

The development of a functional food breakfast and its effects on gluco-regulation, cognitive performance, mood and satiety in adolescents.

**Sarah Kennedy**

Oxford Brookes University

This thesis is submitted in partial fulfilment of the requirements of the award of Doctor of

Philosophy

July 2016

## Acknowledgements

I have been incredibly lucky to have the help and support of many wonderful colleagues and friends who were unbelievably willing to take time away from their own research to assist with the school studies. A quick mention really does not go far enough to express how grateful I am to the following people for their support and friendship:

Marta Campolier, Patricia Shaw, Georgios Saltaouras, Alaaddine El-Chab, Samiah Kokandi, Shelly Coe, Miriam Clegg, Majd Jan, Ameerah Almaski, Kimberly Harcourt, Jon Kennedy, Sarah Graham, Rick Varnham, Ann Fraser, Jilly Martin and Sarah Hillier.

Thank you to all the students and staff from Oxford Brookes who contributed to the development and running of the school study days: Abby Whyte, Sally Morey, Charlie Field, Claudia Preston, Emma Young, Abi Barclay-Watt, Trudy Ing and John Brierley. Pete Robins for the loan of the equipment and Lauren Nicoll for her help with the data entry. Thank you also to Donna Isaac, Jacky Ward and Dean Woodham for being so accommodating and to all the students who took part in the study. Working with adolescents was certainly an interesting and entertaining experience.

I have had the guidance and support of two wonderful supervisors Dr Miriam Clegg and Dr Lisa Ryan. I am forever grateful for all your encouragement, advice and inspiration over the past few years.

A big thank you to all my friends and family who have had to suffer my dashing around, bringing work on holidays and having a general lack of commitment to any social event. Special thanks go to Win Kennedy for her expert help and advice and to Emma Davies for her valuable input.

Lastly, I have to thank my wonderful husband, for his endless help and support and for keeping me fed, laughing and sane. I dedicate this to him, Rowan and Tonylana.

## Abstract

It is well documented that eating a regular breakfast is associated with benefits to markers of metabolic health and cognitive performance. The composition of breakfast differentially affects the metabolic response which may also have implications for cognitive performance. To date, much of the research on gluco-regulation is in adults, and no studies have investigated the effects of a functional-food breakfast (FB) on gluco-regulation and cognitive performance, mood and satiety in adolescents. Therefore, this thesis aims to address this research gap through the implementation of a series of five research studies. The primary aim was to investigate the effects of a FB which included ingredients selected for their potential to improve gluco-regulation (blueberries, baobab, cinnamon and oats) on measures of gluco-regulation (glucose response (GR) and insulin response (IR)) compared to a control breakfast (CB), and ready to eat cereal (RTEC) (adults only).

Secondary aims were to measure: cognitive performance (using a map recall and delayed word recall task), mood (using the 'Activation–Deactivation Check List') and satiety (using VAS scales). Measures were collected at timed intervals over a three-hour period after the three breakfasts. The study was first implemented in healthy adults ( $n=16$ ,  $32.0\pm 10.0$  years) in a controlled laboratory environment and found that consumption of the FB resulted in a lower glucose peak and a lower IR AUC, compared to the CB and RTEC ( $p<.05$ ) (chapter 6). In a school environment, adolescents' ( $n=22$ ,  $13.7\pm 0.5$  years) consumption of the FB reduced peak glucose, peak insulin and IR AUC at 60 and 120 minutes, compared to the CB ( $p<.05$ ) (chapter 7). There were no effects on cognitive performance, mood or satiety regardless of breakfast condition ( $p>.05$ ).

Two preliminary studies (chapter 3 and 4) contributed to aspects of the FB and CB development (chapter 5) and breakfast study design (chapter 6 and 7). In chapter 3, validation of a novel portable indirect calorimeter in adults ( $n=20$ ,  $38.3 \pm 11.2$  years) resulted in the revision of the main hypothesis (chapter 2), where investigations into the effect of the FB on energy expenditure (EE)

was discontinued. In chapter 4, the completion of a breakfast-based questionnaire by adolescents ( $n=434$ , 13-15 years) informed the choice of ready to eat cereal (RTEC) on which the breakfast conditions were based (chapter 5). Additionally, these studies made individual contributions to the literature reporting the use of indirect calorimetry in schools to collect body composition measures from adolescents ( $n=30$ ) (chapter 3) and highlighting implications for the design of breakfast interventions in adolescents (chapter 4).

Findings from this thesis suggest that the addition of functional food ingredients to breakfast has the potential to improve gluco-regulation in healthy adults and adolescents. The inclusion of functional food ingredients as part of breakfast should be considered alongside the promotion of breakfast.

## List of Abbreviations

<b>AD</b>	Active diet	<b>FM</b>	Fat mass
<b>AUC</b>	Area under the curve	<b>FRAP</b>	Ferric ion reducing antioxidant power
<b>ANOVA</b>	Analysis of variance	<b>GI</b>	Glycaemic index
<b>ANCOVA</b>	Analysis of covariance	<b>GL</b>	Glycaemic load
<b>BBB</b>	Blood-brain barrier	<b>GR</b>	Glycaemic response
<b>BDNF</b>	Brain-derived neurotrophic factor	<b>HBSC</b>	Health Behaviour of School-aged Children
<b>BMI</b>	Body mass index	<b>IGT</b>	Impaired glucose tolerance
<b>BMR</b>	Basal metabolic rate	<b>IR</b>	Insulin response
<b>BQI</b>	Breakfast quality index	<b>ISO</b>	International Organization for Standardization
<b>CB</b>	Control breakfast	<b>IWHS</b>	Iowa Women's Health Study
<b>CHO</b>	Carbohydrate	<b>LED</b>	Light-emitting diode
<b>CNS</b>	Central nervous system	<b>LNAAs</b>	Large neural amino acids
<b>CV</b>	Coefficient of variation	<b>LOA</b>	Limit of agreement
<b>CVD</b>	Cardiovascular disease	<b>MCI</b>	Mild cognitive impairment
<b>CHD</b>	Coronary heart disease	<b>MRMR</b>	Measured resting metabolic rate
<b>DT</b>	Deltatrac	<b>NHANES</b>	National Health and Nutrition Examination Survey
<b>DBC</b>	Daily breakfast consumption	<b>NHMRC</b>	National Health and Medical Research Council
<b>DIT</b>	Diet-induced thermogenesis	<b>NO</b>	Nitric oxide
<b>DOH</b>	Department of Health	<b>NMDA</b>	N-methyl-D-aspartate
<b>EB</b>	Breakfast consumption	<b>OB</b>	Breakfast omission
<b>EFSA</b>	European Food Safety Authority	<b>OBU</b>	Oxford Brookes University
<b>EE</b>	Energy expenditure	<b>OR</b>	Odds ratio
<b>FAO</b>	Food and Agricultural Organisation	<b>PA</b>	Physical activity
<b>FB</b>	Functional food breakfast	<b>PBC</b>	Perceived behavioural control
<b>FCR</b>	Folin-ciocalteu reagent	<b>PCA</b>	Principal components analysis
<b>FFM</b>	Fat free mass	<b>PHE</b>	Public Health England

<b>PRMR</b>	Predicted resting metabolic rate		
<b>RCT</b>	Randomised control trial		
<b>RDS</b>	Rapidly digested starch		
<b>REE</b>	Resting energy expenditure		
<b>RMR</b>	Resting metabolic rate		
<b>RQ</b>	Respiratory quotient		
<b>RTEC</b>	Ready-to-eat cereal		
<b>SBP</b>	School-breakfast programmes		
<b>SCFA</b>	Short-chain fatty acids		
<b>SD</b>	Standard deviation		
<b>SDS</b>	Slowly digestible starch		
<b>SES</b>	Socio-economic status		
<b>SEM</b>	Standard error of mean		
<b>SN</b>	Subjective norms		
<b>T2DM</b>	Type 2 diabetes mellitus		
<b>TPB</b>	Theory of planned behaviour		
<b>TRP</b>	Tryptophan		
<b>USDA</b>	U.S. Department of Agriculture		
<b>VAS</b>	Visual analogue scale		
<b>VCO<sub>2</sub></b>	Volume of carbon dioxide		
<b>VO<sub>2</sub></b>	Volume of oxygen		
<b>WHO</b>	World Health Organisation		

## Publications

1. Kennedy S, Ryan L, Fraser A, Clegg ME. A comparison of indirect calorimeters' measures of resting metabolic rate. *Proc Nutr Soc.* 2013; 72 (OCE3):E155.
2. Kennedy S, Ryan L, Fraser A, Clegg ME. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J Nutr Sci.* 2014; 3(e52):1-11 (Appendix 1).
3. Kennedy S, Ryan L, Clegg ME. Associations between breakfast consumption, attitudes towards breakfast and physical activity in adolescents. *Proc Nutr Soc.* 2015; 74 (OCE1):E99.
4. Kennedy S, Ryan L, Clegg ME. The effect of a functional food based breakfast on blood glucose response, insulin response, and satiety. *Abstract/Appetite.* 2015; 91:431-439
5. Kennedy S, Ryan L, Davies EL, Clegg ME. Applying an extended Theory of Planned Behaviour to predict breakfast consumption in adolescents. *Eur J Clin Nutr.* 2017; 71(5):607-613.

## Table of Contents

Acknowledgements .....	2
Abstract .....	3
List of Abbreviations.....	5
Publications .....	7
Table of Contents .....	8
Appendix list.....	16
List of figures .....	17
List of tables.....	17
Chapter 1: Review of the breakfast literature .....	19
1.1 A history of breakfast.....	19
1.2 Definition of breakfast .....	19
1.3 Adolescents .....	20
1.3.1 Defining adolescence.....	20
1.3.2 Health behaviours .....	21
1.4 Breakfast trends in adolescents .....	21
1.4.1 Breakfast frequency .....	21
1.4.2 Factors influencing breakfast behaviours .....	23
1.4.3 Breakfast composition .....	24
1.4.4 Breakfast quality indexes.....	25
1.5 The influence of breakfast on weight status .....	26
1.5.1 Breakfast consumption .....	26
1.5.2 Breakfast composition .....	28
1.5.3 Energy intake and PA levels.....	29
1.5.4 Breakfast habits .....	29
1.5.5 Summary .....	31
1.6 The effect of breakfast on gluco-regulation .....	31
1.6.1 Breakfast consumption .....	31
1.6.2 Breakfast composition .....	33
1.6.3 Summary .....	35



1.7 Measuring cognitive performance .....	35
1.7.1 Cognitive domains .....	35
1.7.2 Cognitive domains in breakfast interventions .....	37
1.7.3 Measuring and selecting cognitive domains .....	38
1.8 A review of the effect of breakfast on cognitive and academic performance .....	41
1.8.1 Breakfast consumption .....	41
1.8.2 Breakfast composition .....	46
1.8.3 Breakfast research in adolescents .....	49
1.8.4 Summary .....	49
1.9 The effect of breakfast on gluco-regulation, cognitive performance, mood & satiety in adolescents	50
1.9.1 Breakfast consumption .....	50
1.9.2 Breakfast composition .....	54
1.9.3 Summary and future work .....	59
1.10 Developing a functional food based breakfast.....	60
1.10.1 Functional foods .....	60
1.10.2 Functional foods in the diet and effects on metabolic health and cognition .....	64
1.10.3 Functional foods and effects on gluco-regulation.....	66
1.10.4 Functional foods and effects on cognitive function.....	67
1.10.5 Energy expenditure.....	71
1.10.6 Summary.....	72
1.11 The development of a functional food breakfast for adolescents.....	72
1.11.1 Blueberries.....	73
1.11.2 Baobab .....	77
1.11.3 Oats ( $\beta$ -glucans).....	77
1.11.4 Cinnamon.....	79
1.11.5 Summary.....	80
Chapter 2: Rationale and hypothesis .....	81
2.1 Validation of indirect calorimeters and the use of a portable indirect calorimeter in a school environment (chapter 3: study 1 and 2) .....	81
2.1.1 Hypothesis.....	81

2.1.2 Aims and sub-hypothesis (study 1) .....	82
2.1.3 Study development (study 1) .....	82
2.1.4 Aims and sub-hypothesis (study 2) .....	83
2.2 Exploring gender differences in the theory of planned behaviour applied to adolescent breakfast consumption: Implications for interventions (chapter 4) .....	83
2.2.1 Hypothesis .....	83
2.2.2 Aims and sub-hypothesis .....	84
2.2.3 Study development .....	84
2.3 The development of a functional food breakfast (chapter 5) .....	85
2.4 The effects of a functional food breakfast on gluco-regulation, cognitive function, mood and satiety in healthy adults (chapter 6) .....	85
2.5 The effects of a functional food breakfast on gluco-regulation, cognitive function, mood and satiety in adolescents (chapter 7) .....	86
2.6 Summary .....	87
Chapter 3: Validation of indirect calorimeters and the feasibility of using a portable indirect calorimeter in a school environment .....	88
3.1 Summary .....	88
3.2 Introduction .....	89
3.2.1 Aims and hypothesis .....	92
3.3 Methods (Study 1) .....	94
3.3.1 Study design .....	94
3.3.2 Subjects .....	94
3.3.3 Indirect calorimeters .....	95
3.3.4 Study protocol .....	96
3.3.5 Determining EE .....	98
3.3.6 Statistical analyses .....	98
3.4 Results (Study 1) .....	100
3.4.1 Measurement acceptability .....	100
3.4.2 Comparison of RMR measures from the GEM and the ECAL to the Deltatrac .....	100
3.4.3 Comparison of RMR measures to determine repeatability of machines .....	105
3.5 Materials and methods (Study 2) .....	107

3.5.1 Study design .....	107
3.5.2 Subjects .....	107
3.5.3 Indirect calorimeter .....	107
3.5.4 Study protocol .....	107
3.5.5 Determining EE .....	108
3.5.6 Prediction equations .....	108
3.5.7 Statistical analyses .....	109
3.6 Results (Study 2).....	110
3.6.1 Measurement acceptability .....	110
3.6.2 ECAL and sample characteristics .....	110
3.6.3 Comparison of ECAL to prediction equations .....	110
3.7 Discussion .....	114
3.7.1 Comparison of accuracy of RMR measures; GEM and ECAL vs. DT (Study 1, part a).....	114
3.7.2 Comparison of RMR measures to determine repeatability of machines (Study 1, part b).....	116
3.7.3 Collection of RMR measures on the ECAL (Study 2, part a) .....	118
3.7.4 Comparison of ECAL to prediction equations (Study 2, part b) .....	119
3.8 Conclusions .....	122
Chapter 4: Exploring gender differences in the theory of planned behaviour applied to adolescent breakfast consumption .....	124
4.1 Summary .....	124
4.2 Introduction.....	125
4.2.1 Aims and hypothesis.....	128
4.3 Methods.....	129
4.3.1 Participants and recruitment .....	129
4.3.2 Design and measures .....	131
4.3.3 Statistical analysis .....	134
4.4 Results .....	135
4.4.1 Correlations.....	137
4.4.2 TPB measures.....	138
4.4.3 Predicting intention to eat breakfast.....	138

4.4.4 Predicting breakfast behaviour.....	140
4.5 Discussion.....	143
4.5.1 Breakfast consumption.....	143
4.5.2 Attitudes.....	144
4.5.3 Predicting intention to eat breakfast.....	144
4.5.4 Predicting breakfast behaviour.....	145
4.5.5 Limitations.....	147
4.6 Conclusion.....	148
Chapter 5: The development of a functional food breakfast.....	149
5.1 Summary.....	149
5.2 Introduction.....	150
5.2.1 Aims and hypothesis.....	151
5.3 Materials.....	152
5.3.1 Chemicals.....	152
5.3.2 Study protocol.....	152
5.3.3 Ferric-ion reducing antioxidant power.....	153
5.3.4 Folin-Ciocalteu.....	153
5.3.5 Muffin preparation.....	154
5.3.6 Nutrient profiles.....	155
5.3.7 <i>In-vitro</i> digestion.....	157
5.3.8 Analysis of reducing sugars released during digestion.....	158
5.3.9 Statistical analysis.....	158
5.4 Results.....	159
5.4.1 Antioxidant and polyphenol content of powdered extracts.....	159
5.4.2 Antioxidant and polyphenol content of muffin samples.....	159
5.4.3 Sugars released from muffin (fresh vs. frozen).....	160
5.5 Discussion.....	164
5.5.1 Antioxidant and polyphenol content of extracts.....	164
5.5.2 Antioxidant and polyphenol content of muffins.....	164
5.5.3 Sugar release and starch digestion.....	164

5.6 Conclusion .....	166
Chapter 6: The effects of a functional food breakfast on gluco-regulation, cognitive performance, mood and satiety in healthy adults .....	167
6.1 Summary .....	167
6.2 Introduction.....	168
6.2.1 Gluco-regulation .....	168
6.2.2 Cognitive performance, mood & satiety .....	169
6.2.3 Breakfast development .....	171
6.2.4 Aims and hypothesis.....	172
6.3 Methods.....	173
6.3.1 Study design .....	173
6.3.2 Participants.....	173
6.3.3 Testing schedule .....	174
6.3.4 Test meals.....	175
6.3.5 Blood samples .....	176
6.3.6 Map task.....	177
6.3.7 Delayed word recall.....	178
6.3.8 Mood .....	179
6.3.9 Satiety and palatability .....	179
6.3.10 Statistical analysis .....	179
6.3.11 Power calculation .....	180
6.4 Results .....	182
6.4.1 Glucose response .....	182
6.4.2 Insulin response .....	183
6.4.3 Cognitive tests: map task and delayed recall .....	184
6.4.4 Mood, satiety and palatability.....	185
6.5 Discussion .....	188
6.5.1 Gluco-regulation .....	188
6.5.2 Cognitive performance, satiety and mood .....	191
6.5.3 Satiety and mood.....	194

6.5.4 Map task and word recall task design .....	195
6.6 Conclusions .....	196
Chapter 7: The effects of a functional food breakfast on gluco-regulation, cognitive performance, mood and satiety in adolescents.....	198
7.1 Summary .....	198
7.2 Introduction.....	199
7.2.1 Gluco-regulation .....	199
7.2.2 Cognitive performance, mood & satiety .....	199
7.2.3 Breakfast development .....	201
7.2.4 Aims and hypothesis.....	202
7.3 Methods.....	203
7.3.1 Study design .....	203
7.3.2 Participants.....	203
7.3.3 Screening schedule .....	204
7.3.4 Testing schedule .....	204
7.3.5 Test meals.....	205
7.3.6 Blood samples .....	205
7.3.7 Map task.....	205
7.3.8 Delayed word recall.....	206
7.3.9 Mood .....	206
7.3.10 Satiety and palatability .....	206
7.3.11 Statistical analysis .....	207
7.3.12 Power calculation .....	207
7.4 Results .....	209
7.4.1 Glucose response .....	209
7.4.2 Insulin response .....	210
7.4.3 Cognitive tests: map task and delayed recall .....	211
7.4.4 Mood, satiety and palatability.....	212
7.5 Discussion .....	216
7.5.1 Gluco-regulation .....	216

7.5.2 Cognitive performance, satiety and mood .....	218
7.5.3 Mood & satiety .....	221
7.5.4 Additional limitations .....	222
7.6 Conclusion .....	223
Chapter 8: General discussion .....	225
8.1 Summary of thesis findings .....	225
8.1.1 Gluco-regulation .....	226
8.1.2 Cognitive performance, mood and satiety .....	228
8.2 Implications of the findings .....	230
8.3 General strengths and limitations .....	231
8.4 Recommendations for future research .....	233
8.5 Overall conclusion .....	234
References .....	235

## Appendix list

Appendix 1	Publication arising from chapter three	274
Appendix 2a	Participant information sheet	285
Appendix 2b	Letter to the head teacher	287
Appendix 2c	Letter to the parents	289
Appendix 2d	Information sheet for teachers	291
Appendix 2e	Information sheet for school counsellor	293
Appendix 2f	Lifestyle habits questionnaire	295
Appendix 3a	Participant information sheet	312
Appendix 3b	Health questionnaire	315
Appendix 3c	Food frequency questionnaire	316
Appendix 3c	Food frequency questionnaire	317
Appendix 3d	Consent form	321
Appendix 3e	Word recall task	322
Appendix 3f	Mood questionnaire	323
Appendix 3g	Appetite and palatability scale (VAS)	327
Appendix 4a	Letter to the head teacher	331
Appendix 4b	Letter to the parents	334
Appendix 4c	Parent consent form	340
Appendix 4d	Participant information sheet	341
Appendix 4e	Health questionnaire	347
Appendix 4f	Mood questionnaire	349



## List of figures

<b>Figure 1.</b> Considerations for how breakfast is defined	21
<b>Figure 2.</b> Theoretical model of pathways through which breakfast may protect against the development of chronic diseases and obesity	33
<b>Figure 3.</b> Domains affecting cognition and contribution from external factors	38
<b>Figure 4.</b> Mechanisms underpinning the effects of dietary flavonoids on memory and learning	70
<b>Figure 5.</b> Study protocol for validation of indirect calorimeters (chapter 3)	98
<b>Figure 6.</b> Animation of measures collected on the GEM and the DT in a supine position	98
<b>Figure 7.</b> Animation of measures were collected on the ECAL in a semi-reclined position	99
<b>Figure 8a.</b> Bland-Altman plot of RMR measures collected on test day 1 (GEM and DT)	103
<b>Figure 8b.</b> Bland-Altman plot of RMR measures collected on test day 2 (GEM and DT)	104
<b>Figure 9a.</b> Bland-Altman plot of RMR measures collected on test day 1 (ECAL and DT)	105
<b>Figure 9b.</b> Bland-Altman plot of RMR measures collected on test day 2 (ECAL and DT)	105
<b>Figure 10.</b> Bland-Altman plot of RMR measures collected on ECAL and predicted by Schofield-HW equations	113
<b>Figure 11.</b> Bland-Altman plot of mean difference in resting metabolic rate measures collected on ECAL and predicted by Oxford equations	113
<b>Figure 12.</b> Flow of participants through questionnaire recruitment (chapter 4)	131
<b>Figure 13.</b> Scree plot of factors retained for PCA analysis	134
<b>Figure 14.</b> Sugars released between the control breakfast fresh and frozen muffins	162
<b>Figure 15.</b> Sugars released between the functional food breakfast fresh and frozen muffins	163
<b>Figure 16.</b> Sugars released between the control breakfast frozen and the functional food breakfast frozen	164
<b>Figure 17.</b> Testing day schedule (chapter 6).	176
<b>Figure 18.</b> Example of spatial memory map task	179
<b>Figure 19.</b> Change from baseline in glucose response	183
<b>Figure 20.</b> Change from baseline in insulin response	184
<b>Figure 21.</b> Tension score recorded at T0, T60, T120 and T180 minutes in all participants	186
<b>Figure 22.</b> Hunger scores measured by VAS scale in all participants	187
<b>Figure 23.</b> Palatability scores rating pleasantness and appearance of the breakfasts	188
<b>Figure 24.</b> School testing day schedule (chapter 7)	206
<b>Figure 25.</b> Mean change from baseline in glucose response to the functional food and control breakfasts	211
<b>Figure 26.</b> Mean change from baseline in insulin response to the functional food and control breakfasts	212
<b>Figure 27.</b> Tension score recorded at T0, T60, T120 and T180 minutes in all participants	214
<b>Figure 28.</b> Desire to eat scores measured by VAS scales in all participants	215
<b>Figure 29.</b> Palatability scores rating pleasantness and appearance of the breakfasts	216

## List of tables

<b>Table 1.</b> Selected memory systems and the major anatomical structures involved	40
<b>Table 2.</b> Reviews of breakfast studies (breakfast vs. no breakfast) published from 1982 to 2016	46
<b>Table 3.</b> Reviews of breakfast studies (breakfast composition) published from 2008 to 2016	51
<b>Table 4.</b> Adolescent studies (breakfast vs. no breakfast) and effects on cognitive performance	56
<b>Table 5.</b> Adolescent studies (breakfast composition) and effects on cognitive performance	59
<b>Table 6.</b> Flavonoid subclasses and structures	65
<b>Table 7:</b> Baseline participant characteristics (chapter 3)	96
<b>Table 8.</b> RMR inter-machine mean difference in all participants for VCO <sub>2</sub> , VO <sub>2</sub> , RMR, RQ, CHO ox & FAT ox collected on the DT, ECAL and GEM during T1 and T2	102
<b>Table 9.</b> Correlations between measures on the DT, ECAL and GEM for VCO <sub>2</sub> , VO <sub>2</sub> , RMR, RQ, CHO ox and FAT ox in all participants on T1 and T2	106
<b>Table 10.</b> Bland Altman analysis of RMR repeated measures of for VCO <sub>2</sub> , VO <sub>2</sub> , RMR, RQ, CHO ox & FAT ox on the DT, ECAL and GEM in all participants on T1 and T2	107
<b>Table 11.</b> Prediction equations derived for use in children and adolescents	110
<b>Table 12.</b> Baseline characteristics of participants (chapter 3, study 2) and correlations with ECAL RMR	111
<b>Table 13.</b> Predicted RMR measures vs. RMR measured using ECAL	112
<b>Table 14.</b> Agreement between RMR measured using ECAL and predicted RMR in adolescents	114
<b>Table 15.</b> Summary of factor analysis results for attitude scale	134
<b>Table 16.</b> Descriptive characteristics of sample (chapter 4)	136
<b>Table 17.</b> Characteristics of participants stratified by frequency of breakfast consumption	138
<b>Table 18.</b> TPB scores by gender (upper table) and breakfast consumption frequency (lower table)	139
<b>Table 19.</b> Hierarchical regression analyses for variables predicting intentions to eat breakfast	141
<b>Table 20.</b> Multinomial logistic regression models predicting breakfast eating	143
<b>Table 21.</b> Comparison of nutritional composition from product packaging (chapter 5)	153
<b>Table 22.</b> Ingredients for control breakfast and functional food breakfast	156
<b>Table 23.</b> Nutrient profiles for control breakfast and functional food breakfast	157
<b>Table 24.</b> FCR and FRAP analysis of baobab extracts 1 (MinVita) and 2 (Aduna)	160
<b>Table 25.</b> FRAP and FCR analysis of fresh vs. frozen muffins	161
<b>Table 26.</b> Nutrient contents of all breakfast meals	177
<b>Table 27.</b> Baseline characteristics of sample (chapter 6)	183
<b>Table 28.</b> Map task scores at T0, T60 & T120 for all breakfasts	185
<b>Table 29.</b> Delayed recall scores at T60, T120 & T180 for all breakfasts	185
<b>Table 29.</b> Baseline characteristics of participants consuming >43g available CHO (chapter 7)	210
<b>Table 30.</b> Map task scores at T0, T60 & T120 for all breakfasts	212
<b>Table 31.</b> Delayed recall scores at T60, T120 & T180 for all breakfasts	213

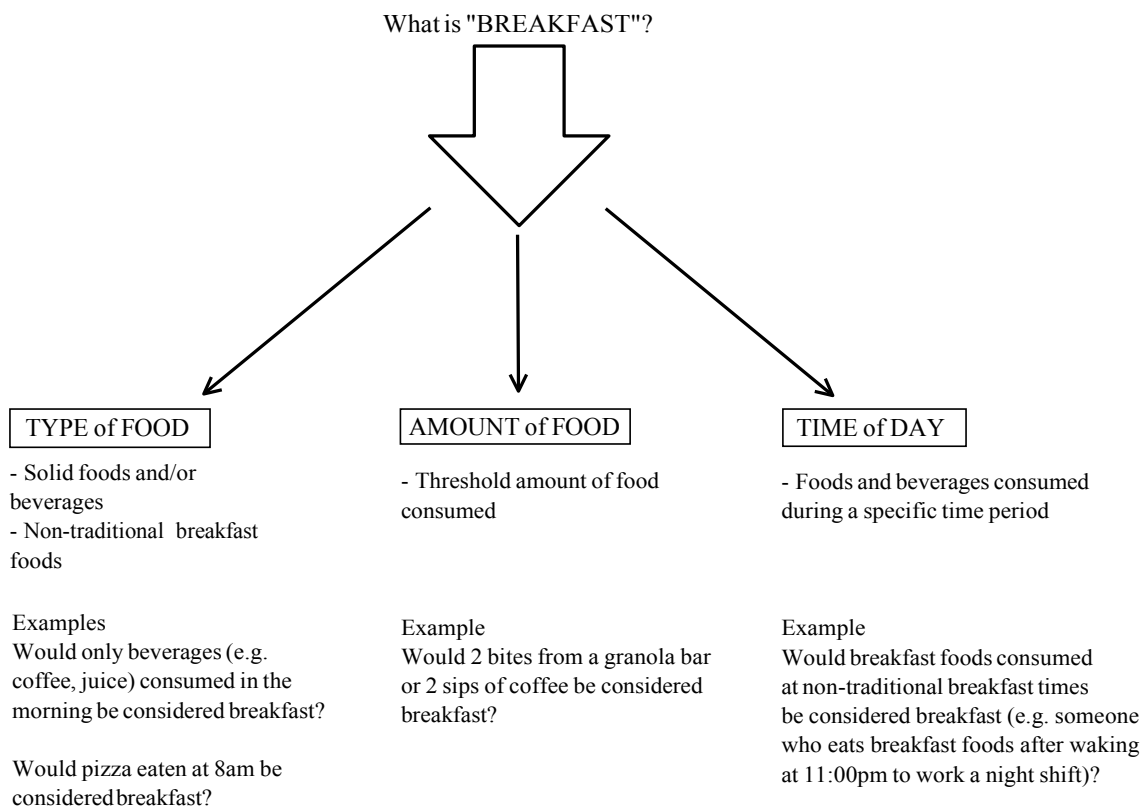
## **Chapter 1: Review of the breakfast literature**

### **1.1 A history of breakfast**

Eating a regular breakfast has been promoted since the 1800s, when Seventh Day Adventists such as John Henry Kellogg promoted breakfast cereals as a cure for what were curiously referred to as ‘the common ills of the day’ (Lawrence, 2008). Small studies emerged through the 1900s reporting potential health benefits (Tuttle, Wilson, & Daum, 1949) and in the 1950s there was a drive to promote a regular, healthy breakfast (fruit or fruit juice, cereal, milk, bread, and butter), instead of the fashionable coffee and cigarette (Ingoldsby, 1951). The Alameda Country Study (Belloc & Breslow, 1972) identified breakfast as one of the ‘seven healthy habits’ associated with increased longevity, and since then research has continued to investigate the benefits of breakfast and its impact on the behavioural, affective and cognitive aspects of health.

