	↓	-
Daily light activity	↓	↑	-
Daily moderate activity	↓	↑	-
Daily vigorous activity	↓	↑	Yes
Skeletal muscle			
Cardiorespiratory fitness	↓	↑	-
Total lean mass	↓	↑	-
Leg lean mass	↓	↑	-
Fat deposition			
Total body fat	↑	↓	-
Android fat	↑	↓	Yes
Gynoid fat	↑	↓	-
Waist circumference	↑	↓	-
Liver fat	↑	↓	-
Fasting metabolic profile			
Insulin	↑	↓	-
HOMA-IR	↑	↓	-
Cholesterol	↑	↓	-
Triglycerides	↑	↓	Yes
LDL cholesterol	↑	↓	-
Post-prandial metabolism (OGTT)			
Glucose AUC	↑	↓	-
Insulin AUC	↑	↓	-
Free fatty acid AUC	↑	↓	-
Matsuda index	↑	↓	-
Muscle insulin sensitivity	↓	↑	Yes

OGTT, oral glucose tolerance test; HOMA-IR, homeostatic model assessment of insulin resistance; AUC, area under curve.

5.4 Discussion

The results of this study demonstrate that even short-term physical inactivity, resulting in a decline in cardiorespiratory fitness, leads to unfavourable changes in body composition with reduced lower limb lean body mass, accretion of body fat, particularly centrally, and of liver fat. These changes are small, but large enough to be clinically significant and pose a serious health threat over a longer term (Thyfault and Krogh-Madsen, 2011). The metabolic sequelae of these changes include whole body insulin resistance and dyslipidaemia (increased triglycerides and LDL-cholesterol). Importantly, FDR+ve of type 2 diabetes demonstrated similar decompensation in whole body insulin sensitivity when compared with FDR-ve. However, some differences were noted: greater rise in plasma triacylglycerol, more android fat deposition and reduced skeletal muscle insulin sensitivity. Although these changes are reversible with resumption of normal physical activity, the pattern of the changes provides mechanistic insight into how chronic physical inactivity and increased sedentary behaviour contributes to development of metabolic syndrome, T2D, non-alcoholic fatty liver disease and potentially cardiovascular disease (Wilmot et al., 2012, Hallsworth et al., 2015).

Previous research has focused on increasing habitual physical activity or structured exercise, yet little is known about the consequences of decreasing habitual physical activity. Validated measures of tissue-specific insulin sensitivity were used to demonstrate that inactivity resulted in decreased skeletal muscle insulin sensitivity. Skeletal muscle insulin sensitivity was significantly altered in both groups; notably the FDR participants failed to return muscle insulin sensitivity to baseline levels on resumption of habitual activity. While this may be an intrinsic defect, the FDR also had lower levels of vigorous activity on resumption of activity. As vigorous activity

induces greater uptake of skeletal muscle glucose (Cassidy et al., 2017), this could have influenced this between-group difference. Increased adipose tissue insulin resistance with lesser suppression of lipolysis and greater release of FFA during the OGTT was evidenced following inactivity. As was the expansion of the adipose tissue mass and small but important increases in liver fat. This peripheral insulin resistance and accumulation of liver fat was accompanied by a rise in plasma triglycerides, LDL-cholesterol and an increase in blood pressure, all components of the metabolic syndrome. These data are consistent with the hypothesis proposed that physical inactivity results in a reduced metabolic demand of skeletal muscle leading to lower glucose uptake (by the development of peripheral insulin resistance) and repartitioning of energy substrates into storage in adipose tissue and in the liver by 'overspill', so that energy will be available when activity resumes (Rector and Thyfault, 2011). This paradigm is supported by measurements of post-prandial muscle and liver glycogen/ lipid ($^{13}C/^{1}H$ magnetic resonance spectroscopy) and *de novo* lipogenesis (deuterated water, $^{2}H_20$), which show that skeletal muscle insulin resistance promotes hepatic steatosis and hyperlipidaemia (Petersen et al., 2007, Jornayvaz et al., 2010). Moreover, this process can be reversed with exercise (Rabol et al., 2011). It is proposed that physical inactivity leads to central and hepatic fat accumulation, development of insulin resistance and emergence of components of the metabolic syndrome, including dyslipidaemia by a similar mechanism.

This step-reduction study somewhat corroborates previous reports regarding the effects of physical inactivity on cardiorespiratory fitness and body composition (Krogh-Madsen et al., 2010, Olsen et al., 2008). The effects of inactivity are amplified when combined with overfeeding (Knudsen et al., 2012), leading to undesirable metabolic changes including impaired insulin sensitivity independent of changes in

body composition developing only 3 days into the intervention. The current findings represents a significant advance for several reasons. Previous step-reduction studies have been limited in sample size, the largest published studies being in 10 young males (Krogh-Madsen et al., 2010, Olsen et al., 2008); the present study is more representative of the general population, involving 45 individuals, both male and female, with a mean age of 36 years. Complete objective monitoring of physical activity throughout ensured that the baseline criteria of >10,000 steps/day was met and likewise ~1500 steps/day during inactivity, habitual activity was monitored during the final 14 days with no instruction or guidance. The time participants spent in domains of activity was objectively determined (categorised by METs) rather than using composite measures. Moreover, the participants involved in the study were comprehensively phenotyped, including all measures of metabolic syndrome, region-specific distribution and quantification of lean muscle and adipose tissue by DXA, and liver fat quantified by ^{1}H-MRS, a highly sensitive non-invasive method (Cuthbertson et al., 2014).

The study is also the first to examine whether FDR of people with T2D are more susceptible to the detrimental effects of reduced physical activity compared to healthy controls. The findings do not suggest that FDR show greater adverse changes with inactivity compared with CON when we consider the effects on the primary outcome, whole body insulin sensitivity. However, there were differences in a subset of measures, with greater increases in triacylglycerol and android fat following step-reduction and a more marked change in cardio-respiratory fitness. These differences may be important, not only associated with an increased risk for future development of insulin resistance and type 2 diabetes, but also long-term for cardiovascular disease and overall mortality (Pinnick et al., 2014). The presence of good metabolic health in

these relatively active individuals at baseline, the decline with increased sedentary time and restoration after resumption of habitual physical activity, all too some extent validate current physical activity recommendations. The findings reinforce the importance of meeting the physical activity recommendations for all participants.

The limitations to the study design must be acknowledged. The gold standard measure of insulin sensitivity is the hyperinsulinaemic euglycaemic clamp. Due to the participant burden of conducting serial clamps, whole body insulin sensitivity was assesses from an OGTT using the Matsuda index which has been shown to correlate with hyperinsulinaemic euglycaemic clamp (Matsuda and DeFronzo, 1999). Calculations of hepatic insulin resistance and skeletal muscle insulin sensitivity have also been validated (Abdul-Ghani et al., 2007). The indices derived from OGTT are valid, repeatable and easily assessable measures of insulin sensitivity which is advantageous for direct comparison with follow-up studies. Future studies may wish to incorporate low and high-dose hyperinsulinaemic euglycaemic clamps to precisely measure hepatic and skeletal muscle insulin sensitivity and consider dynamic measurements of *de novo* lipogenesis and of very low density lipoproteins (VLDL) triglyceride kinetics to determine effects on liver fat and lipoprotein metabolism. These data provide a good rationale to determine the molecular and tissue-specific adaptations in skeletal muscle and subcutaneous adipose tissue in response to short-term physical inactivity. Lastly, against what might be expected, the CON group had a higher fasting insulin and HOMA-IR than FDR. Whilst a lower total body lean mass in CON may account for this another possible explaination is due the variability in CON versus the smaller sample of FDR. A larger cohort of matched (age, gender, body composition and sample number) study groups are needed.

In summary, by taking untrained individuals with a habitually active life style (>10,000 steps/day) and drastically reducing their step count, this study provides direct evidence of a number of unfavourable adaptations to body composition and cardiometabolic risks with increased sedentary behaviour. These changes reversed with resumption of habitual activity. The data suggests a mechanistic framework for understanding the deleterious cardiometabolic adaptations that occur with chronic physical inactivity and sedentary behaviour. Furthermore, FDR and CON are both susceptible to the risks of increased sedentary behaviour. The study findings reinforce the health benefits of meeting physical activity recommendations and emphasise the importance of avoiding prolonged sedentary behaviour.

Chapter 6.