### **1.2 Definition of breakfast**

The lack of a universal definition of breakfast has led to difficulties when making comparisons between breakfast studies. Inconsistencies relate to whether it is the type of food, an amount of food, or the time of day that is understood to represent breakfast (Figure 1) (Rampersaud, 2009). During adolescence, breakfast habits may be affected by maturational changes which lead to later bedtimes, diminished weekday sleeping and extended weekend sleeping (Carskadon, 2011). This may cast doubt on the reliability of self-reported measures to represent an individual’s true breakfast habits depending on whether they are reported on a weekday or weekend. Additionally, the ability to accurately and reliably recall breakfast items may be selectively under-reported, particularly if the foods consumed are high in fat or sugar (McCrary, Howarth, Roberts, & Huang, 2011).



**Figure 1.** Considerations for how breakfast is defined. Reproduced with permission (Rampersaud, 2009)

A systematic review of the context and quality of breakfast consumption found that only eight of 24 studies in adults and young people included a definition of breakfast, which varied between all but two studies (Mullan & Singh, 2010). The latest USA National Health and Nutrition Examination Survey (NHANES) defined breakfast as anything the respondent (adolescent) considered to be breakfast (Deshmukh-Taskar et al., 2010). Although there is limited evidence, qualitative research suggests that in young adults (18-24 years) there are common beliefs concerning the food types and timings that constitute breakfast (Chapman, Melton, & Hammond, 1998). Further research to establish a consistent definition of breakfast is required, but until then all studies should routinely report individual breakfast definitions.

## 1.3 Adolescents

### 1.3.1 Defining adolescence

Adolescence can be identified as the period of growth that occurs from the onset of puberty, and is said to end when a stable, independent adult role in society is attained (Blakemore, Burnett, & Dahl, 2010). With puberty comes profound physiological, psychological, emotional and social changes, influenced to varying extents by internal and external factors including genetics and the environment (Pfeifer & Blakemore, 2012). Consequently, assigning a definitive age range to the period of adolescence is considerably subjective. The World Health Organisation (WHO) defines adolescence as the period of development occurring between the age of 10 and 19 years (WHO, 2011). Based on biological determinants this definition can generally be applied universally, but differences may be apparent at different time points and across cultures and socio-economic circumstances. Therefore, to make valid comparisons across research, studies should define age ranges, or at the very least refer to the stage of adolescence (i.e. early vs. late).

### **1.3.2 Health behaviours**

Increased independence from parents and the importance of approval from peers is reflected in the forming of adolescents' attitudes towards food choices and the need for greater control over food-based decisions (Conner, Norman, & Bell, 2002). Attitudes and health behaviours established during adolescence, including those relating to physical activity (PA) and food choice, are reported to continue into adulthood and impact the development of health conditions (Viner & Macfarlane, 2005; Viner et al., 2012). Therefore, adolescence represents a logical time for intervention, and encouraging health-promoting behaviours could potentially have long-lasting positive effects into adult life (Conner et al., 2002). Furthermore, understanding the underlying factors contributing to adolescent health behaviours is crucial for the development of successful adolescent interventions.

## **1.4 Breakfast trends in adolescents**

### **1.4.1 Breakfast frequency**

In the UK, Australia and USA, health recommendations include regularly eating a healthy, nutrient-dense breakfast (Change4Life, 2014; National Health and Medical Research Council, 2013; United States Department of Agriculture (USDA), 2010), although currently, there is no consensus for a recommendation on a definitive number of days on which breakfast should be eaten to provide health benefits.

Despite efforts to promote breakfast, trends from the USDA combined survey data suggest that breakfast consumption is declining in young people ( $n=24,363$ ; 1-18 years) (Siega-Riz, Popkin, & Carson, 1998). Based on 24-hour dietary recall, from 1965 to 1991, breakfast consumption (defined as any food or beverage consumed from 0500-1000am) declined in adolescents (11-18 years) by between 13 and 20%. Furthermore, the likelihood of breakfast consumption was significantly less in 15-18 year olds than 11-14 year olds, and significantly less for females compared to males.

A decline in adolescents' (11-15 years;  $n=455,391$ ) daily breakfast consumption (DBC) (defined as more than a glass of milk or fruit juice) was also reported in the Health Behaviour in School-aged Children (HBSC) study, between 2002 and 2010 (Lazzeri et al., 2016). Of 19 countries, DBC significantly decreased across 11 countries (Belgium, France, Germany, Croatia, Spain, Poland, Russian Federation, Ukraine, Latvia, Lithuania and Norway), and whilst DBC significantly increased in England, Wales and Scotland from 2002 to 2006, this trend was not apparent in 2010, where almost a third of adolescents were not consuming breakfast daily.

Cross-sectional HBSC data suggests that adolescent breakfast skipping is highest in the USA (Haug et al., 2009), although this may also reflect the large amount of research originating from this area. NHANES data (1999-2006) identified 20% of USA children and young adolescents (9-13 years), and 32% of adolescents (14-18 years), as breakfast skippers (Deshmukh-Taskar et al., 2010), whilst HBSC data reports that 54% of boys and 42% of girls (11-15 years) from the USA eat a regular breakfast (Haug et al., 2009). Across the majority of Central, Southern and Northern European

countries more than 50% of adolescents reported DBC, with the highest consumers ( $\geq 80\%$ ) from the Netherlands and Portugal (Inchley et al., 2016). The HELENA study assessed the frequency and quality of breakfast in 2672 adolescents (12-17 years) across nine European cities (non-UK) and found that whilst 77% consumed some form of breakfast, the overall quality (defined by not including at least one item from all three food groups) was low (Hallstrom et al., 2012). These data highlight the stark differences in reported DBC behaviours collected across countries, and support suggestions of a decline of breakfast consumption in adolescents, which is more prevalent in girls and in older children.

#### **1.4.2 Factors influencing breakfast behaviours**

Variation between countries in breakfast consumption frequencies could be due, in part, to differences in cultural breakfast-norms or family structure. HBSC data suggest that children living in two-parent households are more likely to consume breakfast than children from one-parent households (Lazzeri et al., 2016). There also appears to be a greater tendency to skip breakfast in children from ethnic minority backgrounds (Delva, Johnston, & O'Malley, 2007; Donin et al., 2014; Mullan & Singh, 2010; Niemeier, Raynor, Lloyd-Richardson, Rogers, & Wing, 2006), but research in this area is limited. Mullan and Singh (2010) identified six of 24 studies reporting ethnicity, although five of these studies were USA based, thus limiting the generalisability of the findings. Socio-economic status (SES) may also relate to breakfast consumption, such that children and adolescents from low-income households may be more likely to skip breakfast, potentially due to breakfast items not being made available or where parents have limited time in which to prepare breakfast themselves (Deshmukh-Taskar et al., 2010; Donin et al., 2014). Furthermore, inverse associations between breakfast frequency and overweight or obesity appeared to be more prevalent in adolescents (13-16 years;  $n=39,000$ ) from low-income and ethnic minority backgrounds (Delva

et al., 2007). Therefore, it is important that studies consider the potential for these confounding factors in the design and analysis of breakfast-based studies.

Research investigating the context of breakfast skipping may cultivate understanding of the circumstances under which to target breakfast-eating behaviours during interventions, which are limited in adolescents. Barriers towards regular breakfast habits in adolescents are generally reported to revolve around a lack of time, a lack of food availability, stress, and weight control (Mullan & Singh, 2010), the latter of which appears to be a common misconception, particularly amongst adolescent girls (Field, Haines, Rosner, & Willett, 2010; Neumark-Sztainer, Story, Hannan, Perry, & Irving, 2002). Social considerations of breakfast consumption, including where it is eaten (at home vs. at school) and with whom (alone vs. with family), have also been shown to play an important role, not only in the consumption of breakfast, but also in the quality of the breakfast consumed (Mullan & Singh, 2010).

### **1.4.3 Breakfast composition**

As well as the frequency of breakfast consumption, the composition of breakfast is of increasing interest due to the contribution of breakfast to adolescent intakes of macronutrients, fibre and several micronutrients (Deshmukh-Taskar et al., 2010; Kafatos et al., 2005; McNulty et al., 1996). Ready to eat cereals (RTEC) are reportedly the most commonly consumed breakfast amongst UK adolescents (10-15 years;  $n=70,061$ ), eaten by up to 36% of students (Balding & Regis, 2015). NHANES data suggests a shift in US breakfast patterns, where more adolescents reported consuming 'other' breakfasts (44%) compared to RTEC (36%) (Deshmukh-Taskar et al., 2010). National Diet and Nutrition Survey data may point towards similar trends in the UK, with a significant fall reported in the consumption of non high-fibre cereals in adolescent boys (11-18 years) with no change in consumption rates of high-fibre cereals (Whitton et al., 2011). This raises concerns as higher fibre intakes were observed in adolescents (12-15 years) regularly consuming



RTEC compared with ‘other’ breakfasts or breakfast skippers (Deshmukh-Taskar et al., 2010; Kafatos et al., 2005; McNulty et al., 1996). Furthermore, adolescent fibre intakes are already well below recommended intakes across Nordic countries (Samuelson, 2000).

The fortification of RTECs is a contributing factor to higher micronutrient intakes which are reported in young people (4-18 years) regularly consuming RTECs at breakfast (Gibson, 2003; Kafatos et al., 2005; Michels et al., 2015). It is unlikely that breakfast skippers are able to compensate for this nutrient deficiency from other meals consumed throughout the day (Morgan, Zabik, & Stampely, 1986). Powers and colleagues (2016) investigated this when they compared eating fortified and unfortified RTEC for breakfast and supper and the effects on micronutrient status (Powers, Stephens, Russell, & Hill, 2016). A significant increase in micronutrient status was observed in adolescent girls who ate fortified RTEC whether for breakfast or for supper (16-19 years,  $n=71$ ), but in the supper group this was concurrent with significant weight gain, despite no increase in self-reported overall energy intakes. Efforts to promote the consumption of breakfast in adolescents should highlight the positive impact on micronutrient and fibre status, and the potential for benefits to maintaining weight status when consumed at breakfast time.

#### **1.4.4 Breakfast quality indexes**

A ‘good quality’ breakfast has been defined as consisting of foods from the cereal, dairy, and fruit or vegetable groups (Aranceta, Serra-Majem, Ribas, & Perez-Rodrigo, 2001). Using this as the basis for a breakfast quality index (BQI), Hallstrom and colleagues (2012) designed a BQI and reported that 96% of adolescents across ten European cities were not including foods from all three groups, which was more pronounced in adolescents who were: older, from Southern Europe, and from low-income families (Hallstrom et al., 2012). Although it is useful to define breakfast quality there are a lack of studies, making it difficult to harmonise research findings, and which can be

misleading where serving sizes are not considered, or foods are inaccurately categorised as good or bad (Mullan & Singh, 2010).

## 1.5 The influence of breakfast on weight status

### 1.5.1 Breakfast consumption

Children or adolescents who are overweight or obese have a greater likelihood of developing cardiovascular disease (CVD), respiratory diseases and metabolic syndrome (MetS), including type 2 diabetes (T2DM) in adulthood (Department of Health (DOH), 2011; WHO, 2016). A large amount of the breakfast research relates to its impact on weight status.

Prospective studies support the consumption of breakfast in adolescents and a lower risk of overweight and obesity (Niemeier et al., 2006; Siega-Riz et al., 1998; Timlin, Pereira, Story, & Neumark-Sztainer, 2008) and MetS (Wennberg, Gustafsson, Wennberg, & Hammarstrom, 2014) in adulthood. Monitoring five-year weight change in adolescents ( $15.9 \pm 0.1$  years;  $n=9919$ ) the 'Add Health' study observed a significant decrease in the frequency of breakfast consumption over the study period, associated with concurrent increases in weight gain, and increases in fast food consumption (Niemeier et al., 2006). The 'Project EAT' study ( $n=2216$ ) monitored breakfast frequency and five-year weight change in adolescents ( $14.9 \pm 1.6$  years) (Timlin et al., 2008). They saw an inverse association between frequency of breakfast consumption and change in body mass index (BMI) ( $\text{kg/m}^2$ ) which appeared to be dose-responsive. Weight status was directly associated with weight-related concerns and practices (including dieting), suggesting that overweight individuals were using breakfast skipping in an attempt to control their weight which, conversely, put them at greater risk of becoming overweight. In a Swedish cohort of adolescents ( $n=889$ ; 16 years) poor breakfast habits, defined as skipping breakfast (or eating/drinking something sweet), predicted central obesity and risk factors for pre-diabetes, including high fasting glucose levels, at 27-year follow up (Wennberg et al., 2014).

Analysis of baseline data in the ‘GUT’ study ( $n=14,576$ ) associated breakfast skippers (9-17 years) as more likely to be overweight compared to regular breakfasters; however, conversely, during the three-year follow up, normal-weight breakfast skippers gained weight whereas overweight breakfast skippers lost weight (Berkey, Rockett, Gillman, Field, & Colditz, 2003). Notably, height and weight were self-reported although the subjects were the children of participants from the ‘Nurse’s Health Study’ so guidance was available. The authors proposed the unexpected findings could be linked to differences in resting energy expenditure (REE) between children who were normal-weight and children who were overweight, although this was not measured. It is well established that REE is higher in individuals who are overweight and obese compared with individuals who are not obese, due to increased fat mass (FM) and fat free mass (FFM), and higher demands of weight-bearing activities (Molnár & Schutz, 1997). However, Betts and colleagues (2014) found that there were no significant differences in REE (reported as resting metabolic rate) between lean adults who regularly skipped or consumed breakfast over a six-week period (Betts et al., 2014). Findings suggest that more research is needed to understand the long- and short-term adaptations in REE to breakfast habits, in adults and young people.

Cross-sectional studies tend to support positive associations between regular breakfast consumption and a reduced risk of overweight or obesity in children, young adolescents (9-11 years) (Zakrzewski et al., 2015), and adolescents (11-19 years) (Lazzeri et al., 2014; Nurul-Fadhilah, Teo, Huybrechts, & Foo, 2013), although there are exceptions (Kim & So, 2012). A systematic review of the effects of breakfast consumption on body weight outcomes in young people (7-21 years) (Szajewska & Rusczyński, 2010), reported that breakfast consumption protected against overweight or obesity in 13 of 16 studies. Specific to RTEC, a systematic review (de la Hunty, Gibson, & Ashwell, 2013) reported lower BMIs in regular RTEC eaters (up to 18 years old) compared to irregular RTEC consumers or breakfast skippers; however, it was not established whether effects were due to the

consumption of RTEC, or the consumption of breakfast in general. Furthermore, 13 of the 14 articles included were funded by a breakfast cereal manufacturer, as was the review itself; although this was adjusted for in the analysis and the established guidelines for reporting of such studies were followed.

### **1.5.2 Breakfast composition**

Fewer studies consider the type of breakfast consumed and the impact on weight status. NHANES data showed no difference in overweight prevalence between breakfast skippers and breakfast consumers; however, BMI *z*-score, waist circumference and obesity were significantly higher in those consuming ‘other’ breakfasts (and no breakfast) compared to RTEC (Deshmukh-Taskar et al., 2010). Furthermore, compared with adolescents consuming ‘other’ types of breakfasts, RTEC eaters reported consuming less added sugar, less fat and had lower cholesterol intakes, which may be due to ‘other’ breakfast eaters consuming more fried foods, sweet bread, rolls and pastries. Cho and colleagues (2003) NHANES data analyses associated the consumption of a fibre-rich breakfast (based on whole grains or fruits) in adolescence, with a lower risk of obesity in adulthood (Cho, Dietrich, Brown, Clark, & Block, 2003), potentially due to the satiating effects of fibre, which may reduce overall food intake (Slavin & Green, 2007). Breakfasts higher in whole grains and fibre have been shown to positively influence appetite control in children and adolescents ( $n=28$ ; 9-13 years) compared to refined breakfasts (Pereira et al., 2011), although research reporting appetite control in young people is limited. In university students ( $n=14$ ), a high-fibre, carbohydrate- (CHO) rich breakfast was the least palatable, but most filling meal, and was associated with less food-intake during the morning and at lunch (Holt, Delargy, Lawton, & Blundell, 1999). European adolescents (12.5-17.5 years) who were daily RTEC consumers were 57 % less likely to be overweight than RTEC non-consumers, although they did not differ in glucose or lipid status ( $n=387$ ) (Michels et al., 2015).

### **1.5.3 Energy intake and PA levels**

Two longitudinal studies observed that breakfast consumers had higher energy intakes than breakfast skippers (Berkey et al., 2003; Deshmukh-Taskar et al., 2010); however, in one study this was offset by increased PA levels (Berkey et al., 2003). Associations between regular breakfast consumption and higher PA levels were reported in schoolchildren (10-16 years) (Sandercock, Voss, & Dye, 2010), which was concurrent with a lower BMI. Corder and colleagues (2011, 2014) identified adolescents ( $14.5 \pm 0.5$  years) with high activity levels as being more likely to eat breakfast at the weekend (Corder et al., 2014), and during the morning (girls only) (Corder et al., 2011), independent of body composition, total energy intake and SES, suggesting that habitual breakfast habits may indicate greater participation in physical activity.

A RCT considering causal links between breakfast habits and components of energy balance reported significantly higher energy intakes ( $>500\text{kcal/d}$ ) in breakfast-eating, free-living adults ( $n=33$ ) compared to breakfast skippers (Betts et al., 2014). However, there were no differences between weight-gains over the 14-day intervention, potentially explained by significantly higher PA thermogenesis in the breakfast consumers. The light-intensity activities rather than structured PA were affected, indicating an increase in spontaneous as opposed to conscious decisions to participate in PA. The promotion of a regular breakfast in adolescents could therefore have important implications for adolescents' participation in unstructured activities as a way to maintain PA levels, which generally decline during this time (Riddoch et al., 2007), particularly in girls (Armstrong & Welsman, 2006).

### **1.5.4 Breakfast habits**

Breakfast habits may influence the amount of food consumed at subsequent meals and potentially impact weight status. An acute study reported lower energy intakes (by  $\sim 17\%$ ) in adults who were

habitual breakfast eaters when they consumed breakfast, compared to when they skipped it, suggesting they were compensating for missing breakfast by eating more at lunch (Astbury, Taylor, & Macdonald, 2011). Conversely in children ( $n=21$ , 8-10 years), the majority of whom habitually consumed breakfast, total energy intake was significantly less on the day when breakfast was skipped compared to when breakfast was consumed (by  $\sim 362$ kcal), suggesting that there was no compensation (Kral, Whiteford, Heo, & Faith, 2011). There were no significant differences between intakes at lunch or the rest of the day, but children who skipped breakfast perceived that they were hungrier, less full and could eat more for lunch than when they did eat breakfast. When breakfast composition was considered, less food was eaten at lunch by children and adolescents ( $n=37$ , 9-12 years) following the consumption of low-GI compared to high-GI breakfasts, or low-GI breakfasts with added sucrose (Warren, Henry, & Simonite, 2003).

The lack of experimental studies in young people make it difficult to infer causality between consuming breakfast and positive effects on weight status, particularly as habitual breakfast eaters are more likely to: be non-smokers, consume less alcohol, be more physically active and consume a diet that contains more fibre and micronutrients (Keski-Rahkonen, Kaprio, Rissanen, Virkkunen, & Rose, 2003; Smith et al., 2010). Improvements in nutritional quality may also be associated with higher fruit and vegetable intakes or lower unhealthy snack intakes (Ahadi et al., 2015), which are frequently reported by regular breakfast consumers (Michels et al., 2015; Vereecken et al., 2009). Furthermore, it is hypothesised that initiating a healthy habit in one area (for example, breakfast) could have a positive influence on other healthy habits. A review in young people (5-18 years) by Leech and colleagues (2014) identified a clustering of healthy, or unhealthy behaviours relative to: diet, PA and sedentary behaviours, particularly in older children and adolescent females (Leech, McNaughton, & Timperio, 2014). Therefore, breakfast consumption could be considered as a marker of an overall health-conscious lifestyle (Nicklas, O'Neil, & Berenson, 1998).

### 1.5.5 Summary

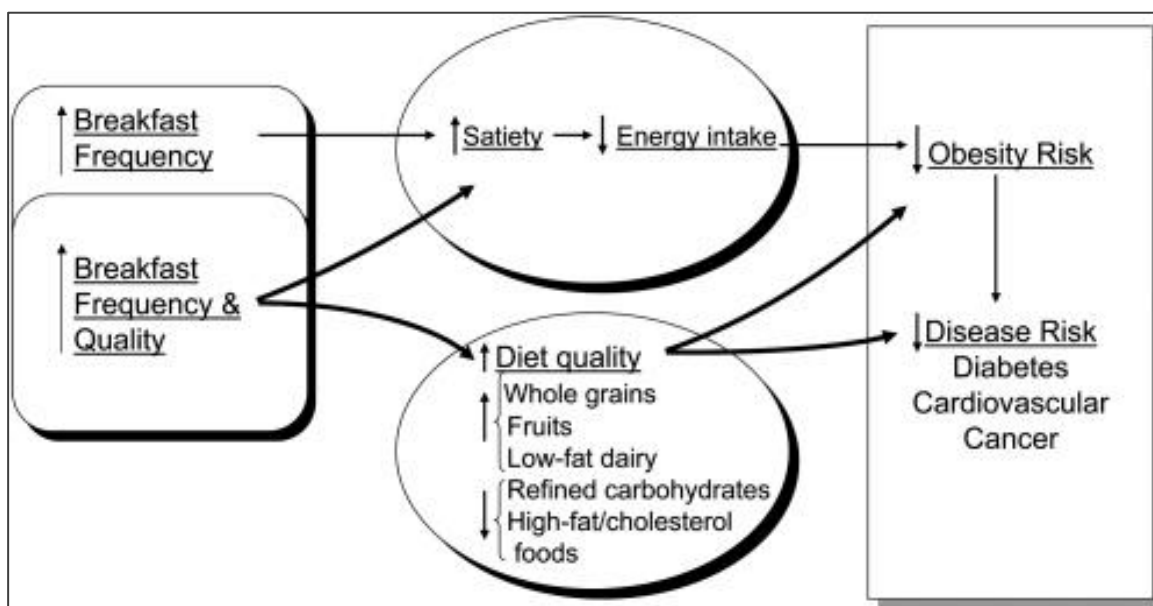
Overall, the literature supports recommendations for the regular consumption of breakfast in adolescents to protect against overweight and obesity, and reduce the risk of impaired gluco-regulation in adulthood; although, a lack of experimental studies and the large number of confounding factors make it difficult to infer causality. Emerging evidence suggests the need to give greater consideration to the type of breakfast consumed by adolescents and how it influences weight status, particularly considering the potential protective effects of consuming a fibre-rich breakfast.

## 1.6 The effect of breakfast on gluco-regulation

### 1.6.1 Breakfast consumption

#### 1.6.1.1 Meal frequency

Eating regular meals, including breakfast, is suggested to improve risk factors for CVD through appetite and diet quality pathways (Figure 2) (Timlin & Pereira, 2007).



**Figure 2.** Theoretical model of pathways through which breakfast (frequency and quality) may protect against the development of chronic diseases and obesity. Presented with permission (Timlin & Pereira, 2007)

During adolescence (13-18 years) meal patterns become increasingly irregular (Höglund, Samuelson, & Mark, 1998; Samuelson, 2000), and in Japanese adolescents (10-19 years) this was associated with an increased prevalence of raised serum cholesterol levels (Murata, 2000). Experimental studies investigating associations between meal frequency and gluco-regulation are currently limited to adult populations only. A RCT in healthy, lean adults ( $n=9$ ) reported that insulin response (IR) and peak IR were higher when participants were following irregular meal patterns compared to regular meal patterns (Farshchi, Taylor, & Macdonald, 2004). When the study was replicated in obese adults ( $n=10$ ) (Farshchi, Taylor, & Macdonald, 2005a), similar effects on IR were observed along with improved lipids and higher postprandial diet-induced thermogenesis (DIT) (the increase in metabolic rate after the ingestion of food (Rothwell & Stock, 1983)), suggesting that optimal CHO and lipid metabolism may also be related to regular meal patterns.

#### ***1.6.1.2 Consuming versus skipping breakfast***

A RCT in healthy adults ( $n=12$ ) investigated the effect on gluco-regulation of consuming versus omitting breakfast, and consumption of a snack and a lunch provided 2.5, and then 1.5 hours later, respectively (Astbury et al., 2011). A higher glucose response (GR) and IR to the snack was reported in the no-breakfast condition; however, there were no significant differences in GR and IR to the lunch, which was in contrast to previous findings in healthy individuals (Liljeberg, Åkerberg, & Björck, 1999). The same research group considered the effects of breakfast consumed over 14 days in healthy adults ( $n=10$ ) and found that IR to a standard meal was higher in the no-breakfast condition (Farshchi, Taylor, & Macdonald, 2005b). Breakfast omission also resulted in significantly higher fasting total and LDL cholesterol levels, and an increase in energy intake. However, DIT response to a standard meal was the same, suggesting that skipping breakfast has no measurable short-term effect on DIT, although it would be informative to consider the effects over a longer period. Comparing the effects of breakfast consumption on metabolic control over six weeks, Betts



and colleagues (2014) reported a more stable GR during the afternoon and evening in breakfast consumers compared to breakfast skippers, although no other benefits to cardiovascular health indexes were observed in either group (Betts et al., 2014).

## **1.6.2 Breakfast composition**

The type of food consumed at breakfast will differentially affect the metabolic response across a range of individuals. Much of the adolescent research around the composition of breakfast makes comparisons between: RTEC, macronutrients, glycaemic index (GI) or glycaemic load (GL). Relative to its effects on gluco-regulation it is most frequently the GI and/or GL, or the fibre content, that is considered. Adolescent studies that have included GR or IR as secondary outcomes (to effects on cognitive performance) are discussed in detail elsewhere (chapter 1.10).

### ***1.6.2.1 Low-GI and high-GI breakfasts***

GI is a method used to classify CHO-rich foods according to their effect on postprandial glycaemia. It can be defined as the incremental area under the two-hour blood glucose curve, after ingestion of 50g of available CHO, calculated as a percentage of the corresponding area following an equivalent amount of CHO from a standard reference product (white bread or glucose) (Atkinson, Foster-Powell, & Brand-Miller, 2008). The extent of postprandial glycaemia depends on both the GI and the amount of CHO consumed; therefore, the concept of GL provides an indication of the total glycaemic effect of the diet and considers the effects of mixed meals, which more closely represent 'real world' situations. GL is the product of the GI and total dietary CHO, divided by 100.68 (Tolfrey & Zakrzewski, 2012).

Low-GI foods release CHO slowly, decreasing the amount of insulin required to clear glucose from the blood, which in turn may: up-regulate insulin receptors, increase insulin sensitivity and improve glucose tolerance (Messier, 2004). Increasing intakes of starch-rich low-GI foods also enhances the

formation of short-chain fatty acids (SCFA) during fermentation in the colon. The SCFA can: reduce hepatic glucose output and serum-free fatty acids, stimulate glucagon-like peptide-1 secretion which modulates insulin secretion and insulin sensitivity, and regulate satiety by inhibiting glucagon secretion (Pereira et al., 2011; Timlin & Pereira, 2007). After a high-GI meal, a rapid rise and peak in plasma glucose concur with a high IR, resulting in rapid glucose disposal which itself can cause blood glucose levels to decrease below the fasting concentration during the postprandial period, potentially worsening glucose control (Wolever, 2003).