Synthesis of findings

Observational approaches were utilised to examine the role of habitual physical activity (PA) and sedentary behaviour on components of metabolic health and liver fat in obese and non-obese individuals. A greater amount of daily sedentary time was strongly associated with obesity and increased liver fat. These data demonstrated a sound basis for the subsequent interventional study, whereby metabolic disruptions caused by reduced physical activity (i.e. sedentary behaviour) were investigated in the context of type 2 diabetes (T2D) development. The primary aim of the intervention study was to determine if those with a first-degree relative (FDR) of a T2D patient was more susceptible to the detrimental effects of physical inactivity than healthy controls (CON). This investigation focused on how inter-organ cross-talk between the liver, skeletal muscle and adipose tissue mediates whole body metabolism. The major findings were: i) short-term physical inactivity induces altered body composition and metabolic decompensation in non-exercising adults that simply avoid physical activity (PA) for 14 days whilst maintaining their usual diet, ii) FDR of T2D demonstrated a greater increase in android fat and a greater rise in plasma triglycerides, and iii) resumption of habitual PA reversed all observed changes in both groups.

6.1 Short-term physical inactivity induces altered body composition and metabolic decompensation in non-exercising adults that simply avoid PA for 14 days whilst maintaining their usual diet

The pooled findings of FDR and CON participants clearly demonstrate the detrimental effects of sedentary behaviour on body composition; increased adipose tissue deposition and a reduction in skeletal muscle mass. The DXA derived results are generally consistent with the only two previous studies within this field (Krogh-Madsen et al., 2010, Olsen et al., 2008). These authors also found that visceral adipose tissue (VAT) increased following physical inactivity which could potentially be a

marker of the observed increase of liver fat in the current investigation. It has however been questioned whether increased VAT is a culprit of liver fat or merely a marker (Despres, 2012). Notably, liver fat, but not total body fat or VAT, has been identified as an independent predictor of insulin resistance (Linder et al., 2014). The results of the current study together with previous investigations have shown that peripheral insulin sensitivity is altered following short-term physical inactivity but significant alterations in hepatic insulin resistance only present when 14 days of physical inactivity are coupled with overfeeding (Knudsen et al., 2012), suggesting that changes in peripheral insulin sensitivity precede hepatic. The outcomes of these data clearly demonstrate that physical inactivity induces central adiposity and insulin resistance, which is known to play a causal role in T2D (Hu et al., 2004). These results indicate that skeletal muscle was the likely cause of reduced insulin action. Identifying how these changes arise in FDR of T2D has important clinical implications for understanding a 'double-edged sword' (genetic predisposition and physical inactivity) in the development of the disease and its associated cardiometabolic complications.

6.2 FDR of T2D demonstrated a greater increase in android fat and a greater rise in plasma triglycerides

The effects of physical inactivity, by reduced ambulatory activity, in FDR of T2D has not been previously studied. There have however been some studies in FDR (young non-obese men) of T2D employing an extreme model of physical inactivity by bed rest. These studies reported that whole body and peripheral insulin sensitivity were not different but a greater increase in hepatic insulin resistance was evidenced in FDR as a result of reduced activity (Alibegovic et al., 2009). Further, changes in adipose tissue metabolism was not different in FDR (Alibegovic et al., 2010) but low grade inflammation which deteriorated to a greater extent was reported (Hojbjerre et al.,

2011). These findings are equivocal but taken with the current data demonstrate some increased risk of physical inactivity in FDR. The current study identified no differences in whole body insulin sensitivity between the groups, post-prandial insulin AUC and free fatty acid AUC was greater in FDR which demonstrates reduced peripheral insulin sensitivity (confirmed by skeletal muscle insulin sensitivity index) with lesser suppression of peripheral lipolysis (confirmed by greater circulating NEFA) following ingestion of carbohydrate. Furthermore, HOMA-IR changes following inactivity were not significantly different between groups following inactivity but fasting insulin was increased to a greater extent in FDR and hepatic insulin resistance had a trend for FDR to exhibit an augmented response. It could be argued that the hepatic insulin resistance index derived from indices of the oral glucose tolerance test (OGTT) was not sensitive enough to detect small changes or differences between groups; the use of a hyperinsulinemic euglycemic clamp may have revealed another insight. However, taking into account previous research, hepatic insulin resistance is likely to be secondary to skeletal muscle.

Moreover, FDR accumulated greater amounts of android fat and shown greater levels of fasting plasma triglycerides. All of the aforementioned have been strongly associated with the development of T2D (Aune et al., 2015, DeFronzo and Tripathy, 2009, Després and Lemieux, 2006, Ferrannini et al., 1983) and should confirm the importance reducing sedentary time in strategies for the prevention of the disease.

When considering inactivity-induced T2D, one plausible paradigm suggests that physical inactivity causes a reduction in skeletal muscle insulin sensitivity, contributing to a repartitioning of energy substrates into storage; increasing central fat accumulation and ectopic storage within the liver and other organs, causing further

insulin resistance (Jornayvaz et al., 2010, Rabol et al., 2011, Rector and Thyfault, 2011, Petersen et al., 2007, DeFronzo and Tripathy, 2009). As peripheral insulin resistance progresses, continued ectopic fat accumulation within the liver and pancreas precipitates development of metabolic syndrome, a progressive decline in ß-cell function and ultimately T2D (Tan and Vidal-Puig, 2008). The data here supports this hypothesis and the early parts of this paradigm; however, precise mechanistic insight of this is yet to be established. The current and previous data (Krogh-Madsen et al., 2010, Olsen et al., 2008) assessed the inactivity responses after 14 days only which provides no time-course of the mechanisms underlying these impairments. With step-reduction and overfeeding, a reduction in insulin sensitivity was observed after 3 days with no change in VAT until day 14 (Knudsen et al., 2012). Molecular study has previously revealed that skeletal muscle insulin signalling is attenuated after reduced PA in humans (Krogh-Madsen et al., 2010) and rodents (Kump and Booth, 2005). Unravelling the sequelae of the combined metabolic and molecular changes would provide pathophysiological insight. Inactivity-induced changes are headed in a pathological direction (Thyfault and Krogh-Madsen, 2011), though it will be difficult to link acute changes to the development of chronic disease. Data from sedentary controls in clinical trials has shown that as little as 4-6 months of a physically inactive lifestyle causes metabolic deterioration (Patel et al., 2011). However, administering mechanistic studies for much longer than 14 days would be ethically difficult.

6.3 Resumption of habitual PA reversed all observed changes in both groups

Small but significant and clinically relevant changes were induced by simply reducing (~80%) average daily steps and avoiding PA. Equally, these changes were reversed when habitual levels of PA and daily average steps were resumed. During the inactivity phase, the observed changes were induced by simply avoidance of PA (e.g.

taxis/public transport, using escalators/elevators, shopping online) and vice versa during resumption of habitual PA by engagement in PA (e.g. commuting by foot or bicycle, taking the stairs, shopping in store). A noteworthy point is that the participants involved did not substantially change their reality throughout (i.e. continued to work, care for dependants and study etc.); they were also non-exercisers and were not provided with any programme for resumption of activity. The effects observed here are as a result of simple habitual PA lifestyle changes where importantly, a reduction of energy demand in skeletal muscle has induced impairments associated with the development of T2D. Some authors will argue the changes observed were due to alterations in energy balance as opposed to PA. Nevertheless, dietary consumption did not change throughout the study so the changes observed relate to a state of positive energy balance derived from decreased energy expenditure rather than increased intake. Future research may wish to reduce energy intake during the inactive phase but then a concomitant dietary restriction will potentially introduce confounding effects.

6.4 Public health message

The public health message of this work is clear - minimise sedentary behaviour. The cross-sectional analysis of healthy versus unhealthy and non-obese versus obese phenotypes can be taken together with regression analysis relating domains of PA and liver fat to show that sedentary behaviour emerges as the most common *domain of activity* associated with a unfavourable health profile. The step-reduction intervention went on to show that physical inactivity induced detrimental changes in body composition (regional and organ specific fat), whole body insulin sensitivity and dyslipidaemia. Together these studies provide compelling evidence that sedentary time is an inherent aspect of a deteriorated cardiometabolic profile which could chronically lead to overt T2D. This thesis also reveals a profound link between physical inactivity

and cardiorespiratory fitness (CRF), which is an independent predictor of mortality risk (Blair et al., 1989, Ekelund et al., 1988). Favourable health parameters have been associated with CRF in the findings of all data chapters. An unhealthy metabolic profile, a greater BMI and higher liver fat were all linked with low CRF in cross-sectional analysis. The striking decrease in CRF following physical inactivity, provides robust evidence for a physically active lifestyle to maintain CRF, and thus optimal health status. Long standing public health messages have promoted exercise and yet adherence to said guidelines are poor. Perhaps it is time to move away from 'idealistic approaches' (i.e. recommendations of moderate-vigorous PA) and adopt a 'compromising approach' promoting subtle and sustainable changes to daily living (e.g. avoidance of sedentary behaviour and increased daily average steps as shown here). In an increasingly sedentary society, behavioural change and innovative strategies may be pivotal.