A review of breakfast composition and its effects on GI and metabolism in young people (children and adolescents), supports the consumption of low-GI compared with high-GI mixed-breakfast meals to: reduce postprandial glycaemia and insulinaemia, improve fat oxidation, and increase satiety (Tolfrey & Zakrzewski, 2012); thus promoting a low-GI breakfast to young people is frequently recommended in the breakfast literature (Rampersaud, 2009; Szajewska & Ruszczynski, 2010). Experimental evidence specific to gluco-regulation in adolescents is largely based on individuals who are overweight or obese and therefore at increased risk of impaired gluco-regulation (Ball et al., 2003; Ludwig et al., 1999), or consider the whole diet (Ebbeling, Leidig, Sinclair, Hangen, & Ludwig, 2003; Spieth et al., 2000). One study which compared differences between overweight and non-overweight adolescents ( $n=20$ , 11-13 years) observed a higher peak GR and IR to a high-GI breakfast, compared to a low-GI breakfast, in the girls who were overweight (Zakrzewski, Stevenson, & Tolfrey, 2012). This was associated with a delayed decline following peak blood glucose, potentially reflecting an increase in metabolic demands and supporting previous suggestions (chapter 1.5) of metabolic differences relative to weight status in adolescents (Sinha et al., 2002).

### **1.6.2.2 Fibre-rich breakfasts**

The viscosity of high-fibre foods promotes delayed gastric emptying and influences the post-prandial GR and IR (Tosh, 2013). The ‘CHASE’ study of children and young adolescents (9-10 years;  $n=4116$ ) considered different RTECs and associated frequent consumption of high-fibre cereals with lower risk markers for T2DM (including a lower GR and IR), compared with: breakfast skipping, consuming a low-fibre cereal, or consuming ‘other’ breakfasts (Donin et al., 2014). Experimental studies comparing fibre intakes are limited in adolescents and children, but a review in adults reported that the consumption of high-fibre breakfast cereals containing at least 4g of  $\beta$ -glucan, can significantly improve the GR and IR to later meals (Tosh, 2013).

### **1.6.3 Summary**

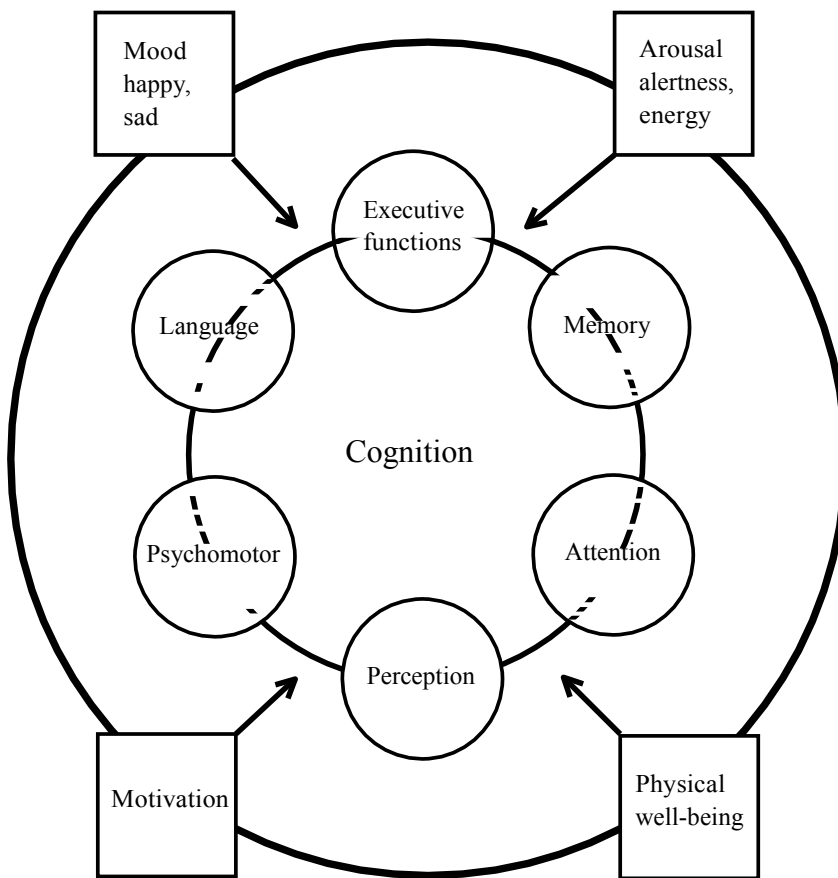
The research to date generally supports the consumption of breakfast compared to no breakfast and improvements to gluco-regulation, potentially due to metabolic benefits associated with eating regular meals. The composition of breakfast may improve gluco-regulation relative to its low-GI and/or high fibre content and this supports current breakfast recommendations in the literature. In adolescents there are limited studies specific to gluco-regulation and most of the research is performed in the context of explaining the effects of breakfast on cognitive (and academic) performance (chapter 1.8).

## **1.7 Measuring cognitive performance**

### **1.7.1 Cognitive domains**

The term cognition refers to the complex mental functions that enable individuals to perform activities critical for daily living and can be identified as any mental process or function mediated by the brain (Wesnes, 2010). There are a number of domains associated with cognitive functioning including: executive functions, memory, attention, perception, psychomotor functions and language skills, as well as external contributing factors including: arousal, mood, motivation and physical

wellbeing (Figure 3) (Schmitt, Benton, & Kallus, 2005). Cognitive domains, for example memory, can be sub-divided to represent separate specific functions, although in reality these processes are interlinked, where efficient functioning in one area is highly dependent on the integrity of other processes (Schmitt et al., 2005).



**Figure 3.** Domains affecting cognition (circles) and contribution from external factors (squares). Reproduced with permission (Schmitt et al., 2005).

Memory includes four major components: episodic or declarative memory, semantic memory, procedural memory and working (or short-term) memory (Table 1) (Budson & Price, 2005).

Clinically, impairments to semantic and procedural memory are less common, whereas working and episodic memory are disrupted in numerous conditions relating to neurological, cardiovascular, nutritional and behavioural disorders (Budson & Price, 2005), making them key research targets.

**Table 1.** Selected memory systems. Reproduced with permission from (Budson & Price, 2005), Copyright Massachusetts Medical Society.

<b>Memory system</b>	<b>Major anatomical structures involved</b>	<b>Length of storage of memory</b>	<b>Type of awareness</b>	<b>Examples</b>
Episodic memory	Medial temporal lobes, anterior or thalamic nucleus, mammillary body, fornix, prefrontal cortex	Minutes to years	Explicit, declarative	Remembering a short story, what you had for dinner last night and what you did on your last birthday
Semantic memory	Inferolateral temporal lobes	Minutes to years	Explicit, declarative	Knowing who was the first president of the United States, the colour of a lion, and how a fork differs from a comb
Procedural memory	Basal ganglia, cerebellum, supplementary motor area	Minutes to years	Explicit or implicit, nondeclarative	Driving a car with a standard transmission (explicit) and learning the sequence of numbers on a touch-tone phone without trying (implicit)
Working memory	Phonologic: prefrontal cortex, Broca's area, Wernicke's area Spatial: prefrontal cortex, visual-association areas	Seconds to minutes: information actively rehearsed or manipulated	Explicit, declarative	Phonologic: keeping a phone number "in your head" before dialling Spatial: mentally following a route or rotating an object in your mind

### **1.7.2 Cognitive domains in breakfast interventions**

Understanding how diet affects cognitive function is currently a key focus to inform the development of drugs which could enhance memory and learning in adults and children, and which in the future may help to lower, or even prevent memory decline (Spencer, 2010b). Research around diet and cognition may also help optimise cognitive function in young, healthy individuals, potentially improving academic performance or behaviour (Adolphus, Lawton, & Dye, 2013).

A review of cognitive measures used in nutrition interventions in adults and children identified: memory (encompassing verbal, visual, spatial, and articulated working memory), selective and sustained attention, executive function, information processing speed, and global cognitive function, as the most commonly tested domains of cognitive function (de Jager et al., 2014). Overall, there

was more evidence for the benefits of nutrition interventions on verbal memory than for any other cognitive domain.

Specific to breakfast, a review by Hoyland and colleagues (2009) reported that the majority of studies in children and adolescents (4-18 years) focused on memory ( $n=9$ ), particularly verbal and spatial memory, and attention ( $n=7$ ), with breakfast consumption (compared to breakfast skipping) being most beneficial to the memory domain (Hoyland, Dye, & Lawton, 2009). This was supported by a more recent breakfast review in children and adolescents (4-18 years) where attention ( $n=21$  studies) and memory ( $n=15$  studies) were the most frequently assessed cognitive domain (Adolphus, Lawton, Champ, & Dye, 2016). The authors concluded that tasks requiring attention, memory and executive function were facilitated more reliably by breakfast consumption relative to breakfast omission; however, specific to adolescents there appears to be fewer consensus on the benefits of breakfast within the attention domain. The effects of breakfast composition on attention ( $n=21$ ) and memory ( $n=15$ ) were also considered and the authors associated a low-GI breakfast with positive effects on overall cognition, although the quality of the evidence was limited so no firm conclusions on which cognitive domains were most frequently improved were provided .

Furthermore, assigning a specific cognitive test to individual cognitive domains was inconsistent between studies, potentially due to the overlapping of cognitive domains (Adolphus et al., 2016). An earlier systematic review including children, adolescents (age not specified) and adults (20-63 years) reported that a lower postprandial GR was beneficial to cognitive performance, particularly memory ability, but it was unclear whether this was due to GI, GL or both (Edefonti et al., 2014).

### **1.7.3 Measuring and selecting cognitive domains**

Cognitive function can be measured objectively and there are a vast number of tests designed to target specific, or a multitude of, cognitive domains and functions. Computerised tests are common and allow for standardised presentation and accurate collection of responses, which can be modified

for use in all populations. Alternatively, pen and pencil tests are still in use which can provide more flexibility in terms of delivery and accessibility, and retain a personalised approach to data collection (Schmitt et al., 2005). Mood should be measured alongside cognitive performance, as one can influence the other, and can be assessed using validated self-rated scales such as visual analogue scales (VAS) and questionnaires which have been validated for use in nutrition interventions (Hammersley, Reid, & Atkin, 2014).

A more harmonious approach to cognitive data-collection and reporting in nutrition interventions is required, and it is important that tests sensitive enough to detect changes relative to the cognitive domain most affected by the nutrition intervention are selected (de Jager et al., 2014). Several reviews offer guidance to researchers selecting cognitive tests (Benton, Kallus, & Schmitt, 2005; de Jager et al., 2014; Philippou & Constantinou, 2014; Schmitt et al., 2005; Wesnes, 2010). Most recommend that nutrition studies use a range of tests to account for the potential interaction effects from other cognitive domains (Benton et al., 2005; Hoyland et al., 2009; Schmitt et al., 2005; Wesnes, 2010); however, unless all tests included are equally sensitive to the nutrition intervention under investigation, this may increase the potential for type one errors (Bell, Lamport, Butler, & Williams, 2015) and is not conclusively recommended (Philippou & Constantinou, 2014).

Ultimately, cognitive tests should be selected based on the relationship between the food and the cognitive domain (de Jager et al., 2014) and should be identified as being:

- Suitable for repeated administration (reliability)
- Sensitive to the nutrient being tested (sensitivity)
- Valid for measuring the selected cognitive domain (validity)
- Appropriate to the sample
- Easy to interpret and administer

Based on the aforementioned reviews aiding the selection of cognitive domains most likely to be influenced by the consumption of breakfast, memory, specifically verbal and spatial memory, were selected for inclusion in the studies performed as part of this thesis (chapter 6 and 7) (de Jager et al., 2014). The implementation and development of the cognitive tests used (map task and delayed word recall task) are discussed in detail in the relevant chapters (chapter 6 and 7).

### *1.7.3.1 Verbal memory*

Verbal memory reflects the ability to store and subsequently retrieve and recognise previously presented verbal information, primarily in the form of anecdotes, stories, facts, serially presented words and declarative statements (de Jager et al., 2014). Tasks can be coded per the time in which information should be retained and are generally referred to as either immediate recall or delayed recall.

Performance on verbal memory tasks can be influenced by the age of acquisition of verbal abilities as well as intelligence, which makes them susceptible to floor and ceiling effects (Benton et al., 2005). A floor effect will produce a small distribution of very low marks and the perceived difficulty of the task results in participants losing motivation to complete it. If the test is too easy a ceiling effect occurs where most of the sample will achieve a small range of high scores. The data will not follow a normal distribution, the mean will be artificially lowered and the standard deviation truncated; therefore, reliability and validity will suffer (Benton et al., 2005). Ceiling effects are a common concern when using memory tests in young, healthy adults (Uttl, 2005).

Word lists can be used to measure immediate or delayed verbal memory recall. To adequately measure verbal memory selected words should be of a similar difficulty and lists matched for: the frequency that the words occur in English, the number of syllables of the words (Wesnes, 2010), and whether the words are concrete or abstract (i.e. the ability to create visual images from the



words). The length of the list will vary, generally being longer in older populations where an increased level of difficulty is required (Benton et al., 2005).

### ***1.7.3.2 Spatial memory***

Spatial memory represents a complex and multifaceted process which is interdependent on attention and memory and which is predominantly moderated by the hippocampus (de Jager et al., 2014). It can be characterised according to processes relating to: spatial working memory, memory for routes and object location memory. It is part of working memory that consists of: subsidiary ‘slave’ systems, the ‘phonological loop’ and the ‘visuospatial sketchpad’, which store specific material in a limited capacity (Fletcher & Henson, 2001). Spatial memory relates to the ability to store and retrieve knowledge about spatial features of the environment and is vital to everyday living, allowing a sense of where critical objects in the environment are located, as well as providing a mental map that can be used for navigation (de Jager et al., 2014).

Spatial memory can be classified into: spatial working memory (the ability to update information to solve a task when contextual conditions are different from one trial to the next), memory for routes (getting from location A to B), and object location memory (the ability to remember the fixed position of objects) (de Jager et al., 2014). Measuring spatial memory can be challenging due to interference from verbal associations; however, using abstract designs or nonsense figures can help minimise verbal mediation (de Jager et al., 2014).

## **1.8 A review of the effect of breakfast on cognitive and academic performance**

### **1.8.1 Breakfast consumption**

Much of the research in children and adolescents (4-18 years) comparing breakfast consumption habits and benefits to cognitive and academic performance comes from experimental studies spanning many years. During this time there have been numerous reviews published in an attempt

to consolidate study findings and provide some insight into the optimal frequency of breakfast consumption (Table 2).

**Table 2.** Overview of reviews of breakfast studies (breakfast vs. no breakfast) published from 1982 to 2016.

Year	Authors	Breakfast studies (total)	Outcome	Sample	Analysis	Conclusion
1982	Dickie & Bender	<30	AP, CF	Children*	Review	Evidence uncertain around benefits of breakfast Limitations: small sample sizes, inconsistent findings, subjective measures
1992	Smith & Kendrick	13	CF	Children & ado*.	Review	Evidence uncertain around benefits of breakfast Some support for benefits of breakfast on memory Limitations: small sample sizes, methodological discrepancies
1994	Roger & Lloyd	6	CF	Children & ado*.	Review	Evidence uncertain around the benefits of breakfast
1998	Pollitt & Matthews	16	CF	Children, ado (9-17 years) & university students*	Integrative summary	No support for long or short term benefits of breakfast on CF or AP
2002	Dye & Blundell	14(24)	CF	University students & adults (18-65 years)	Review	Evidence of effect in breakfast studies Memory improvements in 10/14 studies (not all specific to breakfast)
2008	Ells <i>et al.</i>	15(29)	AP, CF, behaviour	4-18 years	Systematic review	Small benefit of breakfast to some aspects of CF in 10/15 studies Limitations: study quality, methodological issues
2009	Hoyland <i>et al.</i>	45	AP, CF, behaviour	4-18 years (at risk & nourished)	Systematic review	Benefit of breakfast on CF in majority of studies, particularly memory domain Benefit of school breakfast on AP in all except 2 studies Limitations: mixed findings, methodological discrepancies
2013	Adolphus <i>et al.</i>	36	AP, behaviour	<18 years (at risk & nourished)	Review	Positive effect of breakfast on behaviour. Habitual and school breakfast positively influenced AP in children. Limitations: confounding factors not accounted for, methodological weaknesses
2016	Adolphus <i>et al.</i>	24(45)	CF	4-18 years (at risk & nourished)	Systematic review	Effect of breakfast on CF (attention, exec function and memory) especially in 'at risk' Limitations: small sample sizes, insensitive tests, limited research on adolescents

Abbreviations: CF, cognitive function; AP, academic performance; ado, adolescents. \*age range not provided

### *1.8.1.1 Cognitive performance*

From the early reviews there was a general consensus around a lack of evidence (Dickie & Bender, 1982a; Pollitt & Mathews, 1998; Rogers & Lloyd, 1994) attributed to inconsistent findings based on methodological discrepancies, though some support was given to the role of breakfast on memory abilities (Smith & Kendrick, 1992). With later reviews came more support for improvements in memory (Dye & Blundell, 2002; Ells et al., 2008); however, not all studies were specific to young people, some included university students or young adults (Dye & Blundell, 2002; Pollitt & Mathews, 1998), or were generally reluctant to support the overall benefits of breakfast (Ells et al., 2008).

To supersede conflicting and unsystematic reviews, the effects of breakfast on cognitive performance in well-nourished and nutritionally at-risk children and adolescents (4-18 years) were reported (Hoyland et al., 2009). The majority of studies supported a beneficial effect of breakfast over no breakfast on cognitive performance although differences between: cognitive tests used, time points used to collect cognitive measures, and a lack of classification of effects across domains, hindered comparisons. A more recent systematic review (Adolphus et al., 2016) promotes breakfast consumption in children and adolescents for acute benefits on domain-specific cognitive performance, particularly in under-nourished children; however, despite a reasonable number of studies ( $n=24$ ) the authors noted methodological inconsistencies across study designs and findings.

### *1.8.1.2 Academic performance*

Measures of academic performance are often used to assess the benefits of regular breakfast consumption on cognitive function in school-aged children. Hoyland and colleagues (2009) reported improvements in academic performance with the provision of a school breakfast programme (SBP) in all but two (of 13) studies, potentially linked to increases in school attendance (Hoyland et al., 2009). A systematic review including studies on adolescents identified an overall

positive relationship between habitual breakfast consumption and a SBP on aspects of academic performance and behaviour (Adolphus et al., 2013), potentially linked with the quality of breakfast consumed. However, not all studies report benefits (Adolphus, Lawton, & Dye, 2015; Miller, Waldfogel, & Han, 2012), which could relate to how the SBP is implemented (i.e. during lesson time), measured and supervised (i.e. by teaching or non-teaching staff). These factors appear to vary between schools and could impact the behaviour of participants and the overall success of the programme. Inconsistencies may also be due to a lack of consideration for the effect of SES (chapter 1.4.2) as well as differences between how breakfast is defined (chapter 1.2) to participants (Adolphus et al., 2013, 2015; Miller et al., 2012).

### *1.8.1.3 Nutritionally at-risk individuals*

Reviews including children and adolescents (<18 years) who are nutritionally at-risk report that improvements in cognitive and academic performance may be enhanced depending on the nutritional status of the individual (Adolphus et al., 2016; Adolphus et al., 2013; Hoyland et al., 2009). Positive effects of breakfast consumption compared to breakfast skipping were more evident in young people considered under-nourished, defined as <1 standard deviation below normal height or weight for age using US National Centre for Health Statistics (Cueto, Jacoby, & Pollitt, 1998; Pollitt, Cueto, & Jacoby, 1998). Few studies have investigated associations with blood measures. Children ( $10 \pm 0.6$  years) from low-income families who were considered nutritionally at-risk were reported to have greater improvements in cognitive performance compared to children considered no-risk (Cueto et al., 1998). Blood glucose levels were not associated with performance; improvements were suggested to be related to compensatory mechanisms activated from periods of prolonged fasting resulting in an increase in glucose uptake, not attributed to increases in peripheral blood glucose levels (Cueto et al., 1998). These findings suggest that the benefits of breakfast

should be more strongly promoted in interventions targeted towards nutritionally at-risk individuals although more research on the mechanisms underpinning benefits is needed.

## **1.8.2 Breakfast composition**

Fewer reviews consider how the composition of breakfast may affect cognitive and academic performance in young people (Table 3) where only three of five reviews reported exclusively on children and adolescents (Adolphus et al., 2016; Ells et al., 2008; Hoyland et al., 2009), and no studies compared effects on children or adolescents who were nutritionally at-risk.

### ***1.8.2.1 Cognitive performance***

Hoyland and colleagues' review (2009) reported no effect of breakfast composition on cognitive performance when at least two breakfasts were compared across nine studies (Hoyland et al., 2009). Adolphus and colleagues (2016) reviewed 15 studies comparing breakfast composition, nine of which compared breakfasts varying in GI and/or GL content, reporting that a low-GI breakfast was associated with positive effects on: attention ( $n=6$  studies), memory ( $n=4$  studies) and executive function ( $n=2$  studies) (Adolphus et al., 2016). This may have been linked with maintaining a sustained GR, which is often reported following the consumption of a low-GI meal (Wolever, 2003), and which has been associated with improvements in cognitive function in previous systematic reviews (Edefonti et al., 2014; Philippou & Constantinou, 2014). However, previous findings have been inconclusive in children and adolescents, potentially due to the small number of relevant studies included.

### ***1.8.2.2 Academic performance***

Hoyland and colleagues (2009) reported four studies in adolescents (9-17 years) measuring the effect of breakfast quality on academic performance, although only three studies defined the actual composition of breakfast (Hoyland et al., 2009). Overall, academic performance was better with

increasing quality of breakfast identified as the inclusion of dairy, cereals and fruits (Fernandez Morales, Aguilar Vilas, Mateos Vega, & Martinez Para, 2008; Herrero Lozano & Fillat Ballesteros, 2006), or meeting >20% of daily energy requirements (Lopez-Sobaler, Ortega, Quintas, Navia, & Requejo, 2003).

**Table 3.** Overview of reviews of breakfast studies (breakfast composition) published from 2008 to 2016.

Year	Authors	Breakfast studies (total)	Outcome	Sample	Analysis	Conclusion
2008	Ells <i>et al.</i>	3(29)	AP, CF, behaviour	4-18 years	Systematic review	No difference between high and low protein breakfasts Conflicting findings between energy content of breakfast and effect on AP
2009	Hoyland <i>et al.</i>	9(45)	AP, CF, behaviour	4-18 years (at risk & nourished)	Systematic review	Lack of robust evidence to confirm benefits of breakfast quality on CF or AP in nourished Potential effect of breakfast quality on AP in at-risk
2014	Edefonti <i>et al.</i>	15	AP, CF	Children, ado, adults (20-63y)	Systematic review	Some evidence to suggests that lower postprandial GR beneficial to CF Limitations: insufficient quantity, consistency or controlling for confounders
2014	Philippou <i>et al.</i>	10(11)	CF	6-82 years	Systematic review	Low-GI meal beneficial to CF in adults Limitations: inconsistent across studies, methodological issues
2016	Adolphus <i>et al.</i>	15(45)	CF	4-18 years	Systematic review	Lack of evidence for benefits of breakfast composition or SBP Limitations: few studies, inconsistent findings, limited adolescent research, small samples, insensitive tests

Abbreviations: CF, cognitive function; AP, academic performance; ado, adolescents



### **1.8.3 Breakfast research in adolescents**

Recent systematic reviews have highlighted a distinct lack of breakfast studies that focus on adolescents with only 10 (of 45) studies identified (Adolphus et al., 2016; Hoyland et al., 2009). Most cognitive research focuses on children up to 12 years old (Hoyland et al., 2009; Pollitt & Mathews, 1998), potentially due to the increased metabolic demands per unit of brain weight, which are around 50% greater than in adulthood, and which place increased demands on glycogen stores particularly after a long period of fasting (Williams, 2013). However, it is increasingly apparent that adolescence represents a window of opportunity to promote the accumulation of cognitive reserves (Benton, 2010). Using electroencephalogram profiles, peaks of brain growth have been identified at around 7, 12 and 15 years of age (the latter for males only) (Epstein, 1986), suggesting a potential target during which the importance of nutrition and nutritional status should be emphasised.

### **1.8.4 Summary**

There is overall support for the consumption of breakfast compared to no breakfast and positive effects on cognitive and academic performance in children and adolescents; however, inconsistencies relative to the selection of cognitive domains and cognitive tests hinder recommendations for future studies. There is less evidence supporting positive effects of the composition of breakfast on cognitive function, potentially due to the small number of studies in children and adolescents; however, there is some consensus that a low-GI breakfast may improve aspects of cognitive function, potentially attributed to a sustained post-prandial GR.

## **1.9 The effect of breakfast on gluco-regulation, cognitive performance, mood & satiety in adolescents**

Many adolescent breakfast studies measure gluco-regulation alongside cognitive performance to potentially explain performance on cognitive tasks. Mood, palatability and the satiating effects of food can also interact with cognitive performance and although it is recommended that these measures are considered in this context (Dye & Blundell, 2002), not all studies include them.

### **1.9.1 Breakfast consumption**

#### *1.9.1.1 Gluco-regulation and cognitive performance*

Glucose is the main fuel source to the brain and ingesting glucose increases blood glucose levels and its availability to the brain, which enhances cognitive function, particularly during demanding tasks when needs increase (Benton & Owens, 1993; Benton & Parker, 1998; Benton et al., 2003; Gold, 1995). Prolonged blood glucose values above fasting concentrations, as is reported following consumption of a low-GI meal, reflect lighter metabolic stress and facilitate an adequate blood supply to the brain, potentially improving cognitive function in the postprandial phase (Zhao & Alkon, 2001). Therefore, it is reasonable to expect blood glucose levels to correlate with performance on cognitive tasks (Benton & Sargent, 1992); however, as new evidence continues to emerge it is becoming more apparent that there could be a number of interplaying factors through which glucose might influence cognitive function.

Glucose is transported into the brain at the blood-brain barrier (BBB) via GLUT1 and GLUT3 glucose transporters (Messier, 2004). Increases in glucose within the brain region have been linked to activation of frontal-lobe functioning involved in performing memory tasks (Benton, Parker, & Donohoe, 1996). Messier (2004) proposed that uptake of glucose into the brain is driven by neuron activity and not blood glucose concentrations. This suggests that altering blood glucose levels, by

consuming breakfast for example, may not be sufficient to have a measurable impact on brain function, in spite of brain extracellular glucose levels increasing when circulating blood glucose increased. Key neurotransmitters represent another potential mechanism through which glucose may promote cognitive benefits. Exogenous glucose is responsible for the synthesis of a number of neurotransmitters including glutamate, acetylcholine (ACh) and gamma-aminobutyric acid (Messier, 2004). Decreases in concentrations of ACh are a hallmark of progressive dementia; however, even in healthy adults, impairments were associated with substantial deficits in episodic memory (Kopelman, 1986).

Insulin plays a major role as a regulator of blood glucose levels, stimulating the uptake of glucose into the liver, adipose tissue, and muscles (Banks, Owen, & Erickson, 2012). In contrast, glucose uptake in the brain occurs largely independent of insulin. Insulin has a unique role within the central nervous system (CNS) where it can: cross the BBB in areas associated with food intake and cognition (Banks et al., 2012), enhance transport of tyrosine and tryptophan, and increase the transport of leptin, which inhibits hunger (Tagliamonte, DeMontis, Olanas, Onali, & Gessa, 1975).

Poor gluco-regulation puts pressure on metabolic processes which over time can develop into MetS and T2DM. Individuals with MetS experience learning and recall impairment (Hassenstab, Sweat, Bruehl, & Convit, 2010) and this has also been observed in adolescents ( $16.5 \pm 1.9$  years) who are obese and diagnosed with T2DM (Yau et al., 2010). Performance was significantly worse than controls on tests of intellectual functioning, verbal memory and psychomotor efficiency. T2DM involves disruption of insulin transport into the brain affecting areas controlling appetite and hepatic glucose production (Banks et al., 2012). It is proposed then that a resistance at the insulin receptors in the CNS could be termed as Type 3 Diabetes, although further research to understand the exact role of insulin in cognitive function is required (Messier, 2004; Philippou & Constantinou, 2014; Zhao & Alkon, 2001).

In adolescents, all studies reported some benefits of breakfast consumption, compared to no breakfast, on cognitive performance (Table 4), particularly memory (Defeyter & Russo, 2013; Vaisman, Voet, Akivis, & Vakil, 1996; Wesnes, Pincock, & Scholey, 2012; Widenhorn-Müller, Hille, Klenk, & Weiland, 2008) and executive function (Cooper, Bandelow, & Nevill, 2011; Cooper, Bandelow, Nute, Morris, & Nevill, 2012; Defeyter & Russo, 2013). Three studies measuring attention reported no effect of breakfast consumption (Cooper et al., 2012; Wesnes, Pincock, Richardson, Helm, & Hails, 2003; Wesnes et al., 2012).

Only two studies collected blood glucose measures (Cooper et al., 2011; Cooper et al., 2012), although analysis of the no breakfast condition was only reported in one (Cooper et al., 2011). Improvements in the speed of working memory, especially on the more demanding tasks, were found in the breakfast condition attributed to concurrent increases in blood glucose concentrations (Cooper et al., 2011), although this is expected following the consumption of breakfast. The only adolescent study to measure IR (Cooper et al., 2012) did not compare findings between the breakfast and no breakfast condition as a significant difference was expected.

**Table 4.** Acute adolescent studies comparing breakfast consumption vs. breakfast omission and effects on cognitive performance

Year	Authors	Design	Sample <i>n</i> (age)	Breakfast intervention	Cognitive domain	Other measures	Conclusions
1996	Vaisman <i>et al.</i>	School based Randomised, independent grps	569 (11-13yrs)	Standard BF (cornflakes) vs. no BF	Verbal & spatial memory	N/A	Effect of BF at school (30m prior) on immediate recall vs. BF at home (2h prior) or no BF Limitations: unbalanced, time of testing not considered
2003	Wesnes <i>et al.</i>	School based Randomised, 4- way crossover	29 (9-16yrs)	Standard BF (cereal, drink) vs. no BF	Attention Working & episodic memory	Mood Satiety	Effect of BF on memory, attention, mood (alertness and contentment), satiety (fullness) Limitations: age not included as covariate, did not distinguish between memory domains, no post hoc tests
2005	Mahoney <i>et al.</i>	School based Counterbalanced 3-way crossover	<i>n</i> =30 (9-11 yrs)	Oatmeal or RTEC vs. No BF	Immediate & delayed recall Attention	Mood Satiety	Effect of BFs on map task and visual perception. Effect of oatmeal vs. No BF on working memory in girls. Effect of BFs vs. No BF on attention. More motivated after either breakfast. Limitation: differing macronutrient
2008	Widenhorn- Muller <i>et al.</i>	School based Randomised, crossover	104 (13-20yrs)	Standard BF (bread/spread) vs. no BF	Attention Concentration Verbal & spatial memory	Mood	No effect of BF on attention, total memory or verbal memory Effect of BF on accuracy of spatial memory (males only) and mood (varying extents by gender)
2011	Cooper <i>et al.</i>	School based Randomised, crossover	96 (12-15yrs)	Ad-lib BF vs. no BF	Visual perception Attention Working memory	Glucose Mood Satiety	No effect of BF on working memory accuracy Effect of BF on working memory speed (especially more demanding tasks), mood (more energy, less tired, more calm in morning), satiety (less hungry, more full)
2012	Cooper <i>et al.</i>	School based Randomised, crossover	41 (12-14yrs)	Low-GI, high- GI vs. no BF	Exective function Working memory Attention	Glucose Insulin	Effect of BF on attention, working memory and exective function
2012	Wesnes <i>et al.</i>	Internet based	1386 (6-16yrs)	Habitual BF vs. no BF	Attention Episodic memory	N/A	Overall effect of BF on attention and episodic memory (6/7 tests) No effect of BF on accuracy of CRT
2013	Defeyter & Russo.	School based Counterbalanced , crossover	40 (13-15yrs)	Standard BF (all bran) vs. no BF	Verbal & working memory Attention	Mood Satiety	No effect of BF on attention Effect of BF on memory (especially more demanding tasks), mood (more alert and content), satiety (more full)

Abbreviations: BF, breakfast; Ad-lib, Ad libitum

### *1.9.1.2 Mood*

Performing a cognitively demanding task can increase stress hormones, including cortisol and epinephrine, leading to variations in blood glucose which can impact upon memory, either enhancing or impairing performance (Messier, 2004). Mood can also be affected by low blood glucose levels; an activation of the autonomic nervous system attempting to return blood glucose levels to normal has been reported to increase feelings of tension (Benton, 2002).

Studies comparing mood between adolescents who consume breakfast and skip breakfast identified some improvements in the breakfast condition (Cooper et al., 2011; Defeyter & Russo, 2013; Mahoney, Taylor, Kanarek, & Samuel, 2005; Widenhorn-Müller et al., 2008). However, it is well established that eating (palatable) food can improve mood by the release of endorphins, as well as through the alleviation of hunger and dehydration (Hammersley et al., 2014); therefore, conclusions are tenuous when comparisons are only made with a no breakfast condition.

### *1.9.1.3 satiety & palatability*

The palatability of a food can interact with satiety (through the release of endorphins) potentially increasing cognitive efficiency (Hammersley, Reid, & Duffy, 2007). Furthermore, palatability has an important influence on the initiation and termination of eating events suggesting interactions with satiety potentially through a number of mechanisms.

Studies comparing subjective measures of satiety between adolescents who consume breakfast and breakfast skippers report more fullness and less hunger (Cooper et al., 2011; Defeyter & Russo, 2013; Mahoney et al., 2005), which is expected. The palatability of breakfast in these studies was not considered.

## **1.9.2 Breakfast composition**

The composition of breakfast affects post-prandial GR and IR (chapter 1.7) relative to GI (chapter 1.7.1) and fibre properties (chapter 1.7.2). Adolescent breakfast studies generally support the role of maintaining a lower postprandial GR and benefits to cognitive performance (chapter 1.9.2.1) attributed to the effects of low-GI foods and fibre (chapter 1.7.1-2); however, there is some variation between cognitive domains affected, and benefits have been attributed to both high- and low-GI breakfasts (Table 5).

**Table 5.** Acute adolescent studies comparing breakfast composition and effects on cognitive performance

Year	Authors	Design	Sample	BF intervention	Cognitive domain	Other measures	Reported findings
1990	Cromer <i>et al.</i>	Laboratory-based Randomised, independent groups	n=34 (14 yrs)	High vs. low energy BF	Attention Memory Visual perception	Mood BG Beta-hydroxybuterate	No effect of BF condition on CF or mood No correlations with blood measures and CF
1991	Michaud <i>et al.</i>	School-based Randomised, crossover design.	n=319 (13-20yrs)	Habitual BF vs. BF 63% increase	Immediate recall Concentration	Mood BG	Effect of high E BF on immediate recall, concentration impaired No effect of BF on mood or glucose
2003	Wesnes <i>et al.</i>	School based Randomised, 4-way crossover	n=29 (9-16yrs)	Standard BF (2 cereals, glucose drink) vs. no BF	Attention Working & episodic memory	Mood Satiety	Effect of cereal vs. glucose drink on memory and attention, alertness & contentment
2005	Mahoney <i>et al.</i>	School based Counterbalanced 3-way crossover	n=30 (9-11 yrs)	Low-GI oatmeal vs. High-GIRTEC	Immediate & delayed recall Attention	Mood Satiety	Effect of oatmeal vs RTEC on working memory (girls only). No effects on mood or satiety
2008	Smith & Foster	Laboratory-based Randomised independent groups	n=38 (14-17yrs)	Low-GI (Allbran) vs. High-GI (Cornflakes) BF	Verbal memory	Mood Satiety BG	No effect of BF condition on verbal memory, mood, satiety or BG levels Better performance in high-GI group at 90 mins
2010	Micha <i>et al.</i>	School-based Between subjects, 4 independent groups	n=60 (11-14yrs)	High-GL/Low-GI High GL/High-GI Low GL/Low GI Low GL/High-GI	Vigilance Working memory	Mood BG	Effect of BF in 4/7 tests: Low GI, high-GL on attention & memory, High-GI: memory, High-GL: inductive reasoning Mood: positive feelings predicted lower scores on CF tests Satiety: no associations BG: between 90-120 mins associated with high GL
2011	Micha <i>et al.</i>	School-based Independent groups: HGL vs. LGL Crossover: HGI vs. LGI.	n=74 (11-14yrs)	High-GL/Low-GI High GL/High-GI Low GL/Low GI Low GL/High-GI	Vigilance Working memory	Mood BG Salivary cortisol	Effect of BF on CF with low-GI, high-GL eliciting largest effects Mood: Positive effect of low-GI and high-GL BG: High GL & High-GI increased BG levels after CF tests Cortisol: High-GI increased before & after CF tests
2012	Brindal <i>et al.</i>	Laboratory-based Randomised crossover	n=39 (10-12yrs)	High GL Meduim GL Low GL	Short-term & working memory Attention	Satiety BG	No effect of GL on CF or satiety Significant effect of GL on blood glucose
2012	Cooper <i>et al.</i>	School based Randomised, crossover	41 (12-14yrs)	Low-GI, high-GI vs. no BF	Exective function Working memory Attention	BG Insulin	Effect of BF on attention, working memory and exective function GR IAUC higher following high-GI BF
2015	Cooper <i>et al.</i>	School-based Randomised, mixed crossover	n=42 (12.4±0.5 yrs)	Low-GI vs. high-GI	Attention Working memory Executive function	Exercise BG Insulin	Low-GI with exercise enhanced CF

Abbreviations: BF, breakfast; BG, blood glucose



### *1.9.2.1 Gluco-regulation and cognitive performance*

Most adolescent studies ( $n=7$ ) consider the composition of breakfast on cognitive performance relative to the GI and/or GL (Brindal et al., 2012; Cooper et al., 2012; Cooper, Bandelow, Nute, Morris, & Nevill, 2015; Mahoney et al., 2005; Micha, Rogers, & Nelson, 2010; Micha, Rogers, & Nelson, 2011; Smith & Foster, 2008) although findings are equivocal, potentially due to methodological differences relating to meal content.

There were no effects on memory when three isocaloric breakfasts varying in GL were compared over a three-hour testing period (Brindal et al., 2012). Smith and colleagues (2008) reported an improvement in memory after consumption of the high-GI breakfast only (Smith & Foster, 2008) which was not explained by blood glucose levels. Micha and colleagues (2010, 2011) reported varying effects with some improvement in verbal memory, but not executive function, after consumption of the low-GI high-GL breakfasts (Micha et al., 2010; Micha et al., 2011), with some associations between GI/GL and blood glucose levels. Varying effects may have been attributed to differences in energy content of the meals, which were not matched within the Micha studies. This makes comparisons difficult as energy or macronutrient composition can independently affect cognitive performance due to differences in the rate of glucose release into the bloodstream (Fischer, Colombani, Langhans, & Wenk, 2002; Nabb & Benton, 2006), and the duration of elevated blood glucose levels (Benton et al., 2003; Pollitt & Mathews, 1998).

Aspects of working memory and attention were improved in adolescents who consumed a matched, low-GI breakfast compared to a high-GI breakfast (Cooper et al., 2012). This was accompanied by a lower overall GR as well as a trend for a lower IR. Similar benefits to working memory and effects on GR and IR were observed by the same research group when a low-GI (versus high-GI) breakfast was consumed, combined with a bout of exercise (Cooper et al., 2015). Mahoney and colleagues

(2005) reported improvements in girls' short-term memory after the consumption of a low-GI oatmeal cereal compared to a high-GI RTEC, closely matched for energy, CHO and fat (Mahoney et al., 2005). However, no differences were reported between any other cognitive measures and no blood measures were collected.

### ***1.9.2.2 Energy content and RTEC***

The remaining adolescent studies ( $n=3$ ) consider the effect of the energy content of breakfast on cognitive performance (Cromer, Tarnowski, Stein, Harton, & Thornton, 1990; Michaud, Musse, Nicolas, & Mejean, 1991), or the type of breakfast cereal consumed (Wesnes et al., 2003). Only one study collected blood measures (Cromer et al., 1990). Consuming breakfast cereal improved memory and attention compared to a glucose drink (and no breakfast) with larger improvements observed in the high-fibre compared to the low-fibre condition (Wesnes et al., 2003); however, breakfasts were not matched for energy content and differed in fibre and total sugars. Furthermore, participants were spread over a wide age range (9-16 years). Increasing the energy content of breakfast by 63% appeared to enhance memory performance compared to habitual breakfast (Michaud et al., 1991), although concentration levels were impaired. In contrast, Cromer and colleagues (1990) saw no effect of energy content on cognitive performance and blood glucose levels, although a ceiling effect was observed on cognitive tests, which may have potentially distorted true findings (Cromer et al., 1990).

### ***1.9.2.3 Mood and satiety***

The composition of breakfast, particular its CHO content, may improve mood by increasing tryptophan (TRP) levels. TRP is a large neural amino acid (LNAA) which competes with other LNAAs (including tyrosine) for transportation across the BBB where it acts as a precursor to serotonin, which is involved in the regulation of sleep and mood state (Lieberman, Caballero, & Finer, 1986). Ingesting high-CHO meals release insulin, which lowers the plasma levels of other

LNAA relative to TRP, increasing the TRP/LNAA ratio and enabling more TRP to be transported into the brain. However, this theory is often challenged as consuming as little as 5% of protein at the same meal can inhibit the TRP effect (Teff, Young, & Blundell, 1989), and there are conflicting reports on the extent to which the TRP/LNAA ratio must increase to elevate brain serotonin (Dye, Lluich, & Blundell, 2000). It was also hypothesised that only habitual CHO consumers who regularly crave CHOs, or are prone to stress, would improve mood in response to a high-CHO meal; however, so far this has only been evident when using very large portion sizes (Benton, 2002).

In adolescent studies measuring mood, no effect was reported when comparisons were made between: high vs. low energy breakfasts (Cromer et al., 1990), habitual vs. high-energy breakfasts (Michaud et al., 1991), or high- vs. low-GI breakfasts (Mahoney et al., 2005; Smith & Foster, 2008). When the GI and the GL of breakfast were considered there were measurable effects on mood following the consumption of a low-GI, high-GL breakfast (Micha et al., 2011). In support of this there is some evidence to suggest that a refined breakfast increases reported laziness in children and adolescents ( $n=28$ ; 9-13 years) when compared to a high-fibre breakfast based on whole-foods (Pereira et al., 2011).

Breakfasts which are low-GI (Tolfrey & Zakrzewski, 2012) and high in fibre are associated with increased satiety and prolonged satiation (Slavin & Green, 2007) in adults and young people (chapter 1.7.2). So far there is little support in the adolescent literature for an effect of breakfast composition on satiety. There were no effects when comparing a high-GI to a low-GI breakfast (Mahoney et al., 2005; Smith & Foster, 2008), or between breakfasts with a high, medium or low GL (Brindal et al., 2012), although as expected there were significant changes over time where hunger increased over the testing period.

### **1.9.3 Summary and future work**

The experimental evidence to date in adolescents supports suggestions that the consumption of breakfast compared to no breakfast is beneficial to cognitive performance, potentially attributed to the effects of glucose and/or insulin on the brain. The composition of breakfast has shown a measurable effect on GR, and potentially IR (based on one study) and aspects of cognitive performance, particularly memory. However, generalising findings is difficult where differences exist between: cognitive domains measured, time points at which measures are taken, how long measures are collected for, which cognitive tests are used and the matching of breakfasts. Furthermore, improvements in cognitive performance do not always correlate with GR suggesting that benefits additional to those from the GI or fibre properties may be driving positive effects on gluco-regulation, and/or cognitive performance.

## **1.10 Developing a functional food based breakfast**

Functional food ingredients which are rich in antioxidants, polyphenols and fibre are being increasingly researched for their potentially positive effects on gluco-regulation and cognitive function.

### **1.10.1 Functional foods**

A functional food contains bioactive components which demonstrate health benefits beyond their basic nutritional function (Arai, 1996). The term ‘functional food’ was introduced in Japan during the 1980s when the Ministry of Education, Science and Culture, recognising the impending burden of chronic disease within the aging population, stimulated research into whether tertiary benefits (on immune, endocrine, nervous, circulatory and digestive systems) could be gained from certain foods consumed on a regular basis (Arai, 1996). In 1991, the Japanese Ministry of Health and Welfare established a policy whereby functional foods with tertiary benefits were approved as ‘foods for specified health uses’ and foods identified for their anti-oxidative effects included rice, soybean, and ginger. Research since has identified a wide variety of plant foods including fruits, vegetables,

whole grains, juices, plant extracts, tea, wine and chocolate, as naturally rich in polyphenols and these represent major sources of antioxidants in the diet (Miller & Shukitt-Hale, 2012; Zamora-Ros et al., 2013).

Initially, the benefits of functional foods were largely attributed to their antioxidant ability to protect against oxidative damage, a major contributor to CVD (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005); however, it is now recognised that benefits extend beyond antioxidant activities. More complex modes of action, such as the pro-oxidant effects of polyphenols, contribute to: anti-inflammatory, anti-carcinogenic, anti-proliferative, anti-tumorigenic and anti-viral properties (Miller & Shukitt-Hale, 2012), and improve cell survival by inducing apoptosis and preventing tumour growth (Scalbert, A. et al., 2005). Cell protection can also occur through direct interactions with cellular signal transduction pathways in such a way that controls pathogenic processes relevant to the progression of chronic disease (Scalbert, Johnson, & Saltmarsh, 2005; Vauzour, Rodriguez-Mateos, Corona, Oruna-Concha, & Spencer, 2010).

Previous studies in adults have also shown that increasing intakes of functional foods (fruits, vegetables, and wholegrains) in the diet, naturally increases fibre intakes (Tovar, Johansson, & Björck, 2015; Tovar et al., 2012). Consuming a diet rich in fibre is known to protect against risk factors for developing CVD, including being overweight or obese (Slavin, 2008).

#### ***1.10.1.1 Polyphenols***

Polyphenols are plant secondary metabolites which exist as part of natural defence mechanisms to protect the plant against pathogens, toxins and ultraviolet radiation (Kennedy, 2014). They are sensitive to heat, light and air oxidation and are highly soluble in water, which can result in major losses during cooking practices (Spencer, Abd El Mohsen, Miniñane, & Mathers, 2008). The

composition and content of polyphenols can also vary widely within dietary sources due to differences in agricultural, processing and storage practices (Miller & Shukitt-Hale, 2012).

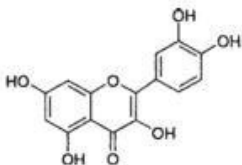
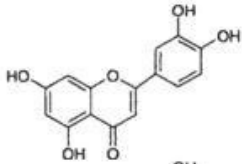
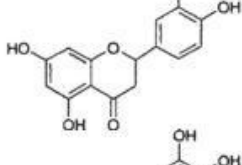
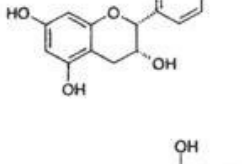
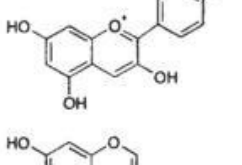
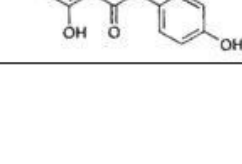
Phenols comprise around 8000 naturally occurring compounds which all possess an aromatic ring with at least one hydroxyl group attached (Leopoldini, Russo, & Toscano, 2011). Phenols are split into polyphenols and simple phenols depending on the number of phenol subunits. Simple phenols have one phenol subunit and include the phenolic acids. Polyphenols with at least two phenol subunits include flavonoids and stilbenes whereas those with three or more subunits comprise the tannins.

Flavonoids make up around two-thirds of polyphenol intake whilst phenolic acids account for much of the rest (Miller & Shukitt-Hale, 2012). Flavonoids share a common underlying structure of two aromatic rings (A and B) linked by three carbon atoms forming ring C, which is an oxygen-containing heterocycle (Vauzour, Vafeiadou, Rodriguez-Mateos, Rendeiro, & Spencer, 2008). According to the degree of oxidation of the ring as well as the hydroxylation pattern of the nucleus and the substituent at carbon three, flavonoids are categorised into flavones, flavonols, flavanols (catechins), flavanones, isoflavones, anthocyanins and proanthocyanidins (Table 6) (Vauzour et al., 2008).

Anthocyanins differ from other flavonoids as they possess a positively charged oxygen atom in the C-ring which gives them potent antioxidant as well as anti-inflammatory capabilities (Leopoldini et al., 2011). Findings from *in-vitro* studies question the bioavailability of anthocyanin components during digestion with other food sources (McDougall et al., 2005). In animal models anthocyanins have been shown to be neuro-available as evidenced by their residing in tissues longer than plasma (Pribis & Shukitt-Hale, 2014), although more studies are needed to elucidate specific mechanisms in humans (Miller & Shukitt-Hale, 2012).

Overall, polyphenol intakes from the diet are estimated to be around 1g per person per day, but intakes and sources vary between countries (Scalbert, Augustin et al., 2005). Higher intakes have been reported in Japan, where coffee and green tea are major contributors (Fukushima et al., 2009), whereas across Europe intakes ranged from around 0.25g in Greek individuals to around 0.5g in UK individuals (Zamora-Ros et al., 2013). As yet, due to a limited understanding of the actions of polyphenols *in-vivo* and their bioavailability, there are no definitive recommendations for optimal intakes of polyphenols (Chiva-Blanch & Visioli, 2012; Scalbert, Augustin et al., 2005).

**Table 6.** Flavonoid subclasses and structures, reproduced with permission (Hooper et al., 2008)

Flavonoid subclass	Structure	Synonyms	Example compounds
Flavonols			Quercetin, kaempferol, myricetin, and isorhamnetin Important glycosides include rutin (quercetin rutinoside).
Flavones			Apigenin, luteolin, and tangeretin
Flavanones			Naringenin and hesperetin Dietary forms are glycosides such as hesperidin and narirutin.
Flavan-3-ols		Flavanols Polymeric forms are called proanthocyanidins (eg, procyanidins and prodelphinidins). Catechins Green tea catechins	(+)-Catechin, (-)-epicatechin, and their polymers (eg, procyanidins-B1, -C1) Tea catechins such as epigallocatechin gallate
Anthocyanidins		Anthocyanins (=glycosylated forms)	Cyanidin, delphinidin, pelargonidin, and malvidin Glycosylated derivatives known as anthocyanins
Isoflavones		Phytoestrogens	Daidzein, genistein, and glycitein Glycosylated forms are daidzin, genistin, and glycitin, respectively.

### ***1.10.1.2 Fibre***

The physiological effects of dietary fibre predominantly relate to its potential to increase satiety and prolong satiation (Figure 2). Fibre-rich foods increase viscosity, which influences satiety by gastric distension and can prolong nutrient digestion and absorption (Dikeman & Fahey, 2006). The bulking properties of fibre require additional work during mastication that can improve satiation, as well as reducing the overall energy density of the diet (Slavin & Green, 2007). Satiety factors are affected due to increases in circulating gut hormones including cholecystokinin, which stimulates the digestion of fat and protein and is important in enhancing satiety (Burton-Freeman, Davis, & Schneeman, 2002). It is suggested that the type of fibre (soluble or insoluble) may alter satiety and hunger cues using different mechanisms (Slavin & Green, 2007). Insoluble fibre is thought to influence satiety in the small and large intestines, which may be linked to changes in gut hormones or transit time through the intestine (Slavin & Green, 2007). Furthermore, the addition of insoluble fibre to white bread was shown to increase insulin sensitivity by altering the patterns of insulin secretion (Weickert et al., 2006). Soluble fibre (of which oat and barley provide a good source) has been shown beneficial for altering the degree of starch breakdown in food and for managing blood glucose and insulin levels (Cavallero, Empilli, Brighenti, & Stanca, 2002; Tappy, Gugolz, & Wursch, 1996; Thondre & Henry, 2009).

### **1.10.2 Functional foods in the diet and effects on metabolic health and cognition**

Experimental studies on functional food diets are limited to adults, but suggest that the daily consumption of a variety of functional food ingredients selected for their health-promoting properties, could reduce risk factors for diet-related disease including: lowering cholesterol levels, lowering blood lipids, lowering GR, improving insulin sensitivity, and increasing fibre intakes (Tovar et al., 2015; Tovar, Nilsson, Johansson, & Björck, 2014; Tovar et al., 2012). Furthermore, their consumption in adults has been associated with improvements in cognitive performance



(Nilsson, Tovar, Johansson, Radeborg, & Bjorck, 2013). In a crossover study, healthy individuals who were overweight but free from disease ( $n=44$ ; 50-73 years), following a multi-functional food 'active' diet (AD) for four weeks, showed: reduced lipid profiles, lower blood pressure, and an overall lower CVD risk score, compared to the control diet (CD), after controlling for weight loss (Tovar et al., 2012). The same study was repeated over eight weeks using a parallel design in healthy individuals (51-72 years) who were overweight or obese (Tovar et al., 2015). Again, individuals consuming the AD ( $n=23$ ) significantly improved blood lipids, particularly LDL cholesterol, and had a lower cardiovascular risk score, compared to the individuals consuming the CD ( $n=24$ ). Participants were typically healthy, but were also mildly hypercholesterolemic; however, reductions in LDL cholesterol following the AD (~35%) were similar to what has been achieved in hypercholesterolemic individuals using medication or following a strict vegetarian diet (Jenkins et al., 2006; Jenkins et al., 2005). In contrast to an earlier study (Tovar et al., 2012), which saw no effect on fasting blood glucose or insulin levels, the AD decreased HOMA-IR values when measured over a longer period of 8 weeks (Tovar et al., 2015), indicating a rise in insulin sensitivity in participants following the AD.

Both diets (AD and CD) provided similar distributions of energy-providing nutrients, but differed where the AD included food items containing active concepts that were not included in the CD. For example, the AD contained polyphenol-rich blueberries and cinnamon, and low-GI oats, rich in antioxidants and soluble fibre (providing 5.8-6.2g  $\beta$ -glucan) (Tovar et al., 2015; Tovar et al., 2012). It is likely, although speculative, that benefits were due to the synergistic actions of the functional ingredients, which were provided in quantities representing how they would be realistically consumed in the diet. Studies comparing individual ingredients are often given in amounts greater than what would be eaten as part of a normal diet, hence limiting the generalisability of findings. The inclusion of functional ingredients naturally increased the fibre content of the AD compared to

the CD (55g/d vs. 22g/d respectively) and lowered dietary cholesterol intake (214mg/d vs. 132mg/d respectively), highlighting additional benefits from the consumption of the AD, although results should also be interpreted in the context of these differences.

The effects of the four-week AD (Tovar et al., 2012) on cognitive processes were also considered (Nilsson et al., 2013). Individuals who had consumed the AD performed better on selective attention and verbal memory tests after a standardised breakfast, compared to the CD, particularly during the later stages of testing. Benefits were attributed to the inclusion of blueberries, low-GI foods, and omega-3 fatty acids in the AD. Cinnamon was also in the AD which, although not selected by the authors for cognitive enhancing properties, has been associated with improvements in working memory in pre-diabetic adults (Wahlqvist et al., 2016). Higher risk markers for metabolic disorders were associated with cognitive performance, suggesting that detriments to cognitive functioning may already be developing in otherwise clinically healthy individuals. A significant effect of test day was observed, which suggests a practice effect of the cognitive tests; however, the randomisation should have accounted for this limitation.

### **1.10.3 Functional foods and effects on gluco-regulation**

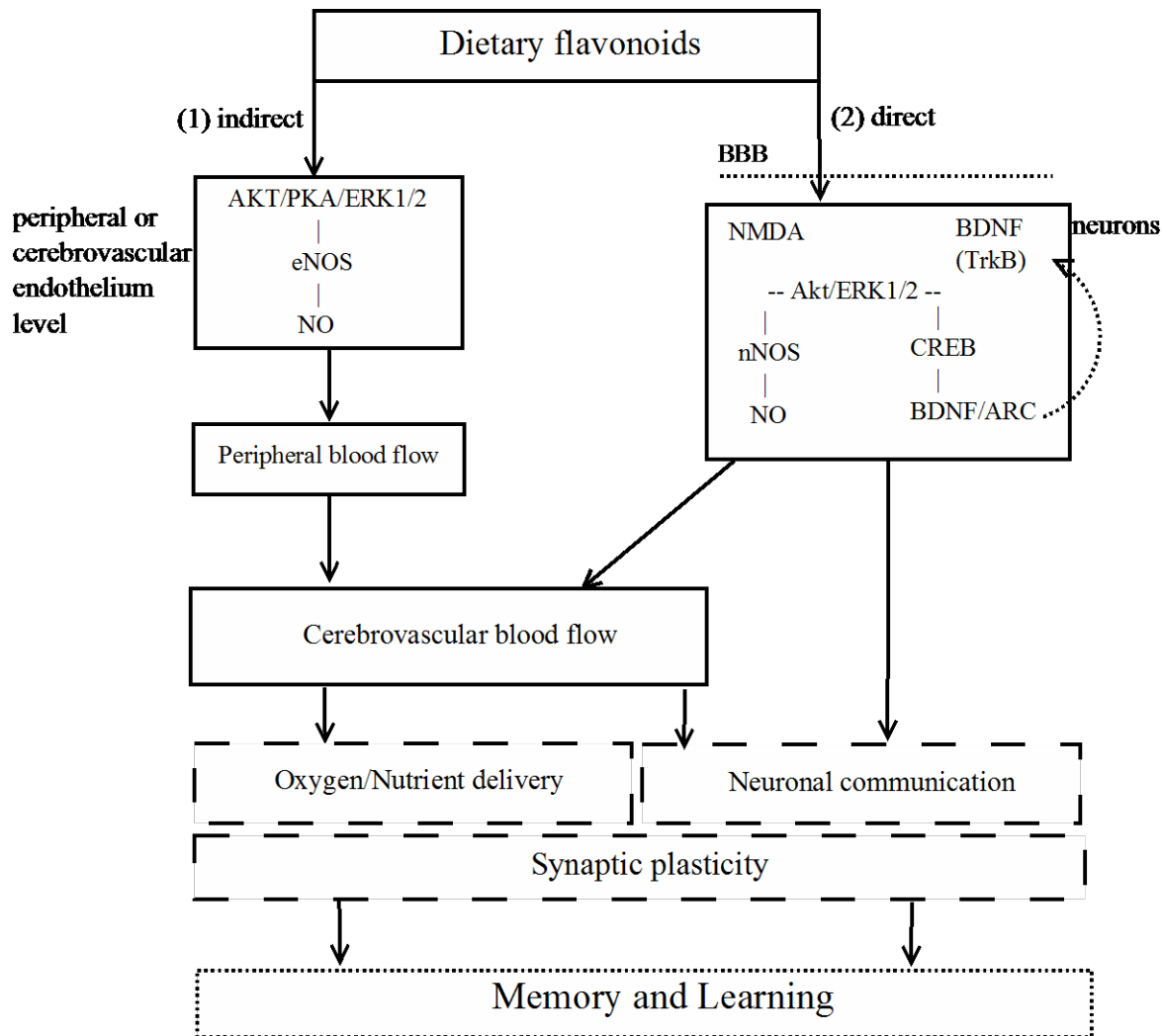
Much of the research on functional foods rich in polyphenols and fibre and their effects on gluco-regulation come from *in-vitro* or animal studies, and a limited number of human studies in adults. Positive findings are generally supported by the epidemiological evidence, where long-term consumption of functional foods (particularly fruits and vegetables ) were associated with benefits to inflammatory markers (Chun, Chung, Claycombe, & Song, 2008), or a reduced risk of T2DM (Mursu, Virtanen, Tuomainen, Nurmi, & Voutilainen, 2014), although some conflicting findings relating to T2DM were reported during longer, but not shorter follow up (Sun et al., 2015).