6.5 Strengths and limitations

The strengths and limitations have been discussed throughout this thesis along with future direction. To summarise, this work has a number of strengths including, objective monitoring of PA, comprehensive assessment of body composition including skeletal muscle and adipose region and organ specific, gold standard measurement of maximal oxygen consumption and validated measures of tissue-specific insulin sensitivity. A major additional strength is the novelty of the step-reduction intervention and the insight it has shed on the potential pathophysiology of physical inactivity. However, as discussed, further research is required. Moreover, are some additional noteworthy limitations, whilst the sample size is greater than that of previous research it is still somewhat small. Further, this research has not investigated other potentially pivotal changes that are associated with the development of T2D, such as pancreatic

fat or β-cell function. Of note, there are some cardiovascular measures (cIMT and FMD) that remain to be analysed in this cohort. To induce a step-reduction intervention for a substantially longer period would pose ethical challenges, however, there are some questions that remain within the realms of 14-28 days. Feasible study design could include investigating the effects of breaking up sedentary behaviour or increased sedentary behaviour without a concomitant decrease in physical activity. A dose-response understanding of sedentary behaviour would be insightful, determining at which point these change become 'irreversible' would aid the provision of recommendations for acceptable levels of sedentary behaviour.

6.6 Future direction

The use of a short-term physical inactivity model provides a mechanistic basis for understanding the consequences of low PA and increased sedentary behaviour. Although this approach cannot be directly linked with chronic disease further investigation is warranted to unravel the pathological processes (Thyfault and Krogh-Madsen, 2011). Future studies should determine the metabolic and molecular mechanisms considering inter-organ cross-talk between skeletal muscle, adipose tissue and liver. The immediate future direction of the current investigation is to examine the skeletal muscle and adipose tissue biopsies have already been obtained. Cells/tissues can modify their phenotype by changing the expression of key proteins (proteomic changes) through epigenetic regulation, a mechanism by which gene expression can be either activated or suppressed without affecting the DNA sequence (i.e. genotype). One such mechanism is through microRNAs that can influence the expression of many genes simultaneously, resulting in changes of the whole proteome and therefore functionality of the cells and tissues. These molecular investigations were postponed due to the time taken to accrue and analyse the data reported here.

Insulin has multiple metabolic functions that differ across organ systems. A multistage hyperinsulinemic euglycemic clamp would be insightful for future research so that simultaneous insulin action can be determined for skeletal muscle, adipose tissue and liver which would define the contribution and/or compensation of each. Moreover, if the observed metabolic changes originate primarily from inactivity-induced skeletal muscle insulin resistance, post-prandial muscle glucose uptake would be blunted which is associated with secondary changes in liver and adipose tissue. Thereby, excess glucose may diverted to the liver and converted to liver fat by *de novo* lipogenesis (DNL). With liver fat accumulation, more lipid is exported as VLDL-triglyceride, accounting for the increased plasma triglyceride. These metabolic mechanisms are yet to be determined by induced physical inactivity *in vivo*. Future studies should i) determine whether short-term, low PA increases DNL leading to an accumulation of liver fat and ii) determine if very low density lipoprotein (VLDL) triglyceride production rates increase, secondary to this liver fat accumulation, causing hyperlipidaemia.

6.7 Conclusion

Sedentary behaviour is independently associated with obesity and liver fat which are major risk factors for the development of T2D. Short-term sedentary behaviour, induced by a step-reduction model, leads to a decrease in cardiorespiratory fitness, central and liver fat accumulation, multi-organ insulin resistance and dyslipidaemia. These changes provides mechanistic insight into how chronic physical inactivity contributes to development of T2D but further research is required to determine how these changes arise. Importantly, the decompensation induced by inactivity are reversible with resumption of normal physical activity. These data must be used as a platform to develop guidelines and strategies aimed at preventing chronic physical inactivity and sedentary behaviour, particularly in FDR. Considering linked epidemics of physical inactivity and T2D new strategies must be developed to promote the importance of a habitually active lifestyle, either as a minimum or in addition to current guidelines.