Polyphenols act as regulators of GR and IR by inhibiting the activities of key enzymes and glucose transporters in the hydrolysis of starch to glucose, thereby inhibiting starch degradation in the GI tract (McDougall & Stewart, 2005; Williamson, 2013) and potentially improving insulin secretion and sensitivity (Hanhineva et al., 2010). The development of Acarbose, a diabetic drug shown to reduce the risk of diabetes over three years, relies on the same mechanism and it is suggested therefore that consuming polyphenol-rich foods over a lifetime may protect against the development of diabetes (Williamson, 2013). A systematic review of interventions comparing effects of combined polyphenol-CHO sources reported that adding polyphenol-rich: foods (berries and different rye breads), drinks (coffee, black tea, fruit juice) and extracts (baobab), to CHO sources (including bread, pancakes and simple sugars) improved gluco-regulation (Coe & Ryan, 2016). Effects were seen where their addition reduced peak and early phase GR, and maintained GR in the later stages of digestion of CHO. To a lesser degree, polyphenol sources reduced peak IR and sustained the IR, but this was dependant on the polyphenol-CHO combination. Furthermore, benefits to GR and IR may have been attributed to other nutrients (including fibre) present in varying amounts across the food sources.

#### **1.10.4 Functional foods and effects on cognitive function**

The consumption of functional foods is suggested to enhance cognitive performance, potentially through improvements to cerebral blood flow, generally attributed to the polyphenol content. The potential of polyphenols to regulate nitric-oxide (NO) dependent cerebrovascular functions at the cerebral endothelium level, stimulates peripheral and then cerebral blood flow (Spencer, 2008), indirectly enhancing cognitive function (Figure 4). Direct effects relate to the ability of flavonoids to cross the BBB. Some flavonoid metabolites may inhibit cell degeneration and inflammation and interact with cell signalling to enhance neuronal communication, encouraging synaptic plasticity by: expressing signalling kinases (ERK1/2, Akt), neuronal receptors (NMDA (important for memory

and learning), TrkB, and neurotrophins (brain-derived neurotrophic factor, BDNF) (Giacalone et al., 2011; Spencer, 2010a; Vauzour et al., 2008). However, identifying how specific flavonoids contribute to individual mechanisms requires further research (Shukitt-Hale et al., 2015).



**Figure 4.** Mechanisms underpinning the effects of dietary flavonoids on memory and learning. Consuming flavonoids might indirectly impact cognitive performance by modulating endothelial NO synthase (eNOS) and Nitric-Oxide (NO) dependent cerebrovascular function at the cerebrovascular level. Flavonoids may cross the BBB to act directly on neuronal receptors (TrkB, NMDA), signalling kinases (Akt, PKA, ERK1/2) and neurotrophins (BDNF) via cellular transcription factors (CREB) and associated proteins (BDNF/ARC) which can lead to changes in synaptic function. Reproduced with copyright permission (Rendeiro, Rhodes, & Spencer, 2015)

Studies suggest that the regular consumption of flavonoid-rich foods is associated with the preservation of cognitive function, but this is mostly based on studies in older adults (Devore, Kang, Breteler, & Grodstein, 2012; Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-

Gateau, 2007). In a large prospective study ( $n=16,010$ ; 74 years), a reduced risk of cognitive decline by up to 2.5 years was reported in women with the highest berry intakes; however, only flavonoids were estimated while other polyphenol groups, such as phenolic acids, were not included (Devore, Jae Hee, Breteler, & Grodstein, 2012). Furthermore, intakes were associated with higher PA levels and higher SES, which suggests that improvements may have been due to an overall healthier lifestyle, although the analysis did make adjustments for these factors.

Not all studies report cognitive benefits of flavonoids in healthy populations. Conversely, higher habitual flavonoid intakes were associated with a greater decline in cognitive flexibility in adults (43-70 years); however, greater intakes of lignans, phenolic compounds found in plant foods including seeds, nuts, fruits and tea, were strongly associated with less cognitive decline, particularly in the memory domain (Nooyens et al., 2015). If lignans were driving cognitive benefits this suggests that they should also be measured in berry studies; however, they are present in very low concentrations in most foods so they may simply represent a marker to an overall healthy lifestyle. In a systematic review of 21 observational and prospective studies, findings were inconclusive due to methodological discrepancies in regards to measures of cognition, dietary assessment methods and reporting of confounding factors (Crichton, Bryan, & Murphy, 2013). Authors concluded that more intervention studies were needed before habitual intakes of antioxidant-rich foods could be attributed to protecting against cognitive decline in adults.

Intervention studies appear more consistent in their findings and a review including thirteen RCTs (Macready et al., 2009) reported a general trend for positive associations between flavonoid intake and cognitive function; however, like the epidemiological data, most studies are based on older adults (>50years). Only two studies included young (<30 years), healthy populations, both reporting improvements in cognitive performance following consumption of soya (File et al., 2001) or cocoa (Francis, Head, Morris, & Macdonald, 2006). Specific to anthocyanins, Hendrickson and Mattes

(2008) investigated whether an acute dose of grape juice would mitigate the deficits in cognition and mood that commonly occur after eating a large meal (Hendrickson & Mattes, 2008).

Approximately 580mg of anthocyanins were served with a standardised lunch to young adult smokers ( $n=35$ , 26 years). There were no significant effects of grape juice on cognitive performance 1h post-prandially when compared to an energy matched placebo control. Positive mood states declined under both conditions, and negative mood states increased, but this did not correlate with cognitive task performance which did not change over time in either condition. Only one cognitive domain (implicit memory) was selected to be measured; however, there were no previous studies cited to support this selection. Caldwell and colleagues also used grape juice to deliver 55mg of anthocyanins in a pilot study in healthy adults ( $n=6$ , 18-35 years) (Caldwell, Charlton, Roodenrys, & Jenner, 2016), but saw no significant improvements at 6h on executive function or memory, although the small sample size and a lack of a considered control suggests the study was likely to be underpowered. A summary of acute studies in young people and children also supports the role of flavonoids to benefit attention, working memory, and psychomotor processing speed in healthy populations (Bell et al., 2015). In young adults ( $n=36$ ; 18-35 years), the consumption of anthocyanin-rich blackcurrants in a juice or a powder were reported to improve attention and executive function compared to a matched control (Watson et al., 2015). Blood measures were also collected suggesting a potential mechanism for the cognitive improvements observed from the juice, although there were no effects on mood or mental fatigue.

Consuming high-fibre foods that promote a prolonged GR may sustain glucose availability to the brain and improve cognitive performance (Messier, 2004). In healthy adults the consumption of fibre-enriched bread was associated with improvements in cognitive performance compared to low-fibre bread (Nilsson, Radeborg, & Bjorck, 2012). In children, a high-fibre (3g) breakfast did not improve cognitive performance compared to a low-fibre (1g) breakfast, although breakfasts were

not matched on all macronutrients (Mahoney et al., 2005). Adolescent studies comparing high- and low- fibre foods and their effects on cognitive performance do so relative to the GI/GL of the meal (chapter 1.9.2).

### **1.10.5 Energy expenditure**

Polyphenol compounds are suggested to play a significant role in the increase of EE and DIT, potentially by altering the metabolic rate and influencing fat oxidation. This has led to increasing interest in their potential in relation to weight maintenance (Hursel et al., 2011), but studies are still limited in this area. DIT may play a role in weight management and the development of obesity (de Jonee & Bray, 1997) and is suggested to influence satiety through increases in body temperature (Westerterp, 2004). DIT varies depending on the nutrient ingested but generally contributes 5-15 % of daily EE when consuming a mixed diet (Westerterp, 2004).

Intakes of tea catechins over 12 weeks were reported to alter the metabolic rate by increasing fat oxidation and DIT in healthy adults (Harada et al., 2005). Green tea is reported to stimulate brown adipose tissue which has a high metabolic rate and therefore may increase EE in adults (Choo, 2003), although the thermogenic effect of caffeine means that benefits might not be solely attributed to the effect of polyphenols (Hursel et al., 2011). Other polyphenol compounds have been reported to affect EE, but studies are limited and include adults or animal models only. Short-term consumption of supplements containing epigallocatechin-gallate and resveratrol increased EE and DIT in overweight adults ( $n=18$ ) compared to a placebo (Most, Goossens, Jocken, & Blaak, 2014). Mice models also support that consuming polyphenols over longer periods increases EE (Stewart et al., 2008). Only one study has considered the effect of polyphenol-rich baobab on EE and DIT (Coe, Clegg, Armengol, & Ryan, 2013). An increase in EE post-prandially was observed following consumption of a baobab drink at varying doses served with bread, compared to a control; however, there were no significant differences between conditions.

### **1.10.6 Summary**

The long-term consumption of functional foods which are low-GI and rich in polyphenols and fibre are suggested to work synergistically to improve markers of metabolic health, including gluco-regulation and performance on cognitive tests (Nilsson et al., 2013; Tovar et al., 2015; Tovar et al., 2012). Individually, polyphenol-rich foods and extracts have been reported to: improve GR and IR, improve cognitive performance and increase EE in healthy adults, attributed to their potential to: act directly on starch digestion, influence neuronal processes and increase DIT respectively.

To date, there are no studies investigating the acute effects of consuming multiple functional food ingredients and effects on gluco-regulation, cognitive performance and EE in young, healthy individuals within normal BMI ranges. Although these individuals should pose no immediate risk of metabolic disease or cognitive decline, identifying the acute benefits which can be obtained from small dietary changes may be easier to implement and sustain over time. Furthermore, optimising the diet of young people represents a modifiable risk factor and encourages a preventative- rather than a treatment-based approach.

### **1.11 The development of a functional food breakfast for adolescents**

Tovar and colleagues (2012) designed a functional food diet based on a range of ingredients that would usually be consumed for breakfast, lunch, dinner and snacks. Breakfast in the AD consisted of ingredients selected to contribute towards:

- cholesterol-lowering effects (soy-based yogurt)
- lowering the GR (oat- or rye-based muesli)
- increasing antioxidant and polyphenol intakes (blueberries, cinnamon, oat- or rye-based muesli)
- improving gut microbiota (probiotic powder)



The authors saw improvements in a range of metabolic measures following consumption of the AD (chapter 1.10.2), but reported no improvements to fasting blood glucose. No acute measures of the GR or IR to individual meals were reported; therefore, it was not possible to determine how the AD meals influenced gluco-regulation. Specific to gluco-regulation, the primary outcome of this PhD, the research to date suggests that in adults (chapter 1.10.3), the consumption of: dark berries which are high in anthocyanins (Coe & Ryan, 2016), oats (Regand, Chowdhury, Tosh, Wolever, & Wood, 2011), baobab (Coe & Ryan, 2015; Coe et al., 2013), and cinnamon (Davis & Yokoyama, 2011) are associated with a lower GR and IR. Furthermore, the addition of oats (Tosh, 2013) and baobab powder (De Caluwé, Halamová, & Van Damme, 2010) would increase the soluble and insoluble fibre content, potentially contributing to maintaining glycaemic control. Additionally, all ingredients would provide a rich source of polyphenols (Anderson, 2008; Coe et al., 2013; Nour, Magboul, & Kheiri, 1980; Ryan, Thondre, & Henry, 2011), which are independently associated with improvements in gluco-regulation and cognitive performance (chapter 1.11.1-4). The selected ingredients could be easily incorporated into a breakfast in amounts that would not compromise the taste or appearance (chapter 6.4.4). Therefore, based on the reviewed literature, the following ingredients were selected (for their potential to improve gluco-regulation and cognitive performance) as the main components in the development of a functional food based breakfast for adolescents:

- blueberries (GR and IR lowering, memory, attention, executive function)
- oats (GR and IR lowering, memory)
- cinnamon powder (GR and IR lowering, memory)
- baobab extract (GR and IR lowering)

### **1.11.1 Blueberries**

The polyphenol composition of blueberries is predominantly anthocyanins (Borges, Degeneve, Mullen, & Crozier, 2010) which, along with flavanols, are the most abundant flavonoids in the diet and are distinguishable by their red, purple and blue pigments (Scalbert & Williamson, 2000). The consumption of anthocyanins delivered through a blueberry extract has been shown to correlate with increases in serum antioxidant capacity in humans (Mazza, Kay, Cottrell, & Holub, 2002) suggesting potential for similar effects when consumed as whole fruits.

#### **1.11.1.1 Gluco-regulation**

Experimental studies considering the effects of blueberries on gluco-regulation are limited to adults, but have shown that the consumption of blueberry-based drinks over six weeks (Riso et al., 2013) and eight weeks (Basu et al., 2010; Erlund et al., 2008) protected against oxidative stress (Riso et al., 2013) and improved selected risk factors for CVD (Basu et al., 2010; Erlund et al., 2008), in at-risk individuals.

The addition of berry polyphenols to simple sugars, including monosaccharides and disaccharides, and starch-rich foods, were reported to significantly impact the GR and IR. In adults, the addition of sucrose to berries (including blueberries) significantly lowered the GR compared to a sucrose load (Törrönen, Kolehmainen, Sarkkinen, Mykkänen, & Niskanen, 2012b; Törrönen et al., 2012a; Törrönen et al., 2010). In starch-based studies, the addition of berries to starch-rich foods (Blacker, Snyder, Eggett, & Parker, 2013; Clegg, Pratt, Meade, & Henry, 2011) had little or no effect on overall GR or satiety. Fewer studies measure insulin, but berries (including blueberries) were found to significantly reduce the IR when consumed with bread (Törrönen et al., 2013). Benefits are generally attributed to anthocyanins, the glycosides of anthocyanidins, which are reported to have inhibitory effects *in-vitro* on pancreatic  $\alpha$ -amylase to varying extents, and intestinal  $\alpha$ -glucosidase to greater extents (Johnson, Lucius, Meyer, & de Mejia, 2011). Anthocyanins may also modulate post-prandial glycaemia by interacting directly with glucose transporters (SGLT1 and GLUT2) thus

decreasing glucose transporter expression (Alzaid, Cheung, Preedy, & Sharp, 2013). Furthermore, anthocyanins have shown insulin-like actions *in-vitro* whereby secretion of insulin is induced from pancreatic cells; however, their low bioavailability suggests this mechanism is unlikely to occur *in-vivo* (McDougall & Stewart, 2005).

#### **1.11.1.2 Cognitive performance**

Much of the research on blueberries relates to effects on cognitive function, dating back to the first study in 1999 which reported benefits to working memory in rats (Joseph et al., 1999). The majority of research continues to be in animal models of aging and neuropathology, but provides substantial evidence to support the protective effects of blueberry consumption on cognitive health, whilst also providing insight into potential mechanisms.

Aged rats consuming blueberry-enriched diets have repeatedly shown improvements in memory, particularly spatial working memory, compared to matched controls (Miller & Shukitt-Hale, 2012). Anthocyanins have been found to pass through the BBB (Figure 4) in rodents and influence areas associated with memory and learning at a molecular level (Andres-Lacueva et al., 2005; Papandreou et al., 2009). Enhancements in spatial memory and a paralleled increase in BDNF are frequently reported, suggesting that the effects of flavonoids on this neurotropic factor may be a crucial contributor to performance in memory tasks. Regional enhancement of BDNF messenger ribonucleic acid expression in the hippocampus, which is known to decrease in aging humans, appeared to be predominantly enhanced by anthocyanin in rodents (Rendeiro et al., 2013; Williams et al., 2008). BDNF is related to brain regions essential for memory and learning and low concentrations have been associated with neurodegenerative disorders (Pribis & Shukitt-Hale, 2014).

Human experimental studies reporting positive effects of polyphenol-rich berries on cognitive performance have so far been methodologically inadequate and are mostly in adults. A systematic review including experimental and epidemiological studies (Lampert, Dye, Wightman, & Lawton, 2012), identified four interventions relating to berries, but only two reported improvements on memory (Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2010a; Krikorian et al., 2010b). Adults with mild cognitive impairment (MCI) showed improvements in a verbal memory task after 12 weeks daily consumption of grape juice ( $n=5$ , mean 75years), compared to a matched control ( $n=7$ ; mean 80years) (Krikorian et al., 2010a). The same study was repeated using blueberry juice, which showed improvements on two verbal memory tasks after 12 weeks of daily consumption ( $n=9$ ; mean 76years), compared to a placebo ( $n=7$ ; mean 80years) (Krikorian et al., 2010b). However, the blueberry control drink was not matched to the intervention; instead, authors used the grape juice control drink from their previous study. Additionally, sample sizes were small and benefits may have been attributed to the intervention group participants being younger, which could have a significant effect on cognitive function in later years (Letenneur et al., 2007), and which limits the interpretation of study findings.

The acute effects of flavonoid-rich foods on cognitive performance are being increasingly considered in children and young adults. A review of 21 intervention studies (3 specific to blueberries) reported that positive effects measured from 0-6 hours post consumption were mostly observed on episodic memory in children and older adults, and on executive function in younger adults, suggesting that differences may depend on brain development stage and function (Bell et al., 2015). In children ( $n=14$ , 8-10 years), there were no effects on spatial memory, but there were significant improvements in verbal memory following the consumption of a blueberry drink, compared to a matched control (Whyte & Williams, 2015). However, no baseline measures were taken to control for variations in performance across test days. In a slightly larger study in children

( $n=21$ ; 7-10 years), consuming blueberries significantly improved aspects of verbal memory in a dose-responsive manner (Whyte, Schafer, & Williams, 2015), although reaction time to an attention task was faster in the control group. However, no blood measures were collected in these studies and the influencing effects of mood or satiety were not considered.

### **1.11.2 Baobab**

Baobab (*Adansonia digitata L.*) is a rich source of polyphenols, particularly tannins and flavonoids (Tanko et al., 2008) and has been found to contain relatively high amounts of both insoluble and soluble dietary fibre (~30g of insoluble fibre and ~30g of soluble fibre / 100g extract) (De Caluwé et al., 2010; Nour et al., 1980). The baobab tree is an African plant traditionally used as famine food due to its nutritional properties, or therapeutically against diarrhoea, measles and smallpox (Tanko et al., 2008). Due to recent increased interest in baobab, powdered extracts are now easily obtainable from a selection of health food shops.

#### **1.11.2.1 Gluco-regulation**

Until now, baobab has not been studied for its effects on cognitive performance; however, its potential to reduce starch digestion and benefit gluco-regulation is of increasing interest, although studies are limited to adults. *In-vitro* and *in-vivo*, baobab was found to significantly reduce the overall GR to white bread (Coe et al., 2013). In diabetic rats, baobab lowered the GR suggesting insulin-like actions which may promote the uptake of glucose or inhibit hepatic gluconeogenesis (Tanko et al., 2008). However, both studies used relatively high doses, therefore the benefits to GR at lower doses are yet to be determined. In a later study the addition of baobab to white bread (at smaller doses) was found to reduce overall IR compared with green tea enriched bread and control bread, but no differences on GR or satiety were observed (Coe & Ryan, 2015).

### **1.11.3 Oats ( $\beta$ -glucans)**

A number of studies have found oat-containing products to have cholesterol-lowering effects, which led to the USA and UK approving a cholesterol-lowering health claim whereby the inclusion of 3g of oat  $\beta$ -glucan per day in foods could help to reduce blood cholesterol and the risk of coronary heart disease (European Food Safety Authority (EFSA), 2011; Othman, Moghadasian, & Jones, 2011; Xu, 2012).

Oats provide a natural source of soluble fibre. Oat soluble fibre consists of  $\beta$ -glucan (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan, a non-starch polysaccharide containing mixed linkage glucose molecules, varying in molecular weight (Wood, 1986). Analysis of oat-based breakfast cereals have also identified oats to be a significant source of polyphenols and antioxidants (Ryan et al., 2011).

#### **1.11.3.1 Gluco-regulation**

The functional properties of oat  $\beta$ -glucans relate to their bulking ability, which increases viscosity and solubility in the GI tract and are associated with improvements in GR, IR and satiety (Beck, Tosh, Batterham, Tapsell, & Huang, 2009a; Dikeman & Fahey, 2006; Regand et al., 2011; Thondre, Shafat, & Clegg, 2013). A review of studies comparing oat (or barley)  $\beta$ -glucans reported that meals providing between 0.3-12.1g of  $\beta$ -glucans significantly reduced overall GR compared to a suitable control (Tosh, 2013).

Breakfast studies comparing varied amounts of  $\beta$ -glucan (2-6g) found GR (Tappy et al., 1996) and IR (Beck et al., 2009a) significantly decreased, and satiety significantly increased (Beck et al., 2009a) in a dose-responsive manner. Oatmeal consumption in young adults ( $n=48$ ; 18 years) was reported to lead to greater feelings of satiety compared to a RTEC (Rebello et al., 2013); however, no glucose measures were taken, and despite being a well-designed study in many aspects, macronutrients were not matched between breakfasts, making comparisons difficult to interpret. Furthermore, *in-vitro* results suggested a possible interaction between the polyphenols in oat-based porridges and a reduction in starch digestibility (Thondre, Ryan, & Henry, 2011).

### ***1.11.3.2 cognitive performance***

The effects of oats on cognitive performance are limited to just one study in children (Mahoney et al., 2005) or in adults to the aforementioned study where oats were included as part of a functional food active diet (Nilsson et al., 2013). In children ( $n=30$ , 9-11years) spatial memory and attention significantly improved following consumption of an oat breakfast compared to no breakfast, although there were no differences between the RTEC and the oat breakfast conditions, making it difficult to separate the effects of breakfast consumption versus breakfast composition (Mahoney et al., 2005). The same study was repeated in younger children ( $n=30$ ; 6-11 years) where improvements in aspects of spatial memory and satiety were found compared with a RTEC or no breakfast (Mahoney et al., 2005). There were no effects of breakfast on mood in either age group.

## **1.11.4 Cinnamon**

### ***1.11.4.1 Gluco-regulation***

Early *in-vitro* studies (Khan, Bryden, Polansky, & Anderson, 1990) identified cinnamon (*Cinnamomum cassia*) as stimulating insulin activity, suggesting a role for its use in alleviating signs and symptoms of diabetes and CVD (Anderson, 2008). A meta-analysis of the effects of cinnamon concluded that whole cinnamon or cinnamon extract significantly lowered fasting blood glucose in pre-diabetics or diabetics (Davis & Yokoyama, 2011). Reviews since then have been inconclusive (Leach & Kumar, 2012) or cautiously supporting benefits, due to poorly designed studies on cinnamons' effectiveness on glycaemic targets (Medagama, 2015).

### ***1.11.4.2 cognitive performance***

Cinnamon is a rich source of polyphenols and is associated with having anti-inflammatory, antimicrobial, antidiabetic and antitumor properties (Ho, Chang, & Chang, 2013). Cinnamon has also been identified as a potential contributor to reducing the risk of neurodegenerative disease (Ho et al., 2013) and it was recently found to enhance working memory in pre-diabetics (Wahlqvist et

al., 2016). Animal models have shown cinnamon extract to reduce  $\beta$ -amyloid oligomerisation and cognitive impairment, suggesting great potential for its use in treating Alzheimer's disease (Frydman-Marom et al., 2011) and improving cognitive function.

#### **1.11.5 Summary**

There is considerable support for consuming individual functional food ingredients (blueberries, baobab, oats and cinnamon) rich in polyphenols and fibre, and potential benefits to gluco-regulation and cognitive performance. Polyphenol- and fibre-rich functional foods are also associated with increasing EE, improving mood and increasing satiety. In real-life situations a number of foods are consumed at each meal; however, only a few studies report the effects of combining functional food ingredients on gluco-regulation, and to date there are no studies reporting the effects of a functional food breakfast on gluco-regulation in adolescents.



## **Chapter 2: Rationale and hypothesis**

This thesis reports five studies (chapters 3-7) where the results of one study informed the development of the next study. Each study was designed to contribute to the development of the FB and CB, and guide the implementation of the final intervention study in a school-setting to adolescents (chapter 7).

Initially, the central hypothesis of this thesis proposed that the consumption of a FB based on functional food ingredients rich in polyphenols and fibre, would improve gluco-regulation (GR and IR) (primary outcome), enhance: cognitive performance (using a map task and delayed recall task), mood, satiety and EE (measuring DIT) (secondary outcomes), compared to a CB. However, over the course of studies the central hypothesis was revised and one of the secondary outcomes (EE) was removed. This rationale process is briefly described below.

### **2.1 Validation of indirect calorimeters and the use of a portable indirect calorimeter in a school environment (chapter 3: study 1 and 2)**

#### **2.1.1 Hypothesis**

Polyphenol compounds are associated with increased EE (chapter 1.10.5); therefore, part of the central hypothesis of this thesis proposed that the consumption of the FB, which was higher in polyphenols than the CB (chapter 5), would increase DIT in adolescents (chapter 7).

DIT is measured using indirect calorimetry (Rothwell & Stock, 1983); however, changes are difficult to detect unless machines are very accurate. Indirect calorimeter validation studies determine the accuracy and repeatability of measures of resting metabolic rate (RMR) when compared to a standard reference machine (Weissman, Sardar, & Kemper, 1990; Wells & Fuller, 1998); however, there are no portable indirect calorimeters that have been validated for use in the field. The ability to measure RMR and DIT accurately in schools would contribute to understanding the thermic effect of functional foods, which are rarely reported (chapter 1.10.5), as well providing

further insight into the metabolic adaptations to breakfast habits between regular and irregular breakfast consumers (chapter 1.5.1), thus contributing to an emerging area of research. To address these hypotheses two studies were undertaken.

### **2.1.2 Aims and sub-hypothesis (study 1)**

Study 1 aimed to validate a novel portable indirect calorimeter (the ECAL) against the Deltatrac (DT), which is considered to be the gold standard (chapter 3.3.3), for RMR and DIT measures. Successful validation for RMR and DIT measures would provide a rationale for the use of the ECAL to measure adolescents' RMR and subsequent DIT response to the FB and CB in the final study (chapter 7), thus contributing to one of the secondary outcomes of the central hypothesis.

It was hypothesised that the ECAL would be accurate enough to measure small changes associated with collecting DIT measures based on there being non-significant differences between machines and would give accurate and reliable measures of RMR (chapter 3.2).

### **2.1.3 Study development (study 1)**

Early analysis of individuals' RMR and DIT data showed that the ECAL would not be sensitive enough to detect changes in DIT measures; therefore the collection of DIT measures was discontinued. The termination of DIT measures led to a revision of the central hypothesis, and the consideration of the effect of the FB on DIT was removed.

Study 1 continued to collect RMR measures as the findings would contribute to the literature reporting on the development of novel methods to measure RMR. Despite finding that the ECAL was not an accurate alternative to the DT, based on the reliability of repeatable measures and questions surrounding the accuracy of the DT, the ECAL was taken forward for use in schools (study 2).

### **2.1.4 Aims and sub-hypothesis (study 2)**

A fundamental aspect of the proposed breakfast study (chapter 7) was implementing the study in a school environment to adolescents, which partially relies on compliance from participants with study protocols.

Study 2 aimed to determine the feasibility of using the ECAL in a school to collect RMR measures from adolescents (as per the manufacturer's protocol) assessed by: observing participants' understanding and completion of measures, identifying adverse effects, and collecting informal feedback from participants. A secondary aim was to compare ECAL values to currently recommended prediction equations. It was hypothesised that the ECAL would be acceptable to adolescents and that ECAL values would not be significantly different from prediction equations (chapter 4.2).

Although collecting RMR data would not contribute directly to the new central hypothesis of the thesis, reporting the use of novel methods to collect RMR would contribute towards a greater understanding of body composition during this critical period of growth, and potentially reduce the need for using prediction equations which can be inaccurate. Furthermore, as RMR measures would be collected during a screening session (chapter 7.3.3), this would provide an opportunity for participants to practice adhering to protocol measures (i.e. fasting and following protocol instructions).

## **2.2 Exploring gender differences in the theory of planned behaviour applied to adolescent breakfast consumption (chapter 4)**

### **2.2.1 Hypothesis**

The frequency and quality of breakfast consumption declines in adolescence (chapter 1.4) and this can be influenced by gender and SES (chapter 1.4.2). It was hypothesised that adolescents

completing a questionnaire would report infrequently consuming breakfast, and this would be more evident in girls than boys, and in those holding negative attitudes towards breakfast.

### **2.2.2 Aims and sub-hypothesis**

The primary aim was to investigate the factors underpinning the breakfast choices of adolescents, considering demographics and gender, which could potentially inform future interventions to promote consumption of a healthful breakfast in adolescents.

A secondary aim was to contribute to the development of the FB and CB (chapter 5). The questionnaire collected data on participants' usual breakfast habits (frequency and composition). The literature suggests that RTECs are the most commonly consumed breakfast by adolescents (chapter 1.4.3), but there is little information on the type of RTECs which are popular. It was hypothesised that RTECs would be the most popular breakfast choice in adolescents.

Finally, the study aimed to establish relationships with local schools and teachers and recruit schools 'in principal' to the final school study (chapter 7).

### **2.2.3 Study development**

Analysis of the questionnaire data confirmed that RTECs were the most commonly consumed breakfast by adolescents, specifically 'chocolate Weetos'. This was used in the development of the FB and CB (chapter 5), and as one (of three) breakfast conditions in the adult study (chapter 6.4). Although the questionnaire results showed that almost a quarter of adolescents did not consume breakfast (chapter 4.4), it would have been unethical to include a 'no breakfast' condition in the study. Firstly, it did not directly contribute to answering the central hypothesis; secondly, working with schools during the questionnaire study highlighted the difficulties with recruitment, and the tight time constraints the schools were under, thus it was not included to help reduce participation burden on the schools.

## **2.3 The development of a functional food breakfast (chapter 5)**

### **2.3.1 Hypothesis**

It was hypothesised that *in-vitro*, the FB would contain a significantly higher amount of polyphenols, and would be superior in its potential to reduce the amount of sugars released, compared to the CB.

### **2.3.2 Aims and sub-hypothesis**

The primary aim was to design a FB using ingredients selected for their potential to improve gluco-regulation, cognitive performance, mood and satiety (blueberries, baobab, oats and cinnamon; chapter 1.11), and compare levels of total antioxidants, polyphenols and sugar release with the CB, which did not contain any of the selected functional food ingredients (chapter 5).

A secondary aim was to inform the storage procedures used in the proceeding breakfast studies (chapter 6 and 7) by comparing the effects of freezing on total antioxidant, polyphenol and sugar release.

### **2.3.3 Study development**

The *in-vitro* findings confirmed that the FB was a richer source of total antioxidants and polyphenols, and released sugar more slowly compared to the CB. This justified the potential of the FB to elicit improvements to GR and IR *in-vivo* (chapter 1.11.1-4), and the interventions were implemented in healthy adults (chapter 6) and adolescents (chapter 7).

## **2.4 The effects of a functional food breakfast on gluco-regulation, cognitive function, mood and satiety in healthy adults (chapter 6)**

### **2.4.1 Hypothesis**

It was hypothesised that the inclusion of functional foods ingredients in the FB would work synergistically to improve postprandial gluco-regulation and enhance cognitive performance, mood

and satiety, compared to the CB and the RTEC. It was further hypothesised that there would be no difference in the effects on gluco-regulation between the CB and the RTEC as ingredients were matched and both breakfasts were devoid of the selected functional food ingredients.

#### **2.4.2 Aims and sub-hypothesis**

The primary aim was to compare the FB with the CB and the RTEC and measure the effects on gluco-regulation, in a laboratory setting with healthy adults. A secondary aim was to compare the effects of breakfast conditions on cognitive performance, mood and satiety (chapter 6.1).

Additionally, performing the study in adults in a laboratory setting would inform the implementation of the study protocol before being taken into schools (chapter 7).

#### **2.4.3 Study development**

As hypothesised, the results showed that the FB improved gluco-regulation compared to the CB and the RTEC. Furthermore, there were no differences between the CB and the RTEC and the effects on gluco-regulation; therefore, in order to reduce participation burden on the schools, the RTEC condition was removed from the final adolescent study (chapter 7). There were no effects of breakfast condition on any other measure.

### **2.5 The effects of a functional food breakfast on gluco-regulation, cognitive function, mood and satiety in adolescents (chapter 7)**

#### **2.5.1 Hypothesis**

It was hypothesised that consumption of the FB would improve gluco-regulation compared to the CB, as suggested by previous findings in adults (chapter 6). It was further hypothesised that the FB would enhance cognitive performance, satiety and mood in adolescents, based on a larger sample size and some minor amendments and piloting of the cognitive tests and mood questionnaire.

#### **2.5.2 Aims and sub-hypothesis**

The primary aim was to deliver the study protocol in a school environment and compare the FB to the CB for measures of gluco-regulation, cognitive performance (map task and delayed recall performance), mood and satiety in adolescents.

### **2.5.3 Study development**

As hypothesised, the results showed that the FB improved gluco-regulation compared to the CB.

There were no effects of breakfast condition on any other measure.

## **2.6 Summary**

Taken together this series of studies contributed to the development of a FB that showed potential to improve gluco-regulation in adults and adolescents compared to a CB (and RTEC in adults only).

There were no effects on cognitive performance, mood or satiety.

The discussion and implications of these findings are addressed in detailed in chapter 8.

## **Chapter 3: Validation of indirect calorimeters and the feasibility of using a portable indirect calorimeter in a school environment.**

### **3.1 Summary**

Assessing resting metabolic rate (RMR) provides insight into the metabolic adaptations which can occur in individuals and is used to assist with the management of overweight and obesity. In a clinical setting equations predicting basal metabolic rate (BMR) are relied upon, but these can over- or under-estimate in certain populations. Indirect calorimeters provide accurate measures, but a frequently employed indirect calorimeter, the DT, is no longer commercially available, so there is a need for new calorimeters that will facilitate the collection of objective measures, particularly in the field. The aims of this study were to:

- (i) Compare the accuracy and reliability of two new indirect calorimeters (the GEM and the ECAL) using the DT as a standard reference for measures of RMR.
- (ii) Investigate the feasibility of the ECAL portable calorimeter to collect RMR measures from adolescents in a school environment and compare these with the recommended prediction equations.

The GEM and the ECAL were not accurate replacements for the DT, but these machines were reliable for repeated measures. The ECAL was acceptable for use in a school environment and measures were collected successfully, but prediction equations significantly underestimated RMR compared to RMR measured on the ECAL. The findings suggest the GEM and ECAL are reliable indirect calorimeters, but determining the accuracy of measures requires further research.

Comparing measured RMR with predicted RMR highlights the difficulties facing researchers selecting prediction equations, considering the range of variation at an individual level.



## 3.2 Introduction

BMR represents the energy used to maintain basic physiological functions of the body at rest, under strictly defined conditions, and can account for 45% to 70% of daily total EE (FAO, 2001).

Difficulties adhering to the strict measurement protocol of BMR led to the development of prediction equations to estimate BMR. In a systematic review comparing prediction equations, the authors refer to RMR throughout, pointing out that one of the most commonly used equations (Harris & Benedict, 1918) was measured under resting and not basal conditions (Frankenfield, Roth-Yousey, & Compher, 2005). Referring to prediction equations as measuring RMR and not BMR was also preferentially reported by EFSA (EFSA, 2013) and will therefore be referred to in this way from now on.

RMR refers to energy expended at rest when no additional energy is used for muscular effort and tends to be higher than BMR by between 10% and 20%. For practical reasons, RMR is commonly measured and reported in research studies (Otten, Hellwig, & Meyers, 2006). RMR is related to body size, being most strongly correlated with the measure of the FFM (Otten et al., 2006). Changes in RMR occur over a person's life time, with steady declines observed through adolescence (9.5-16.5 years) potentially due to the increasing proportion of muscle mass, which relative to the major organs, has a lower RMR (Holliday, 1971). Furthermore, there is a general decline of metabolic activity of FFM with age (Molnár & Schutz, 1997). Differences in proportions of muscle mass or in the metabolic activity of certain organs may further explain higher RMR in adolescents, even when compared with adults of the same FFM (Weinsier, Schutz, & Bracco, 1992). Understanding RMR and its relative contribution to metabolic activity is fundamental to nutritional assessments in clinical and research settings, and requires accurate and reliable measures of EE. However, there are relatively few studies measuring EE in young people and consequently few suitable methods for measuring RMR outside of laboratory settings.

Currently, in clinical settings, energy needs are calculated with prediction equations using minimally invasive measures including: height, weight, gender and age. Prediction equations are generally acceptable at a population level, but their accuracy may vary by as much as 20% for an individual (Frankenfield et al., 2005), depending on factors including ethnicity (Lawrence, Lee, Kim, & Kim, 2009) and fat mass (Hasson, Howe, Jones, & Freedson, 2011). A systematic review comparing predicted RMR to measured RMR in adults, recommended using equations that predict to within 10% of measured RMR; however, it acknowledged that older individuals and ethnic minorities are still largely under-represented by prediction equations and in these situations taking a direct measure of RMR is strongly recommended (Frankenfield et al., 2005).

Prediction equations in young people were considered in EFSA's latest scientific opinion 'Dietary Reference Values for Energy' (EFSA, 2013). They compared six prediction equations derived from healthy-weight children and adolescents and concluded that two equations, Schofield height and weight (Schofield, 1985) and Henry's Oxford equations (Henry, 2005), were equally valid for predicting RMR, due to the large sample sizes and diverse age range that the equations were derived from (EFSA, 2013).

Indirect calorimetry allows an objective measure of RMR to be collected. RMR is estimated based on the assumption that the energy produced in the body by substrate oxidation is coupled to oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ) (Ferrannini, 1988). For over 24 years the DT™ II Metabolic Monitor (Datex-Ohmeda Inc., Helsinki, Finland) has been considered the standard reference tool in indirect calorimetry. It has been validated *in-vitro* and *in-vivo* for use in clinical and research settings (Weissman et al., 1990; Wells & Fuller, 1998) and is frequently used as a reference standard against which new machines are compared (Alam et al., 2005; Blond et al., 2011; Compher, Hise, Sternberg, & Kinosian, 2005; Frankenfield & Coleman, 2013; Graf et al., 2013; Littlewood, 2002; Roffey, Byrne, & Hills, 2006; St-Onge, Rubiano, Jones, & Heymsfield,

2004; Stewart, Goody, & Branson, 2005; Wahrlich, Anjos, Going, & Lohman, 2006). Now that the DT is no longer commercially available there is increasing interest in validating alternative indirect calorimeters which consist of hooded or portable options. Portable calorimeters circumvent some of the challenges associated with the hooded calorimeters relating to expense and transport, and represent an important step towards improving the accuracy of measures in non-clinical settings (Dhurandhar et al., 2014). However, results of validation studies have been conflicting, with some studies finding agreement between methods (Blond et al., 2011; Compher et al., 2005; Roffey et al., 2006; St-Onge et al., 2004; Stewart et al., 2005; Wahrlich et al., 2006) and other studies rejecting agreement (Cooper et al., 2009; Frankenfield & Coleman, 2013; Graf et al., 2013; Littlewood, 2002).

Making comparisons between machines can be complicated due to differences in how RMR measures are collected between the portable and hooded calorimeters. Indirect calorimeters using a ventilated hood, such as the DT, are often set to generate data from  $VO_2$  and  $VCO_2$  samples at one-minute intervals. In contrast, portable calorimeters using a facemask, such as the Cosmed K4 b<sup>2</sup>, are able to perform a measurement on every breath cycle (Littlewood, 2002). Furthermore, handheld calorimeters such as the MedGem RMR<sup>®</sup> (Microlife, Florida, USA), collect  $VO_2$  and subsequently calculate  $VCO_2$  using an assumed respiratory quotient (RQ) value of 0.85 (Compher et al., 2005).

In clinical settings the precision of measurements is critical as these data are primarily used for determining the caloric requirements of patients to prevent under- or over-feeding (Frankenfield et al., 2005). In research or weight-loss settings users often measure differences in EE over time (Gibbons, Henry, Ulijaszek, & Lightowler, 2004) or following an intervention (Clegg, Golsorkhi, & Henry, 2013). In these circumstances the accuracy of repeated measures on the same machine (i.e. repeatability) is important.

A potential replacement for the DT is the GEM (GEM Nutrition Ltd, Cheshire, UK). Similar to the DT, the GEM is an open-circuit calorimeter using a ventilated hood. A clear plastic hemispherical canopy is placed over the individual's head and the exhaled air is drawn through a Nafion tube into a mixing chamber. Here, it is diluted with the room air before  $\text{VO}_2$  and  $\text{VCO}_2$  sensors collect a sample. In contrast to the DT, which assumes a constant flow of air, the GEM measures the actual flow rate through the hood, which can be varied in the range of 20 to 80 l/min. Like the DT, the GEM measures  $\text{VO}_2$  using a paramagnetic oxygen sensor and  $\text{VCO}_2$  via an infrared sensor. Both machines report data at one-minute intervals.

A potential portable replacement to the DT is the ECAL (Energy Testing Solutions, Queensland, Australia). The ECAL is a novel open circuit portable calorimeter that measures both  $\text{VO}_2$  and  $\text{VCO}_2$  using a small mixing chamber. The ECAL was primarily designed to be used by health professionals and is therefore low maintenance and requires little technical expertise. The machine uses a proprietary mouth piece and nose clip.  $\text{VO}_2$  is measured using a galvanic fuel cell oxygen analyser.  $\text{VCO}_2$  is measured using a patented ultra-low power  $\text{VCO}_2$  analyser which uses light emitting diode (LED) and detector technology in a novel non-dispersive near infra-red absorption sensor. Data on the ECAL is reported for each breath cycle.

To date, there are no studies comparing the ECAL or the GEM against a known reference standard for accuracy and reliability of RMR measures. Additionally, previous use of the ECAL is limited to adults in weight loss clinics and its use in a school environment with adolescents has not been considered.

### **3.2.1 Aims and hypothesis**

Two studies were developed with the following objectives:

#### Study 1

- a. Compare the ECAL and the GEM indirect calorimeters to the DT for measures of accuracy in a laboratory setting in adults.
- b. Compare the repeatability of the machines and the potential of the ECAL to be used in study 2 in a school environment.

Hypothesis:

It was hypothesised that the ECAL and the GEM would give accurate and reliable measures of RMR based on variability ranging up to 20% (accuracy) (Fields, Kearney, & Copeland, 2006; Sundstrom, Tjader, Rooyackers, & Wernerman, 2013) and 10% (repeatability) (Compher, Frankenfield, Keim, & Roth-Yousey, 2006)

Study 2

- a. Determine the feasibility of collecting RMR measures using the ECAL with adolescents in a school environment.
- b. Compare measured RMR on the ECAL with two EFSA recommended prediction equations in adolescents.

Hypothesis:

It was hypothesised that the ECAL would be acceptable to adolescents and that ECAL values would not be significantly different from prediction equations.

## **3.3 Methods (Study 1)**

### **3.3.1 Study design**

Study 1 was a randomised crossover study with repeated measures on two days within a two-week period.

### **3.3.2 Subjects**

Twenty healthy participants (4 males and 16 females) (Table 7) were recruited for the study by means of advertisements displayed throughout Oxford Brookes University (OBU) and classified advertisements on a local website. Before inclusion potential participants were briefed on all aspects of the study and were given the opportunity to ask questions. Individuals fulfilling all eligibility criteria (age 18-55 years, BMI between 18-30kg/m<sup>2</sup>, blood pressure 110-120/75-85mmHg, not on prescription medication, no known genetic or metabolic diseases, no food allergies or intolerances) were included in the study. The study was conducted in the laboratory at OBU. On the day prior to each test, participants were asked to abstain from alcohol and caffeine, and to refrain from strenuous PA. Participants arrived between 7-9am after an overnight fast (10-12 hours before testing time). A record was made of their evening meal and they were asked to repeat this the evening prior to their next testing session. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University Research Ethics Committee at OBU. Written informed consent was obtained before testing.

**Table 7:** Baseline participant characteristics

		Males (n=4)		Females (n=16)		All (n=20)	
		Mean	SD	Mean	SD	Mean	SD
Age	(years)	38.3	11.2	24.1	3.5	26.9	8.0
Height	(m)	1.8	0.04	1.7	0.1	1.69	0.1
Weight	(kg)	81.8	7.1	62.3	9.4	66.2	11.9
BMI	(kg/m <sup>2</sup> )	24.4	1.4	22.6	2.6	22.9	2.5
Fat mass	(kg)	12.7	5.9	18.1	5.9	17.0	6.1
Fat free mass	(kg)	69.1	7.5	44.2	4.0	49.2	11.2
Body fat	(%)	15.4	6.6	28.4	5.3	25.8	7.6

Abbreviations: BMI, body mass index.

### 3.3.3 Indirect calorimeters

#### *Deltatrac*

The DT was calibrated once in the morning after a 30-minute warm up. Gas was calibrated using standard calibration gas (Quick Cal, Helsinki, Finland) to 5% CO<sub>2</sub> and 95% O<sub>2</sub>. Pressure was measured then adjusted to match ambient air pressure as reported in the daily weather forecast. Measurements were collected using a ventilated hood and artifact suppression was switched off. The flow rate was fixed at 40 l/min therefore no manual adjustment was required.

#### *GEM*

The GEM was calibrated after a 20-minute warm up. Calibrations were performed; before each use, when the machine was left idle for more than 20 minutes, and after every 2 hours of continuous use (as per the manufacturer's recommendation). Gas was calibrated using standard calibration gases (BOC, Surrey, UK) to 1% CO<sub>2</sub> and 20% O<sub>2</sub>. Flow was manually adjusted at the time of calibration to approximately 40 l/min and measurements were collected using a ventilated hood. Monthly ethanol burn tests were performed on the GEM as a quality check for the calibration.

#### *ECAL*

The ECAL was calibrated once in the morning after a 10-minute warm up. Gas was calibrated using standard calibration gas (Calgaz, Cheshire, UK) to 4% CO<sub>2</sub> and 16% O<sub>2</sub>. Flow was calibrated using

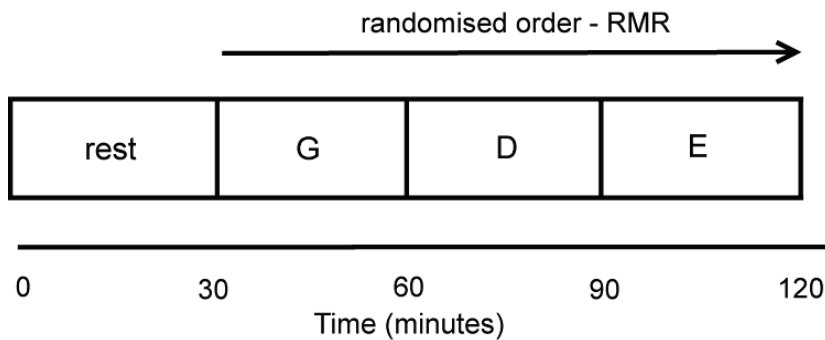
a one-litre calibration syringe (Hans Rudolph Series 5540). ECAL uses a proprietary method for calculating individualised flow rates with a set upper limit of 4 l/min.

With the exception of the ECAL all calibrations were performed in line with the manufacturer's recommendations. The ECAL user manual (ECAL, 2013) recommends regular calibration of the sensors (i.e. weekly) are performed; however, in this study, calibrations were performed on every test day to maintain consistency between protocols. Calibration values were checked either directly with the manufacturers (GEM and ECAL) or against manufacturer's guidelines (DT) and were found to be within acceptable ranges.

### **3.3.4 Study protocol**

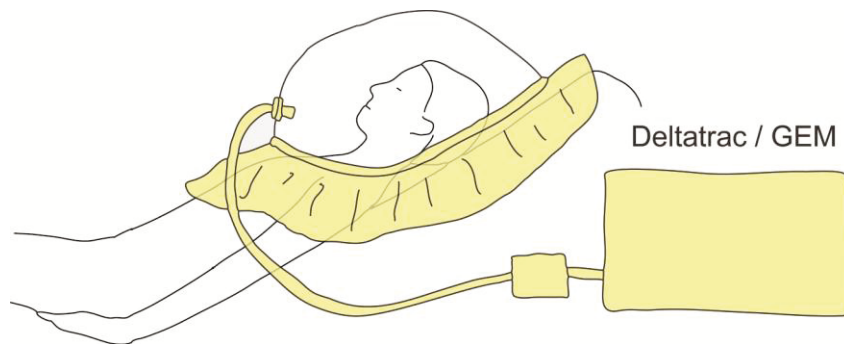
On arrival at the laboratory anthropometric measures were taken. Body weight and percentage body fat were collected using the Tanita BC-418 MA (Tanita UK Limited, Yiewsley, UK). Height was measured to the nearest centimetre using a stadiometer (Seca Ltd, Birmingham, UK). Participants were rested in a supine position for 30 minutes during which their blood pressure was checked using a digital blood pressure monitor (A&D UA-767, Oxfordshire, UK). Following randomisation using a random order generator (Oxford-Brookes-University, 2011) RMR was consecutively measured for 30 minutes on the GEM (G), the DT (D) and the ECAL (E) (Figure 5).





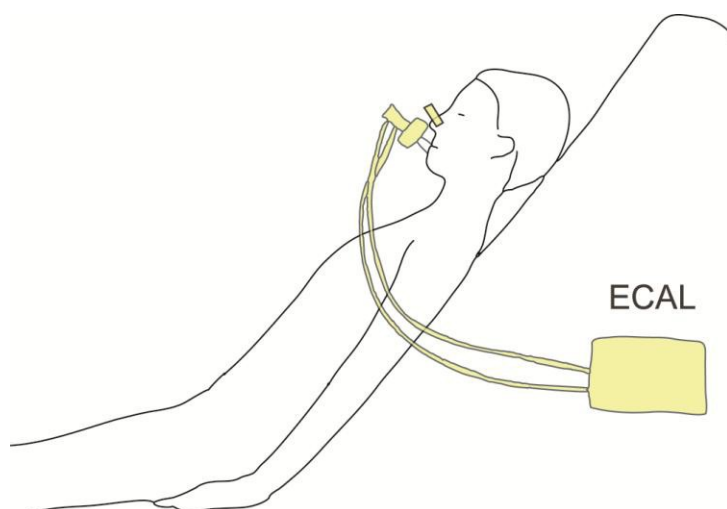
**Figure 5.** Study protocol. On arrival at the laboratory (time 0) participants rested (rest) for 30 minutes. In a randomised order RMR was consecutively measured for 30 minutes on the GEM (G), the DT (D) and the ECAL (E).

During testing, participants were requested to minimise movements and remain awake. Participants could read or watch films on a laptop which, if required, was positioned on a small table placed over the bed. The DT and GEM measurements were collected with the participant in a supine position (Figure 6).



**Figure 6.** Measures were collected on the GEM and the DT in a supine position

ECAL measurements were collected with the participant in a semi-reclined position (Figure 7) with a cushion supporting the mouthpiece connector so it was not necessary for the participant to hold it. Participants were allowed to remove the mouthpiece momentarily during testing if they needed to swallow, but were told to replace it as soon as possible. Temporary removal of the ECAL mouthpiece creates an abnormal airflow and consequently those data points are not recorded. Room temperature was maintained throughout at 22-24°C and blankets were available on request.



**Figure 7.** Measures were collected on the ECAL in a semi-reclined position.

### **3.3.5 Determining EE**

$VCO_2$  and  $VO_2$  data generated from the DT, GEM and ECAL were collected and RMR, fat oxidation, CHO oxidation and RQ were calculated using the Lusk equations (Lusk, 1928). The equations based on Lusk's calorific factors have been reported as appropriate for use in normal conditions (McLean & Tobin, 1987). To allow for stabilisation with the mouthpiece or within the hood the first 10 minutes of every 30-minute time period were discarded (Compher et al., 2006). The averages of the remaining twenty minutes of data were used. A steady-state coefficient of variation (CV) for  $VO_2$  and  $VCO_2$  during these 20 minutes was set to within 20% (Fields et al., 2006; Sundstrom et al., 2013). This level was specified to account for the fluctuations observed in breath samples collected from a mouthpiece compared to samples collected from a ventilated hood.

### **3.3.6 Statistical analyses**

Outcome measures consisted of  $VO_2$ ,  $VCO_2$ , RMR, RQ, fat oxidation and CHO oxidation. Accuracy of the machines (inter-machine variability) was assessed by comparison of RMR measures between machines using a one-way repeated measures analysis of variance (ANOVA) with Bonferroni adjustment for multiple comparisons. Repeatability of machines (intra-machine

variability) was assessed using paired samples *t*-tests. Significance was set at  $p < .05$ . The relative variability of measures was calculated using the CV. Individual data were examined for the percentage difference between mean measures. The strength of relations was determined using Pearson's correlation coefficient and Bland Altman plots were used to determine agreement of machines compared to the DT (Bland & Altman, 1986). The 95% limits of agreement (LOA) were calculated as the mean difference plus or minus ( $\pm$ ) 2 standard deviations (SD) of the differences, within which 95% of differences between measurements by the two methods are expected to lie. Normality was assessed using the Shapiro-Wilk test.

An *a priori* power calculation predicted that a sample size of 19 volunteers would be sufficient to achieve 90% power based on a SD of 63.1 kcal/d and a change in mean RMR of  $\pm 50$  kcal/d (Roffey et al., 2006). Statistical analyses were performed using IBM SPSS Statistics 21.0 (2013; SPSS Inc., Chicago, IL, USA) and data and figures were processed in Microsoft Excel 2010 (Reading, UK). Values are presented as mean  $\pm$  SD or standard error of the mean (SEM).

## 3.4 Results (Study 1)

### 3.4.1 Measurement acceptability

Study 1 was successfully completed by 20 participants on test day one (T1) and test day two (T2). Analysis of steady-state data revealed that a number of ECAL measures ( $n=10$  VO<sub>2</sub>,  $n=11$  VCO<sub>2</sub>) were not within the target 20% CV. As this would have excluded a large amount of ECAL data it was decided that all results should be included. Therefore, RMR analysis includes data from all participants.

### 3.4.2 Comparison of RMR measures from the GEM and the ECAL to the Deltatrac

There were significant differences between machines for: VCO<sub>2</sub> on T1

( $F(2,38)=10.512, p<.001, \text{partial } \eta^2=.356$ ) and T2 ( $F(2,38)=15.537, p<.001, \text{partial } \eta^2=.450$ ), VO<sub>2</sub> on T1 ( $F(2,38)=11.365, p<.001, \text{partial } \eta^2=.374$ ) and T2 ( $F(2,38)=31.596, p<.001, \text{partial } \eta^2=.624$ ), RMR on T1 ( $F(2,38)=11.228, p<.001, \text{partial } \eta^2=.371$ ) and T2 ( $F(2,38)=29.257, p<.001, \text{partial } \eta^2=.606$ ), fat oxidation on T1 ( $F(2,38)=6.778, p<.005, \text{partial } \eta^2=.263$ ) and T2 ( $F(2,38)=14.581, p<.001, \text{partial } \eta^2=.434$ ), CHO oxidation on T1 ( $F(2,38)=4.066, p<.05, \text{partial } \eta^2=.176$ ) and T2 ( $F(2,38)=6.742, p<.005, \text{partial } \eta^2=.262$ ).