Chapter 7.

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Chapter 8.

Appendices

Table 8.1 Demographics of those who had MRI versus those who did not (Chapter 3).

	MRI (*n*=72)	**No-MRI (*n*=24)**	***P* value**
Gender	M *n*=40; F *n*=32	M *n*=12; F *n*=14	0.135
Age (years)	40 (37, 43)	37 (32, 42)	0.335
Weight (kg)	77.5 (73.6, 81.4)	82.6 (76.0, 89.3)	0.181
BMI (kg/m^2)	26.2 (25.1, 27.3)	27.8 (26.5, 31.1)	0.137

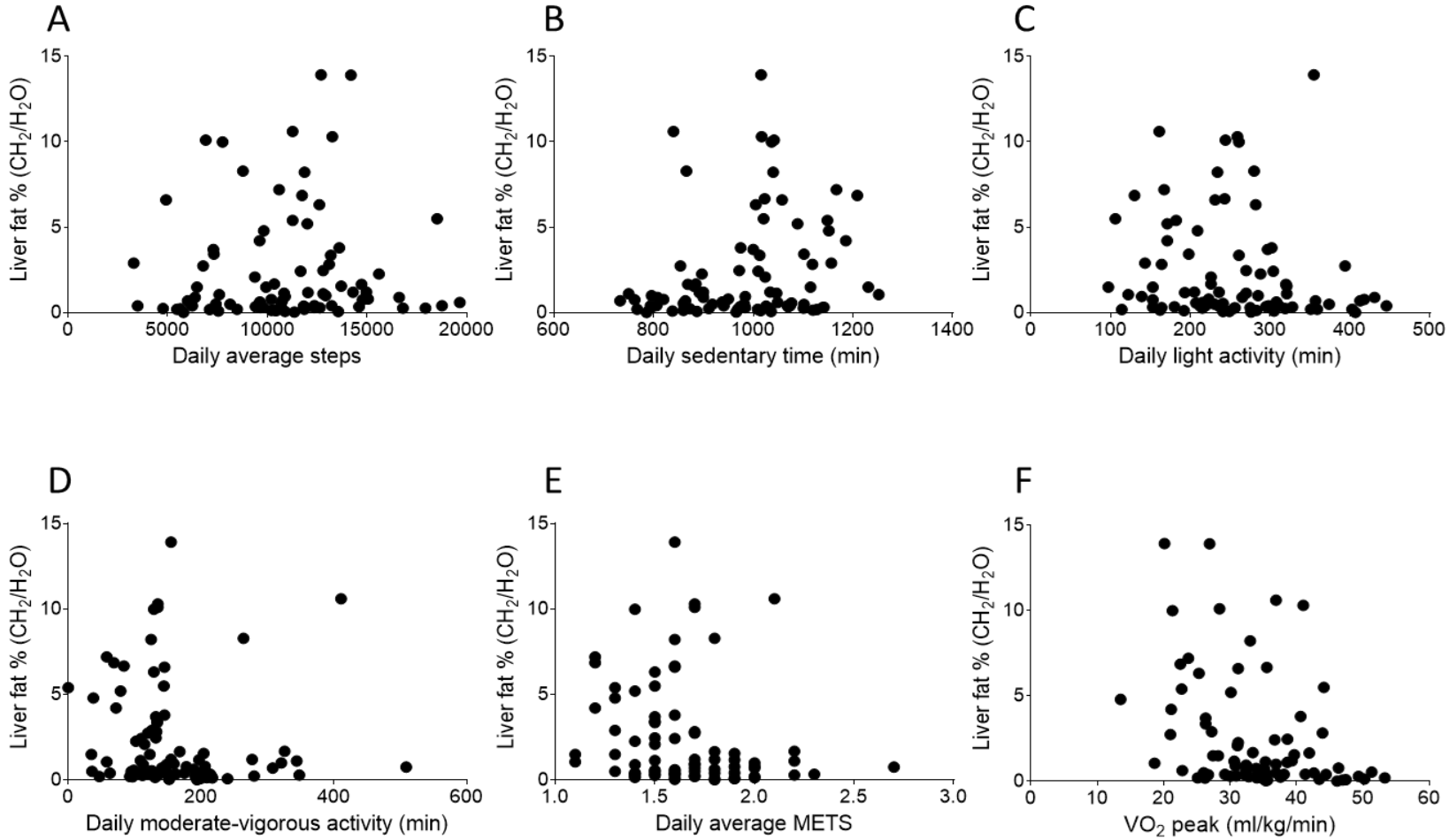

Figure 8.1 Association of absolute liver fat percentage (%) with A) daily average steps, B) sedentary time, C) light activity, D) moderate-vigorous activity, E) metabolic equivalents (METS) and F) $\dot{V}O_2$ peak.

Table 8.2 Univariate and multivariate regression for visceral adipose tissue (VAT) (Chapter 4).

	Univariate			Multivariate		
	β coefficient	**95% CI**	***P***	**β coefficient**	**95% CI**	***P***
Age (yrs)	1.03	1.01, 1.07	**<0.0005**	1.01	1.01, 1.04	**0.031**
BMI (kg/m^2)	1.11	1.03, 1.28	**<0.0005**	1.10	1.04, 1.26	**<0.0005**
Steps (1,000)	-0.94	-0.85, -0.95	**0.027**	-0.99	-0.94, 1.02	0.565
Sedentary time (hr)	1.10	1.08, 1.31	**0.023**	1.10	1.04, 1.42	0.218
METS (0.1)	-0.94	-0.83, -0.94	**0.044**	-0.40	-0.37, -2.51	**0.049**
$\dot{V}O_2$ peak (ml/kg/min)	-0.96	-0.90, -0.98	**<0.0005**	-0.98	-0.95, 0.99	0.094
Light activity (hr)	-0.88	-0.69, -0.89	**0.041**	1.07	-0.99, 1.34	0.353
Moderate activity (hr)	-1.01	-0.87, 1.18	0.937			
Vigorous activity (hr)	-0.79	-0.41, 0.96	0.275			