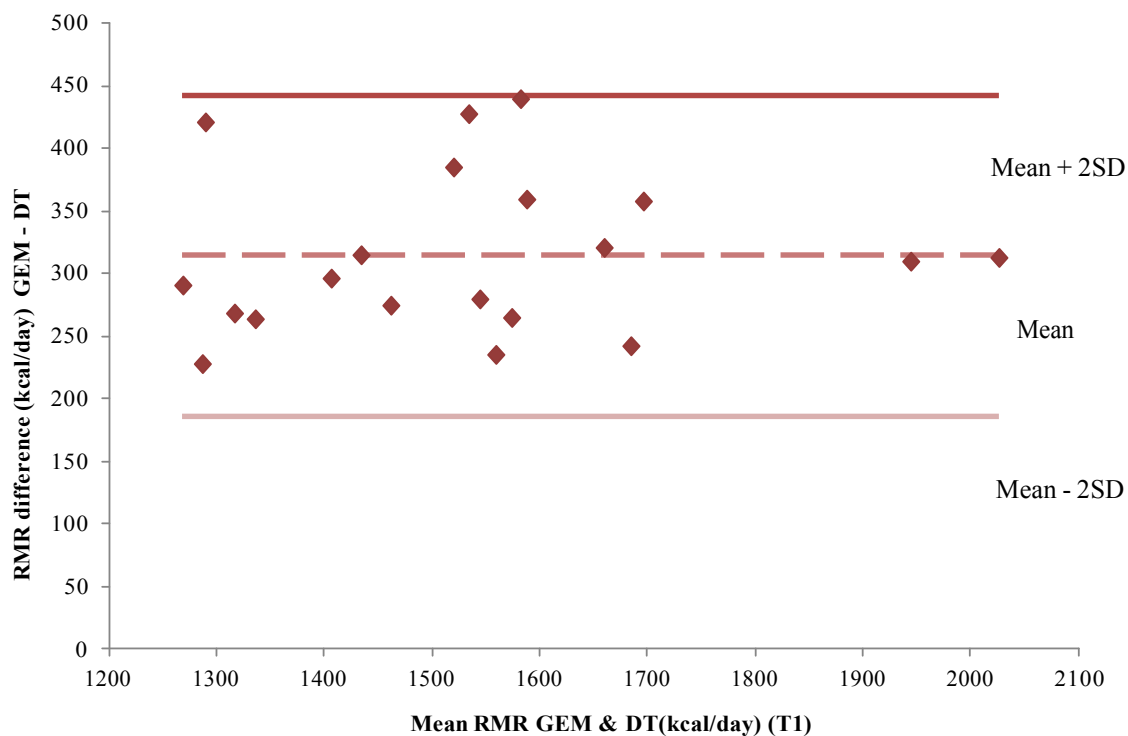
Post hoc analysis with a Bonferroni adjustment revealed that the GEM was significantly higher than the DT on T1 and T2 for VCO<sub>2</sub>, VO<sub>2</sub>, RMR and fat oxidation ( $p<.005$ ) (Table 5) and significantly higher than the ECAL for VCO<sub>2</sub> on T1 and T2 ( $p<.05$ ) and RQ and CHO oxidation on T2 only. The ECAL was significantly higher than the DT for measures of VO<sub>2</sub>, RMR and CHO oxidation on T2 and RQ and fat oxidation on both test days ( $p<.05$ ). Variation was greatest on the ECAL with higher SEM than the DT and the GEM on all measures.

**Table 8.** RMR inter-machine mean difference in all participants ( $n=20$ ) for  $VCO_2$ ,  $VO_2$ , RMR, RQ, CHO ox & FAT ox collected on the DT, ECAL and GEM during T1 and T2. Significance was calculated using repeated measures ANOVA with Bonferroni adjustment for multiple measures.

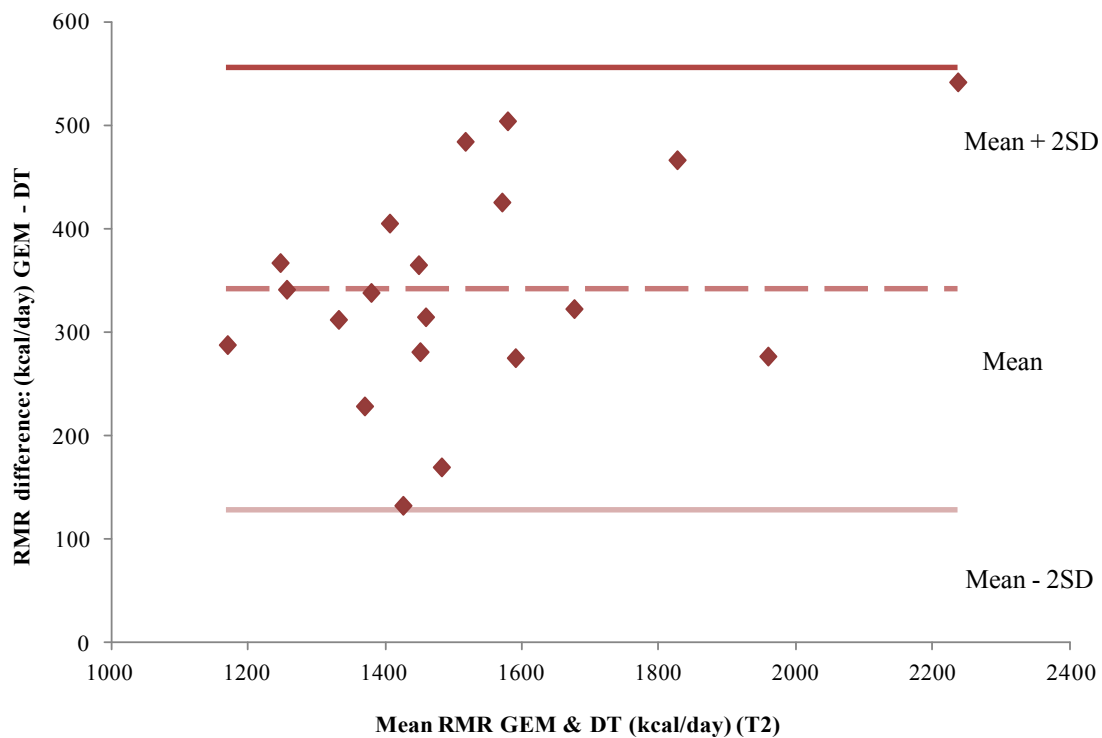
	$VCO_2$ (ml/min)						$VO_2$ (ml/min)						RMR (kcal/day)					
	DT - ECAL		GEM - DT		GEM - ECAL		DT - ECAL		GEM - DT		GEM - ECAL		DT - ECAL		GEM - DT		GEM - ECAL	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
T1	-1.54	9.74	33.00**	1.99	31.49*	9.97	-18.86	11.77	46.50**	2.25	27.62	12.04	-109.66	80.72	314.05**	14.39	204.41	82.79
T2	-11.76	7.86	35.40**	2.90	23.67*	7.45	-41.86**	8.100	50.90**	3.72	9.07	7.79	-254.41**	55.31	342.78**	23.99	88.36	53.48
	RQ						CHO ox (kcal/min)						FAT ox (kcal/min)					
	DT - ECAL		GEM - DT		GEM - ECAL		DT - ECAL		GEM - DT		GEM - ECAL		DT - ECAL		GEM - DT		GEM - ECAL	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
T1	0.06*	0.02	-0.03*	0.01	0.03	0.02	0.16	0.08	0.00	0.03	0.16	0.08	-0.24*	0.09	0.22**	0.03	-0.02	0.08
T2	0.10*	0.02	-0.03	0.01	0.07*	0.02	0.25*	0.09	-0.01	0.04	0.24*	0.08	-0.43**	0.10	0.25**	0.05	-0.18	0.08

Abbreviations: RMR, resting metabolic rate; DT, Deltatrac; SEM, standard error of mean; T1, test day 1; T2, test day 2; RQ, respiratory quotient; CHO ox, carbohydrate oxidation; FAT ox, fat oxidation. Statistically significant \*\* $p<.005$ , \*  $p<.05$ .

Bland Altman analysis of RMR measures between the GEM and the DT indicated a consistent bias towards overestimation on the GEM with a mean difference of 314 ( $\pm$  64) kcal/d on T1 and 343 ( $\pm$  12) kcal/d on T2 (Figure 8a; Figure 8b).

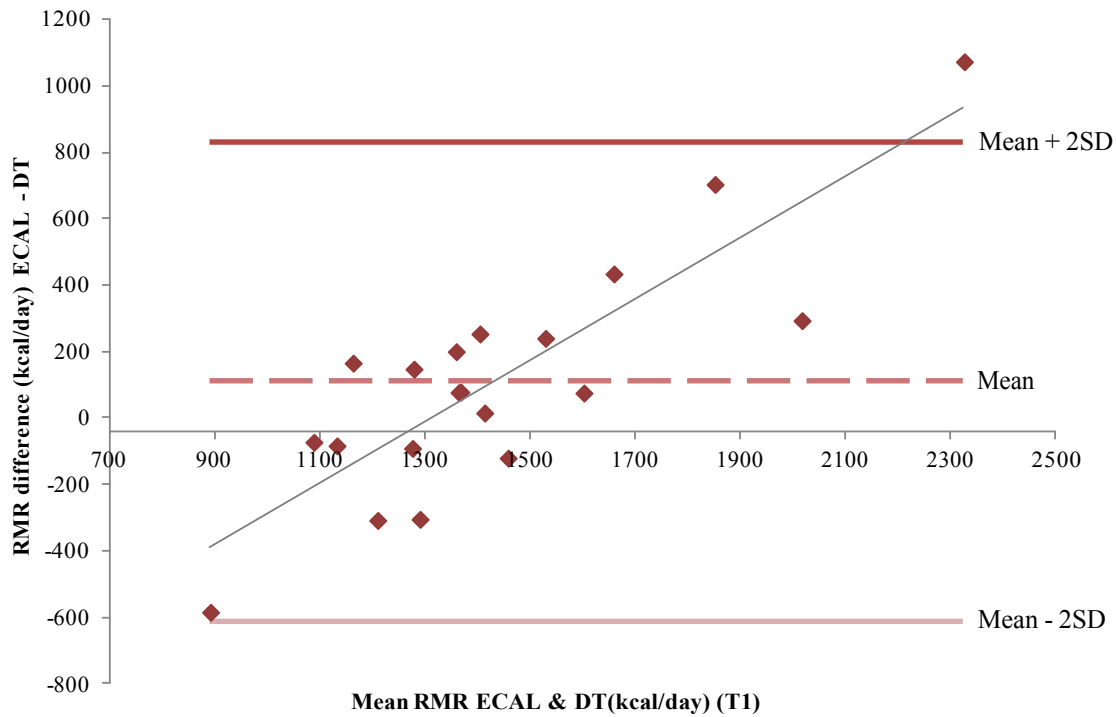


**Figure 8a.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the GEM and the DT on test day 1 (T1). The lower 95% LOA was 185 kcal/day and the upper 95% LOA was 443 kcal/day. Difference was calculated as GEM minus DT

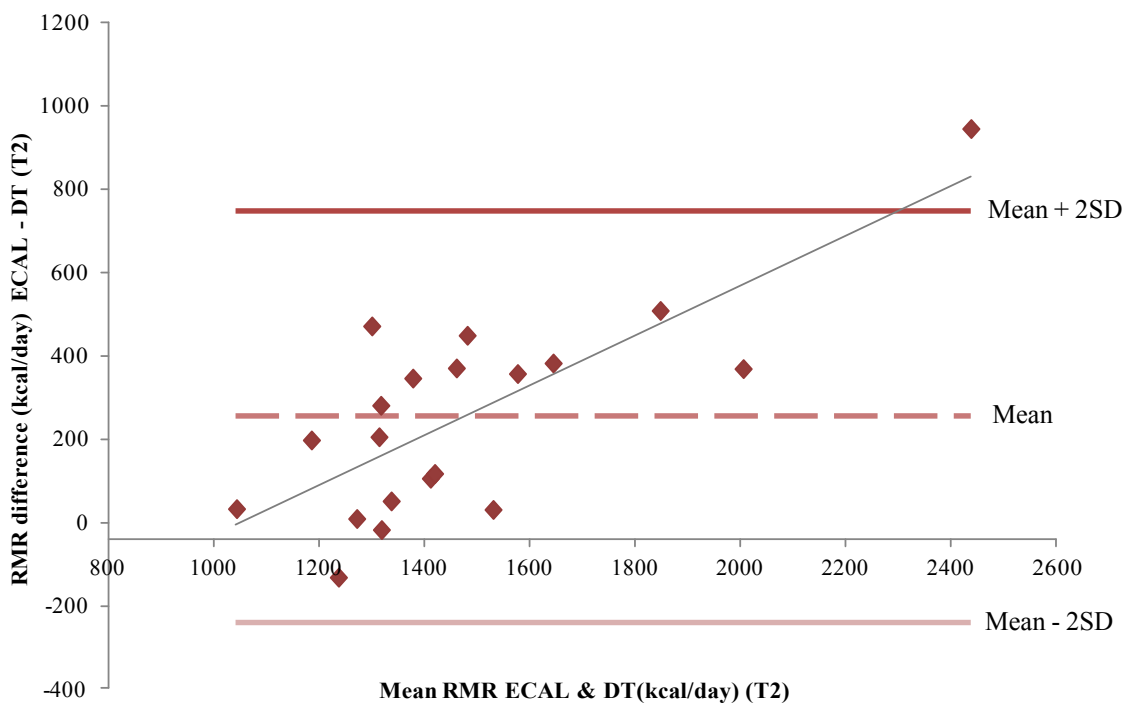


**Figure 8b.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the GEM and the DT on test day 2 (T2). The lower 95% LOA was 128 kcal/d and the upper 95% LOA was 557 kcal/d. Difference was calculated as GEM minus DT.

A smaller bias towards overestimation on the ECAL was observed with a mean difference of 110 ( $\pm$  361) kcal/day on T1 and 254 ( $\pm$  247) kcal/day on T2. However, LOA were wider and a proportional bias was observed suggesting that at higher RMRs the difference between the two machines was greater (Figure 9a; Figure 9b).



**Figure 9a.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the ECAL and the DT on test day 1 (T1). The lower 95% LOA was -612 kcal/day and the upper 95% LOA was 832 kcal/day. Difference was calculated as ECAL minus DT.



**Figure 9b.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the ECAL and the DT on test day 2 (T2). The lower 95% LOA was -240 kcal/day and the upper 95% LOA was 749 kcal/day. Difference was calculated as ECAL minus DT.



### 3.4.3 Comparison of RMR measures to determine repeatability of machines

There were no significant differences between repeated measures of VCO<sub>2</sub>, VO<sub>2</sub>, RMR, RQ and substrate oxidation within the GEM or the DT. Within the ECAL only VO<sub>2</sub> measures were significantly different between test days ( $t(19) = (-2.642), p < .05, d = (-.591)$ ). Significant correlations ( $p < .005$ ) were observed for repeated measures of VCO<sub>2</sub>, VO<sub>2</sub> and RMR on all machines (Table 9). Substrate oxidation and RQ were significantly correlated on the GEM ( $p < .05$ ) and the ECAL ( $p < .005$ ); however, this was in contrast to the DT for which low, non-significant correlations between RQ and substrate oxidation repeated measures were observed.

**Table 9.** Correlations between measures on the DT, ECAL and GEM for VCO<sub>2</sub>, VO<sub>2</sub>, RMR, RQ, CHO ox and FAT ox in all participants ( $n=20$ ) on T1 and T2.

	Intra-machine					
	DT		GEM		ECAL	
	T1, T2		T1, T2		T1, T2	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
VCO <sub>2</sub> (ml/min)	0.83	0.005**	0.81	0.005**	0.80	0.005**
VO <sub>2</sub> (ml/min)	0.87	0.005**	0.80	0.005**	0.86	0.005**
RMR (kcal/day)	0.87	0.005**	0.80	0.005**	0.85	0.005**
RQ	-0.15	0.496	0.50	0.018*	0.72	0.005**
CHO ox (kcal/min)	0.06	0.766	0.56	0.009**	0.71	0.005**
FAT ox (kcal/min)	0.17	0.480	0.57	0.009**	0.74	0.005**

Abbreviations: DT, Deltatrac; T1, test day 1; T2, test day 2; RMR, resting metabolic rate; RQ, respiratory quotient; CHO ox, carbohydrate oxidation; FAT ox, fat oxidation;  $r$  = Pearson's correlation value.

Statistically significant \* $p < 0.05$  and \*\* $p < 0.005$  (2-tailed).

Bland Altman analysis of RMR repeated measures estimated agreement within the GEM of 2 ( $\pm$  168) kcal/day [LOA -332 and 337 kcal/day] and within the DT of 31 ( $\pm$  116) kcal/day [LOA -203 and 265 kcal/day]. The greatest bias occurred within the ECAL with a mean difference of 114 ( $\pm$  259) kcal/day and wide LOA [LOA -631 and 404 kcal/d] (Table 10).

**Table 10.** Bland Altman analysis of RMR repeated measured on each machine in all participants (n=20) on T1 and T2 .

	GEM			DT			ECAL		
	(T1, T2)			(T1, T2)			(T1, T2)		
	MD	SD	LOA	MD	SD	LOA	MD	SD	LOA
VCO <sub>2</sub> (ml/min)	-1.2	21.2	-43.5 41.1	1.2	16.9	-32.6 35.0	-9.0	36.6	-82.1 64.1
VO <sub>2</sub> (ml/min)	0.8	24.3	-47.8 49.5	5.3	16.8	-28.2 38.8	-17.7	36.5	-90.7 55.3
RMR(kcal/day)	2.4	167.5	-332.5 337.4	31.1	116.9	-202.6 264.9	-113.6	258.8	-631.3 404.1
RQ	-0.01	0.05	-0.10 0.08	-0.01	0.05	-0.12 0.09	0.02	0.08	-0.14 0.19
CHOox(kcal/min)	-0.03	0.17	-0.38 0.32	-0.04	0.19	-0.41 0.33	0.05	0.34	-0.62 0.72
FATox(kcal/min)	0.03	0.19	-0.34 0.41	0.06	0.17	-0.29 0.41	-0.13	0.31	-0.74 0.49

Abbreviations: DT, Deltatrac. T1, test day 1. T2, test day 2. MD, mean difference. SD, standard deviation. LOA, limits of agreement. RMR, resting metabolic rate. RQ, respiratory quotient. CHO ox, carbohydrate oxidation. FAT ox, fat oxidation.

The CV was calculated as 4 ( $\pm$  5.3) % on the DT, 4.9 ( $\pm$  4.5) % on the GEM and 11.2 ( $\pm$  12.1) % on the ECAL. Differences within individuals' repeated RMR measures were averaged to give a mean within individual difference of 5.4% on the DT, 6.9% on the GEM and 13.1% on the ECAL.

## **3.5 Materials and methods (Study 2)**

### **3.5.1 Study design**

Adolescents recruited to the school breakfast study (chapter 6) attended a pre-study screening session during which RMR measures were collected on one morning. In the preceding 24 hours to screening, unusual vigorous exercise, alcohol, nicotine and caffeine were avoided and after 21:00 hours only water was consumed.

### **3.5.2 Subjects**

Recruitment of participants to the study and characteristics are described in detail elsewhere (chapter 7.3). Written parental consent and informed consent was obtained prior to screening sessions and a health questionnaire was completed to confirm that all participants were in good health. Thirty adolescents (16 girls and 14 boys; 13-15 years) were recruited and all were eligible to take part. Study procedures were approved by the Ethical Advisory Committee at OBU according to the guidelines laid down in the Declaration of Helsinki.

### **3.5.3 Indirect calorimeter**

Measures were collected using the ECAL indirect calorimeter (Energy Testing Solutions Limited, UK). Three machines controlled by three trained researchers were used to facilitate efficient testing. Calibrations were performed in accordance with the ECAL manual (ECAL, 2013) as described previously (chapter 3.3.3).

### **3.5.4 Study protocol**

On arrival at school anthropometric measures were collected. Weight and body fat composition were measured using the Tanita BC-418 MA (Tanita UK Limited, Yiewsley, UK). Height was measured to the nearest centimetre using a stadiometer (Seca Ltd, Birmingham, UK). BMI ( $\text{kg}/\text{m}^2$ )

was calculated and converted to *z*-scores using online software (Pan & Cole, 2012) based on UK reference data (Cole, Freeman, & Preece, 1995).

The ECAL manual (ECAL, 2013) recommends measures are collected for ten consecutive minutes with the participant in a semi-reclined position, but in this study participants sat instead in a relaxed position on upright chairs. Before testing, RMR collection measures were explained and participants practised 'normal' breathing to familiarise them with breathing through their mouth into the mouthpiece. Participants rested for five minutes, and once comfortable and making minimal movements, a sterilised mouthpiece and single-use nose clip were provided and connected to the ECAL. Participants sat away from other students whilst measures were taken and were reminded to minimise movements during data collection. During testing, the mouthpiece could be removed momentarily if they needed to swallow but was to be replaced as soon as possible. Room temperature was not controlled but was measured to be within 22-24°C and participants confirmed that body temperature was comfortable before measures were taken.

### **3.5.5 Determining EE**

RMR was calculated using the Lusk equation (Lusk, 1928), from  $VO_2$  and  $VCO_2$  data recorded by the ECAL. Measurements were collected for ten consecutive minutes. The first two minutes of data were discarded to allow for acclimatisation and five minutes of steady-state data with a CV of <20% were identified and used for analyses as previously described (chapter 3.3.5).

### **3.5.6 Prediction equations**

The Schofield-HW (Schofield, 1985) and Oxford (Henry, 2005) equations were selected (Table 11) to compare with measured RMR values from the ECAL, based on current recommendations from EFSA (EFSA, 2013).

**Table 11.** Prediction equations derived for use in children and adolescents

	Year	Gender	<i>n</i>	Age	RMR prediction equation (kcal/day)
Schofield-HW <sup>a</sup>	1985	male	734	10-18y	[16.25 * wt] + [137.2 * ht] + 515.5
		female	575	10-18y	[8.365 * wt] + [465 * ht] + 200
Oxford Equations <sup>b</sup>	2005	male	863	10-18y	[15.6 * wt] + [266 * ht] + 299
		female	1063	10-18y	[9.40 * wt] + [249 * ht] + 462

Abbreviations: wt, weight (kg); ht, height (m)

<sup>a</sup> (Schofield, 1985), <sup>b</sup> (Henry, 2005).

### 3.5.7 Statistical analyses

Comparisons between predicted and measured RMR were assessed using paired samples *t*-tests. Significance was set at  $p < .05$ . The strength of relations was determined using Pearson's correlation coefficient and Bland Altman methods were used to determine agreement between prediction equations and ECAL (Bland & Altman, 1986). The 95% LOA were calculated as the mean difference  $\pm 2$  SD of the differences, within which 95% of differences between measurements by the two methods are expected to lie. Accuracy of prediction equations were estimated as the percentage of subjects with predicted RMR within 10% of the measured RMR (Frankenfield et al., 2005). To determine how precise an equation was, 95% confidence intervals for the bias were calculated. Gender differences in baseline characteristics were assessed using Mann-Whitney U. Repeated measures analysis of covariance (ANCOVA) determined the interaction between methods, adjusting for gender, BMI and age. Post-hoc comparisons were performed using Bonferroni adjustments to account for multiple comparisons. Statistical analyses were performed using IBM SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, USA). Values reported are mean  $\pm$  SD unless otherwise indicated. Normality was assessed using the Shapiro-Wilk test.

## 3.6 Results (Study 2)

### 3.6.1 Measurement acceptability

Ten minutes of data were collected from all 30 participants. One participant's data were excluded as they had consumed breakfast.

### 3.6.2 ECAL and sample characteristics

Baseline characteristics were split by gender and significant differences were observed; girls were lighter and shorter than boys and had a higher body fat percentage, higher FM and a lower FFM ( $p < .05$ ) (Table 12). Several anthropometric measures were significantly associated with measured resting metabolic rate (MRMR). Weight and height form the basis of most prediction equations and these were both strongly associated with MRMR, but FFM was the most strongly associated with MRMR ( $p < .001$ ).

**Table 12.** Baseline characteristics of participants completing RMR measures and correlations ( $r$ ) of measures with RMR measured using indirect calorimetry (ECAL).

		All ( $n = 29$ )	$r$	Boys ( $n = 14$ )	Girls ( $n = 15$ )	Gender differences $p$ -value
	Unit	Mean $\pm$ s.d.		Mean $\pm$ s.d.	Mean $\pm$ s.d.	
Age	years	13.7 $\pm$ 0.5	0.03	13.7 $\pm$ 0.5	13.7 $\pm$ 0.5	0.847
Weight	kg	57.2 $\pm$ 11.0	0.58**	62.3 $\pm$ 13.4	52.4 $\pm$ 4.7	0.020*
Height	m	1.7 $\pm$ 0.1	0.67**	1.7 $\pm$ 0.1	1.6 $\pm$ 0.1	0.002**
BMI $z$ -score	$z$ score	0.7 $\pm$ 0.9	0.25	0.9 $\pm$ 1.0	0.5 $\pm$ 0.7	0.355
Bodyfat	%	24.0 $\pm$ 6.3	-0.46*	20.0 $\pm$ 6.0	27.6 $\pm$ 4.0	0.001**
FM	kg	13.8 $\pm$ 5.3	0.03	13.1 $\pm$ 7.0	14.6 $\pm$ 2.9	0.029*
FFM	kg	43.4 $\pm$ 8.2	0.76**	49.3 $\pm$ 7.75	37.8 $\pm$ 3.1	0.001**
MRMR	kcal/d	1999 $\pm$ 523	-	2413 $\pm$ 384	1613 $\pm$ 282	0.001**

Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat free mass; MRMR, measured RMR.

Statistically significant \*\*  $p < .005$ , \*  $p < .05$  (2-tailed)

### 3.6.3 Comparison of ECAL to prediction equations

Significant correlations were observed between MRMR and Schofield-HW equations and between MRMR and Oxford equations ( $p < .001$ ); however, there were significant differences between the

absolute values obtained ( $p < .001$ ) (Table 13). These differences remained when the sample were analysed by gender, but the findings suggest that both equations predicted RMR (PRMR) more accurately in girls (14-16% difference to MRMR) compared to the boys (27-29% difference to MRMR). When accuracy was investigated at an individual level, 21% of participants (17% girls, 4% boys) had PRMR values by Schofield-HW within 10% of MRMR values on the ECAL. For the Oxford equations as few as 10% of participants had PRMR values within 10% of MRMR, also with a higher proportion being girls. After adjusting the analysis for FFM, age and BMI  $z$  score, using a Bonferroni adjustment for multiple comparisons, significant differences in RMR measurement methods between males and females remained ( $F(1,24)=15.01$ ,  $p < .001$ , partial  $\eta^2 = .385$ ).

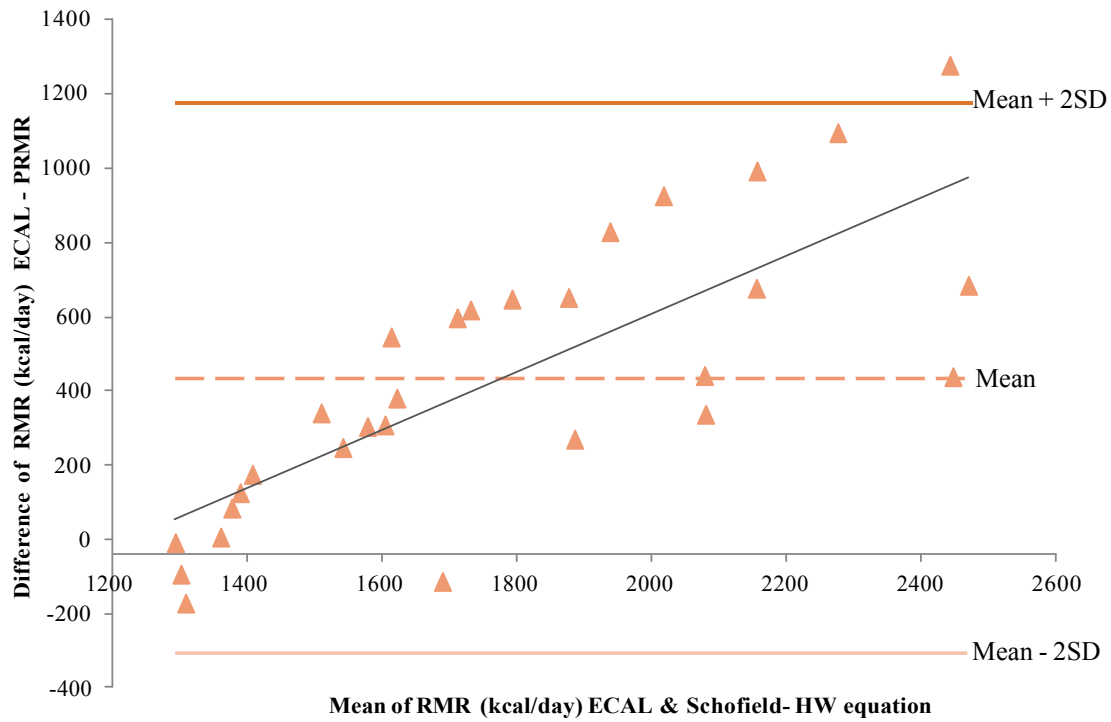
**Table 13.** Comparisons between predicted RMR measured with two equations and measured RMR using ECAL

	All subjects ( $n=29$ )	Boys ( $n=14$ )	Girls ( $n=15$ )
ECAL			
RMR (kcal/day)	1998.9 ± 523.2	2412.5 ± 384.2	1612.8 ± 282.4
Schofield-HW <sup>a</sup>			
RMR (kcal/day)	1564.0 ± 251.3***	1762.2 ± 226.0***	1379.1 ± 53.9**
Correlation ( $r$ )	0.76***	0.37	0.65**
% difference to ECAL	22	27	14
% within ± 10%	21	3	17
Oxford equations <sup>b</sup>			
RMR (kcal/day)	1531.5 ± 246.9***	1724.6 ± 225.4***	1351.2 ± 50.8***
Correlation ( $r$ )	0.75***	0.39	0.65**
% difference to ECAL	23	29	16
% within ± 10%	10	3	7

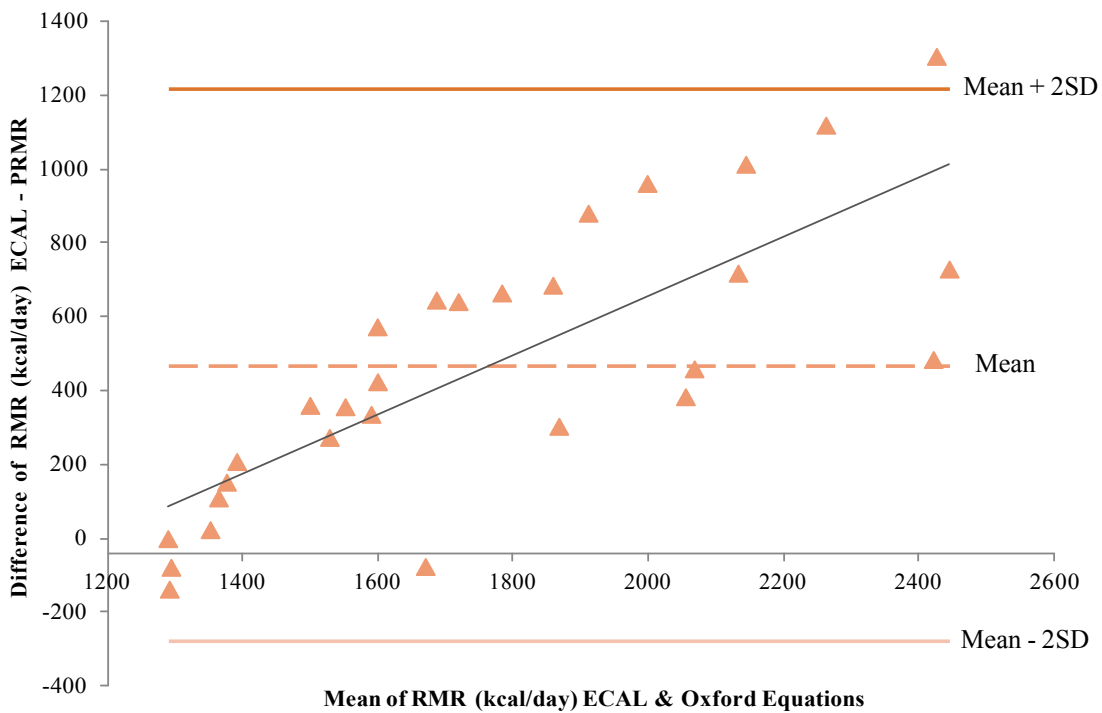
Abbreviations: RMR: resting metabolic rate. \*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$

<sup>a</sup> (Schofield, 1985), <sup>b</sup> (Henry, 2005)

Bland Altman analysis confirmed a consistent bias towards underestimation of the prediction equations with a mean difference of 434 (± 372) kcal/day for Schofield-HW (Figure 10) and 467 (± 374) for Henry (Figure 11).