MVPA (hr)	-0.99	-0.86, 1.10	0.840			
Lying time (hr)	-1.00	-0.85, 1.15	0.947			
Sleep duration (hr)	1.02	-0.89, 1.23	0.769			

Data was transformed and analysed using 1og10; data presented here is back transformed to original units. BMI, body mass index; METS, metabolic equivalents; $\dot{V}$O2 peak, cardiorespiratory fitness; MVPA, moderate-vigorous physical activity.

Table 8.3 Univariate and multivariate regression for subcutaneous adipose tissue (SAT) (Chapter 4).

	Univariate			Multivariate		
	β coefficient	**95% CI**	***P***	**β coefficient**	**95% CI**	***P***
Age (yrs)	0.070	-0.088, 0.227	0.379	-0.194	-0.323, -0.066	**0.004**
BMI (kg/m^2)	1.227	0.883, 1.570	**<0.0005**	1.188	0.780, 1.597	**<0.0005**
Sedentary time (hr)	1.705	0.745, 2.665	**0.001**	0.082	-1.834, 1.998	0.932
Light activity (hr)	-2.216	-3.636, -0.769	**0.003**	-0.052	-1.888, 1.785	0.955
METS (0.1)	-1.374	-2.052, -6.957	**<0.0005**	-2.391	-13.520, 8.738	0.669
$\dot{V}O_2$ peak (ml/kg/min)	-0.563	-0.794, -0.332	**<0.0005**	-0.376	-0.612, -0.140	**0.002**
Steps (1,000)	-0.352	-0.980, 0.277	0.268			
Moderate activity (hr)	-1.347	-3.224, 0.529	0.157			
Vigorous activity (hr)	-4.864	-9.918, 0.191	0.059			
MVPA (hr)	-1.334	-2.841, 0.173	0.082			
Lying time (hr)	0.918	-0.842, 2.678	0.302			
Sleep duration (hr)	1.490	-0.446, 3.425	0.129			

BMI, body mass index; METS, metabolic equivalents; $\dot{V}O_2$ peak, cardiorespiratory fitness; MVPA, moderate-vigorous physical activity.