**Figure 10.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the ECAL and predicted by Schofield-HW equations



**Figure 11.** Bland-Altman plot of mean difference in resting metabolic rate (RMR) measures collected on the ECAL and predicted by Oxford equations



Limits of agreement were wide (Table 14) and a proportional bias was observed such that there were greater differences between measures at higher RMR, suggesting that RMR may be underestimated more strongly by the prediction equations in these individuals.

**Table 14.** Agreement between measured RMR (ECAL indirect calorimeter) and predicted RMR in adolescents ( $n=29$ )

	Mean diff (MRMR-PRMR)	SD	LOA (mean diff $\pm$ 2SD)	95% confidence interval (for the bias)
	kcal/day	kcal/day	kcal/day	kcal/day
Schofield-HW <sup>a</sup> equation	435**	372	1179 and -309	293 to 576
Oxford <sup>b</sup> equation	467**	374	1215 and -281	325 to 610

Abbreviations: MRMR, measured RMR; PRMR, predicted RMR; SD, standard deviation

<sup>a</sup>(Schofield, 1985); <sup>b</sup>(Henry, 2005).

To consider the effect of outliers the analyses were repeated with the CV reduced from 20% to 15% which removed 5 participants from the analysis. This was repeated with CV reduced to 10% based on a 4-minute steady state which excluded 6 participants, however, on both occasions, differences between MRMR and PRMR were still significant ( $p<.005$ ) (data not shown).

## 3.7 Discussion

### 3.7.1 Comparison of accuracy of RMR measures; GEM and ECAL vs. DT (Study 1, part a)

The GEM and the ECAL were not accurate alternatives to the DT for measures of RMR to within the 20% limit hypothesised. Measures were consistently higher on the GEM and higher and more variable on the ECAL.

Due to a lack of published data on the GEM and the novel aspect of the ECAL it was not possible to make direct comparisons with previous research; however, comparable indirect calorimeters have been validated against the DT and it is these which are used here for comparison. Three studies compared the **QuarkRMR**<sup>®</sup> (Cosmed<sup>®</sup>, Rome, Italy), a hooded indirect calorimeter similar to the GEM, with the Deltatrac, reporting mixed conclusions (Blond et al., 2011; Graf et al., 2013; Sundstrom et al., 2013). All observed similar differences between mean RMR measures ranging from 24 to 39 ( $\pm 110$ ) kcal/day; however, the Quark was rejected as an accurate alternative by two studies (Graf et al., 2013; Sundstrom et al., 2013) based on high LOA (LOA -196 and 244 kcal/d and -325 to 430 kcal/day respectively). In contrast, one study (Blond et al., 2011) found the Quark's LOA were within those observed on the DT and therefore accepted it as a valid alternative. Two of the aforementioned studies (Graf et al., 2013; Sundstrom et al., 2013) compared another hooded indirect calorimeter, the **CCMExpress**<sup>®</sup> (Medgraphics<sup>®</sup>, Minneapolis, USA) to the DT and again found no agreement between methods. Similar to the GEM, the CCMExpress<sup>®</sup> was found to overestimate compared to the DT with unusually large differences on the CCMExpress<sup>®</sup> of more than 1000 kcal/day reported, potentially due to measurement error (Sundstrom et al., 2013). In the second study smaller differences than the present study were observed (111 ( $\pm 130$ ) kcal/day) but the authors rejected it as an accurate alternative based on high LOA [LOA -367 and 150 kcal/day] (Graf et al., 2013). Despite the GEM reporting higher than the DT by a significantly large amount,

the variation within measures on each test day was remarkably low (SD 12 on T1, SD 64 on T2) and LOA were within acceptable ranges (Blond et al., 2011), therefore, it would be pertinent for future studies to validate the accuracy of this machine.

Of three validation studies (Graf et al., 2013; Littlewood, 2002; Wahrlich et al., 2006) comparing portable calorimeters which, similar to the ECAL, can measure  $VO_2$  and  $VCO_2$ , only one found agreement with the DT (Wahrlich et al., 2006). The **Cosmed K4 b<sup>2</sup>**<sup>®</sup> **facemask** (Cosmed Srl, Rome, Italy) was rejected due to differences in RMR compared to the DT of 268 ( $\pm$  702) kcal/day, a similar magnitude to the ECAL in this study (Littlewood, 2002). The CCMExpress<sup>®</sup> face mask underestimated by a small non-significant amount, however, as with the ECAL, there was high variability and it was rejected based on wide LOA (5 ( $\pm$  201) kcal/day [LOA -397 and 407 kcal/day]) (Graf et al., 2013). The **VO2000**<sup>®</sup> **facemask** (Medgraphics, Minneapolis, USA) was the only machine accepted, based on measures within 5% of those on the DT (Wahrlich et al., 2006). The majority of comparison studies tend to use the **MedGem RMR**<sup>®</sup>; a portable calorimeter which uses a mouthpiece to measure  $VO_2$ , but they report mixed conclusions. In a review of eight portable calorimeter studies only two studies reported agreement to the DT (Frankenfield & Coleman, 2013), based on there being low, non-significant differences between the machines (St-Onge et al., 2004; Stewart et al., 2005). Conflicting results between studies may be, in part, down to differences in study design. Some validation studies do not include a repeated measure (Compher et al., 2005; Graf et al., 2013; St-Onge et al., 2004; Stewart et al., 2005) or use healthy participants in a non-fasted state (Graf et al., 2013). Comparisons made under non-standardised conditions make it even harder to determine if the variation is due to physiological factors or machine variation (Cooper et al., 2009). Additionally, some studies reject new methods without defining what an acceptable variation from the old method would be (Alam et al., 2005; Fields et al., 2006; Graf et al., 2013; Littlewood, 2002; Sundstrom et al., 2013). Setting limits for acceptable variation between indirect

calorimeters is subjective; nevertheless, studies should at least provide a rationale for the basis of their conclusions.

Expectations for machines to agree are confounded by the variation that exists not only biologically within individuals, but also between the function and design of the machines. The adaptability of the mouthpiece allows it to be used in a wide range of settings; however, increased levels of discomfort, changes in breathing patterns and the energy cost of being in an upright position are suggested as some of the reasons for the increased values commonly observed in mouthpiece measures (Frankenfield & Coleman, 2013; St-Onge et al., 2004; Stewart et al., 2005). In the current study using a facemask may have improved on some of these factors, but as the ECAL was designed to be used with a mouthpiece, the manufacturer's protocol was followed. The additional energy cost of a mouthpiece is around 70 kcal (Compher et al., 2006). This value is not consistent with the differences observed in the present study of 110 kcal (T1) and 254 kcal (T2) suggesting the differences were due to additional factors. A mouthpiece can introduce the potential for leakages if the individual does not form a complete seal around it, but the results gave no indication of this. Additionally, all participants successfully completed 30 minutes with relative ease.

### **3.7.2 Comparison of RMR measures to determine repeatability of machines (Study 1, part b)**

The machines showed good reliability for repeated RMR measures with the GEM and the DT CV within 5.0%, within the hypothesised value of 10%. The ECAL was higher at 11.2%, however, if one participant's data was excluded this came down to 8.9%.

When comparisons between indirect calorimeters and an established reference standard are made it is not possible to be certain which machine is reporting accurately; therefore, agreement of methods is measured with the assumption that the reference standard is accurate. Inaccessibility to new parts meant it was not possible to perform monthly methanol burns on the DT as a way to measure the

accuracy of readings. Furthermore, the DT is an old machine and poor consistency between RQ values on repeated measures were observed, implying the possibility that the DT may not be a reliable reference, a conclusion also suggested in another, recent study (Acheson, 2014). It may be possible to compare trends between machines as the magnitude of difference should in theory be similar even if the values themselves are not (as observed on the GEM in the current study).

A more consistent measure of a machine's validity is its reliability of repeated measures. Whether measuring individuals for research purposes or for health, it is the consistency of the machine that will enable changes over time to be measured. If the reliability of a machine cannot be proven this would suggest that agreement between methods is also likely to be poor (Bland & Altman, 1986). Therefore, for every validation study both accuracy and repeatability should be measured (and regularly checked), but studies that include both are limited. This is surprising as acceptable differences in repeated measures of RMR within the same individual are well established in the literature, with the DT variation reported to be within 5% (Shetty, Henry, Black, & Prentice, 1996; Ventham & Reilly, 1999). A systematic review concluded that day-to-day subject variation ranges from 3% to 5% over a 24-hour period, increasing to around 10% when measured over weeks or months (Compher et al., 2006). When the current study calculated the mean difference on an individual level it found that differences in RMR on the GEM were 6.9%, and 5.4% on the DT. This was slightly above the 5% observed in previous DT studies (Shetty et al., 1996; Ventham & Reilly, 1999) but within the 10% variation observed in studies of a similar length (Compher et al., 2006). When the individual data was analysed further it was found that 50% of measures on the GEM and 60% of measures on the DT were within 5%, increasing to 75% and 90% respectively being within 10%.

A study comparing intra-individual variation of a portable calorimeter, the **MOXUS Modular VO<sub>2</sub>**<sup>®</sup> (AEL Technologies, Naperville, IL, USA), to the DT (Roffey et al., 2006), concluded that

after five days of separate testing the CV of the MOXUS was  $7.3 (\pm 2.3) \%$  versus  $5.3 (\pm 1.2) \%$  on the DT. The current study found the CV of the ECAL to be  $11.2 (\pm 12.1) \%$ ; however, if one participant was excluded this came down to  $8.9 (\pm 6.5) \%$ . When analyses of the individual differences on the ECAL were made it was found that 40% of participants had repeated RMR measures within 10%. Two other mouthpiece studies that looked at individual values reported 43% of measures coming within 5% (Frankenfield & Coleman, 2013) and 80% of measures coming within 10% (Compher et al., 2005).

Although the ECAL was designed for measuring RMR over a 10-minute period, in the present study data were collected from subjects for 30 minutes to ensure consistency of protocol. To investigate the effect this may have had on the results the first 10 minutes were analysed separately (data not shown); however, there was no indication of any differences from the findings reported using the 20-minute data. Towards the end of the study it emerged that RQ values on the ECAL had dropped below 0.70 on the final six sessions, which is below physiological normal (McClave & Snider, 1992). The ECAL was designed for health professionals assuming little technical expertise, so there is no facility to perform a methanol burn. Investigations by the researchers and manufacturers were unable to identify the cause at that time; therefore, all subjects were included in the final analysis. Results excluding these participants were analysed separately (data not shown) and as expected, RQ values were no longer significantly different to the DT and there was an improvement in repeatability measures with a lower CV of 8.1%.

A limitation of the current study was the uneven number of male and female participants, limiting the ability to perform gender analysis. Some intra-individual variation can potentially be reduced by taking simultaneous measures; however, this was not attempted in the current study as there have been conflicting results using this method (Littlewood, 2002).

### **3.7.3 Collection of RMR measures on the ECAL (Study 2, part a)**

All anthropometric measures with the exception of age, BMI and FM were significantly correlated with MRMR with the strongest associations between FFM and MRMR. RMR is most strongly related to the magnitude of FFM, which is more metabolically active than adipose tissue (Weinsier et al., 1992), primarily representing skeletal muscle and highly metabolic major organs (Buchholz, Rafii, & Pencharz, 2001).

Boys had a significantly higher RMR than girls regardless of which method was used (ECAL or prediction equations), which may be due to differences in metabolically active tissues between genders; boys gain more lean tissue, especially skeletal muscle, compared to girls, who gain more adipose tissue (van Mil, Westerterp, Kester, & Saris, 2001). During puberty relative changes in FM and FFM could influence RMR; however, there are few studies, and when adjusted for body composition, stage of puberty was not a significant determinant of RMR (Bitar, Fellmann, Vernet, Coudert, & Vermorel, 1999; Molnár & Schutz, 1997). Differences in RMR may also relate to the phase of the menstrual cycle (Buchholz et al., 2001). In the current study menstrual phase data were collected, but this was not analysed due to the small number of females.

#### **3.7.4 Comparison of ECAL to prediction equations (Study 2, part b)**

Agreement between MRMR and PRMR values were poor, with between 10-21% of the predicted values being within 10% of measured values. Compared to MRMR both prediction equations systematically underestimated RMR, with stark differences at higher RMR and greater differences between PRMR and MRMR observed in boys compared to girls.

A larger study ( $n=116$ ) comparing PRMR to MRMR on a hooded calorimeter in young people (7-16 years) reported good agreement with prediction equations, including Schofield-HW, also used in the current study (Rodriguez, Moreno, Sarría, Fleta, & Bueno, 2002). The authors found the Schofield-HW equation to predict particularly well in girls and non-obese individuals and this may,

in part, help explain findings of greater accuracy in girls in the current study. Absolute differences between PRMR and MRMR were  $<4$  kcal/d, much lower than the current study ( $>400$  kcal/day) with narrower LOA [-293 and 300 kcal/day]. Based on previous comparisons between indirect calorimeters (chapter 2.4.2) greater accuracy could be expected from a hooded calorimeter, which strengthens the use of the Schofield-HW equations in adolescent populations. Additionally, Rodriguez and colleagues recruited a large sample size which would be expected to narrow LOA (Giavarina, 2015). A review collating information from 90 adolescents (12-15 years) reported that, similar to the current study, PRMR under-estimated compared to MRMR using a ventilated hood in non-obese boys but not girls (van Mil et al., 2001). Differences were still lower than the current study, ranging between 55-248 kcal/day; however, Bland Altman analysis was not performed therefore no LOA were reported. Having wide LOA was not unexpected in the current study as there are increased energy costs associated with sitting upright (Frankenfield & Coleman, 2013; St-Onge et al., 2004; Stewart et al., 2005); additionally, using three different devices may have introduced some inter-device variation.

A study comparing adult prediction equations to the Medgem, a handheld portable calorimeter potentially more representative of the ECAL, also found poor agreement between methods, with 41-46% of overweight female adults ( $n=39$ ) having predicted RMR values within 10% of measured values (Spears, Kim, Behall, & Conway, 2009). As in the current study a proportional bias was observed such that PRMR was underestimated in individuals with a higher RMR. The same relationship has been reported in a much larger study ( $n=1307$ ) where the accuracy of the WHO equation was compared with a hood, a mouthpiece and a metabolic chamber (Müller & Bosy-Westphal, 2003).

It is well established that applying prediction equations to individuals can produce large errors as they are not able to accurately predict extremely high and low values (Frankenfield, 2013). Higher



metabolic demands of overweight and obese individuals led to the development of equations specifically for use in the overweight and obese. When a selection of these equations were compared in obese adolescents ( $n=80$ , 7-18 years) to the **Korr** portable calorimeter they also revealed wide variations between PRMR and MRMR and significant differences were observed between the Korr data and all but one equation (Henes et al., 2013). However, accuracy to within 10% of MRMR was still higher than the current study, ranging from between 40-65%, although again, agreement between methods was not assessed. A smaller study in overweight and obese adolescents ( $n=19$ , 17-19 years) used a portable device and a hood (Henes et al., 2015). There were no significant differences between calorimeters and there was good agreement with prediction equations. Using a portable calorimeter requires that the machine itself demonstrate an acceptable level of accuracy, and including a measure of RMR from a hood strengthens the assumption of accuracy; however, as demonstrated by Henes and colleagues (2015), this may compromise sample size.

When analysed by gender there were significant differences in the accuracy of prediction equations, with larger underestimations in boys compared to girls, independent of age, FFM or BMI. A limitation of prediction equations is their inability to account for inherent gender differences in their estimation of RMR (Rodriguez et al., 2002) and this contributes to better predictions in boys or girls, as observed in the current study. In a study with obese adolescents significant gender differences were also observed; however, in contrast to the current study the prediction equations significantly overestimated RMR in boys compared to girls (Henes et al., 2013), which may be a reflection of the differences between prediction equations used.

Indirect calorimetry is not without limitations, particularly if measures are not collected with the participant in a true rested state.  $\text{CO}_2$  exchange is less constant than  $\text{O}_2$  exchange and can be altered by deep breathing, in which case, the amount of  $\text{CO}_2$  released in the lungs may not be a true

reflection of what is being produced in the tissues (Wilmore, Costill, & Kennedy, 2007). Despite a consistent protocol and supportive researchers there was a trend in the data for greater differences between MRMR and PRMR as levels of CO<sub>2</sub> increased (data not shown), suggesting that some adolescents found it hard to adapt to breathing normally through the mouthpiece. Furthermore, Bland Altman analysis identified differences in predicted energy needs that could result in clinically relevant under- or over-nutrition if applied in the long term, highlighting the need for caution in the application of prediction equations to individuals.

### **3.8 Conclusions**

Measuring and monitoring RMR can make a fundamental contribution to understanding the metabolic adaptations which occur within and between individuals (chapter 1.6.1). Developing and validating novel methods to objectively measure RMR is challenging but essential, and would reduce the need for reliance on prediction equations, which can be unreliable (Acheson, 2014). The current study could not recommend the ECAL or the GEM as accurate alternatives to the DT; however, it cannot be discounted that the DT itself may not have been a reliable comparison. Including a measure of repeatability was a strength of the current study and showed the reliability of the machines to perform repeated measures. This should be considered in all validation studies as there is a greater consensus on intra-individual variation within the literature.

Portable indirect calorimeters attempt to bridge the gap between prediction equations and objective measures of RMR, and the ECAL was acceptable to adolescents and practical to use in a school setting. Further validation research of the ECAL is warranted in a larger sample size, or where an additional measure of accuracy may be introduced. Accuracy is an important feature of portable indirect calorimeters if they are to be used to investigate the postprandial effects of food. Identifying the fuel source from foods consumed which is being utilised by the body, particularly during cognitively demanding tasks, could provide additional insight into the mechanisms related to the

effects of food on cognition. However, due to the large variation observed in the current study the ECAL was identified as reliable for collecting measures of RMR only.

The accuracy of the recommended prediction equations were poor compared to the ECAL, particularly at higher RMRs, highlighting the challenges faced with estimating RMR at an individual level.

## Chapter 4: Exploring gender differences in the theory of planned behaviour applied to adolescent breakfast consumption

### 4.1 Summary

Breakfast skipping increases during adolescence and is associated with lower levels of PA and weight gain. Theory-based interventions promoting regular breakfast consumption in adolescents are limited and do not consider gender differences between theory components. This study aimed to:

- (i) Utilise the Theory of Planned Behaviour (TPB) to investigate breakfast frequency in adolescents and identify the relative importance of the TPB constructs in the prediction of breakfast consumption for boys and girls.
- (ii) Identify a RTEC regularly consumed by adolescents to inform the development of the breakfasts (chapter 5.5.5) and the breakfast intervention studies (chapters 6 and 7).

Questionnaires were completed by 434 students (mean  $14 \pm 0.9$  years) measuring breakfast consumption (0-2, 3-6 or 7 days), PA levels and TPB measures towards breakfast. Data were analysed by breakfast frequency and gender using regression analyses. Breakfast was consumed every day by 57% of students with boys more likely to eat a regular breakfast, report higher PA levels and more positive attitudes towards breakfast than girls ( $p < .001$ ). The TPB predicted 59% of the variation in intentions, but TPB variables were more successful at predicting intentions in girls, than in boys. For the whole sample, the model was predictive of breakfast frequency explaining 62% of the variation, but again, behaviour was predicted better in girls than in boys ( $p < .001$ ). Findings confirm that the TPB is a successful model for predicting intention to eat breakfast and breakfast behaviours in adolescents; however, efforts to promote breakfast consumption should consider differences in TPB constructs between boys and girls in the design and implementation of interventions.

## 4.2 Introduction

Participation in healthy behaviours, including being physically active <sup>(Public-Health-England, 2013)</sup>, and eating a regular breakfast (Siega-Riz et al., 1998), decreases during adolescence, as does the quality of breakfast consumed (Hallstrom et al., 2012) (chapter 1.4). There appears to be a greater tendency for children from ethnic backgrounds or low-income families to skip breakfast (Mullan & Singh, 2010), as well as differences by gender, with skipping prevalence consistently higher in adolescent girls compared to boys (Timlin et al., 2008) (chapter 1.4.2). Adolescence is an important transitional period during which attitudes towards food choices are formed (Conner et al., 2002) and can potentially persist into adulthood (Viner et al., 2012) (chapter 1.3). Regular breakfast consumption in adolescents has been positively associated with improvements in diet quality (Rampersaud, Pereira, Girard, Adams, & Metz, 2005) and PA levels (Berkey et al., 2003) as well as a reduction in the risk of obesity (Timlin et al., 2008). This emphasises the importance of breakfast, and adolescent behaviour, as key targets for health interventions.

Theory-based interventions have been shown to be more effective than interventions without a theory component (Michie, Abraham, Whittington, McAteer, & Gupta, 2009). Applying theories can help to identify causal determinants of behaviours which can then be targeted in interventions. One of the dominant theories in health behaviour is the TPB (Ajzen, 2012). The theory proposes that intention is the most important precursor to perform (or not perform) a behaviour and there are numerous large meta-analyses supporting its use (Armitage, 2001; McEachan, Conner, Taylor, & Lawton, 2011) around healthy eating (Chan & Tsang, 2011; Fila & Smith, 2006; Grønhøj, Bech-Larsen, Chan, & Tsang, 2013), PA (Duncan, Ravis, & Jordan, 2012) and breakfast consumption (Conner, Hugh-Jones, & Berg, 2011; DeJong, van Lenthe, van der Horst, & Oenema, 2009; Kothe, Mullan, & Amaratunga, 2011; Mullan, Wong, & Kothe, 2013; Mullan, Wong, Kothe, & MacCann, 2013; Ravis, Sheeran, & Armitage, 2006; Wong & Mullan, 2009).

The TPB has been successfully applied in children and adolescents; explaining between 50-60% of the variance in diet-related intentions, and 6-19% of the variance in behaviours (Riebl et al., 2015). Attitudes were most strongly associated with intention to perform a diet-related behaviour, whilst intention was most strongly associated with behaviour, (Riebl et al., 2015) consistent with a previous meta-analysis including adolescents (McEachan et al., 2011). Only five studies were specific to breakfast (Berg, Jonsson, & Conner, 2000; Conner et al., 2011; Gummesson, Jonsson, & Conner, 1997; Hewitt & Stephens, 2007; Mullan, B. et al., 2013) where two found attitudes most strongly predicted intention to consume healthy items at breakfast (Berg et al., 2000; Gummesson et al., 1997). Intention to consume breakfast, measured in only one study (Mullan, B. et al., 2013), was most strongly predicted by PBC, followed by attitudes. In line with TPB assumptions, intentions most strongly predicted all breakfast behaviours, followed by PBC; however, attitudes also strongly correlated with breakfast behaviours (Riebl et al., 2015).

To explain a greater proportion of the variation in breakfast intentions and behaviours, studies are increasingly interested in the individual components of TPB constructs, such as attitudes and SNs, to directly predict behaviour (Conner et al., 2011; Martens, van Assema, & Brug, 2005; Moore, Moore, & Murphy, 2009), and the potential effects of gender, age and socioeconomic status (SES) (Berg et al., 2000; Conner et al., 2011). Conner and colleagues (2011) reported that intention to consume healthy items for breakfast in adolescents was most strongly predicted by descriptive norms and affective attitudes, whilst descriptive norms also directly predicted healthy eating behaviours (Conner et al., 2011). Considering breakfast consumption frequency in adolescents, attitudes were the strongest predictor over and above all other TPB constructs (Martens et al., 2005); however, to date, there are no studies investigating how the individual components of attitudes are associated with breakfast consumption frequency in adolescents. Gender differences are rarely considered (Riebl et al., 2015) and both studies reported no gender differences in

intentions or behaviours associated with eating a healthy breakfast (Berg et al., 2000; Conner et al., 2011).

Attitudes can consist of three underlying components: affective (feelings towards the behaviour), behavioural (action tendencies with respect to the behaviour) and cognitive attitudes (beliefs about the behaviour) (Ajzen, 2005). Typically, the more favourable the attitudes and SN, and the greater the PBC, the stronger the intention to perform the behaviour (Ajzen, 2006). Understanding the nature of attitudes suggests an appropriate target in adolescent interventions to increase the frequency of breakfast consumption, but there are few TPB breakfast interventions in young people reporting mixed findings, and key attitude components are not specified (Gharlipour et al., 2015; Hosseini, Aghamolaei, Gharlipour Gharghani, & Ghanbarnejad, 2015; Kothe et al., 2011). In first year university students (mean age 19.9 years) an intervention using implementation intentions to increase attitudes and PBC towards breakfast consumption was measured using a standard TPB questionnaire; however, there were no changes in TPB scores or breakfast behaviours at follow up (Kothe et al., 2011). In a school-based educational intervention targeting all TPB variables there were significant improvements in adolescents' ( $n=97$ , mean  $12.5 \pm 0.5$  years) TPB scores (except SN) in the control and intervention groups, but no significant increase in breakfast consumption was reported (Gharlipour et al., 2015). In contrast, a smaller educational intervention in adolescents ( $n=88$ , mean 13.8 years) (standard deviation not reported) reported significant increases in knowledge and TPB scores, concurrent with significant increases in breakfast consumption in the intervention group (Hosseini et al., 2015).

Scales to specifically measure the individual components of attitudes towards breakfast (affective, cognitive and behavioural) were developed to evaluate the effectiveness of a free school breakfast initiative in children (Tapper et al., 2008), where children who held more positive attitudes reported being less likely to skip breakfast, but their use has not yet been reported in adolescents.

Understanding the nature of attitudes and how differences in gender might be represented could help inform future interventions to increase the frequency of breakfast consumption.

#### **4.2.1 Aims and hypothesis**

The current study had four aims:

- (i) To utilise the TPB to identify the relative contribution of TPB constructs, particularly the components of attitudes, in the prediction of intention to eat breakfast and breakfast consumption frequency in adolescents.
- (ii) To determine whether demographic factors, particularly gender, affects the relationship between TPB variables, intention and behaviour.
- (iii) To identify a commonly consumed breakfast in adolescents to inform the development of breakfasts (chapter 5) and breakfast interventions (chapters 6 and 7)
- (iv) To establish relationships with local schools and teachers and recruit schools 'in principal' to the final school study (chapter 7).

#### Hypothesis:

It was hypothesised that adolescents completing a questionnaire would report infrequently consuming breakfast, and this would be more evident in girls than in boys, and in those holding negative attitudes towards breakfast. Furthermore, it was hypothesised that RTECs would be the most popular breakfast choice in adolescents.

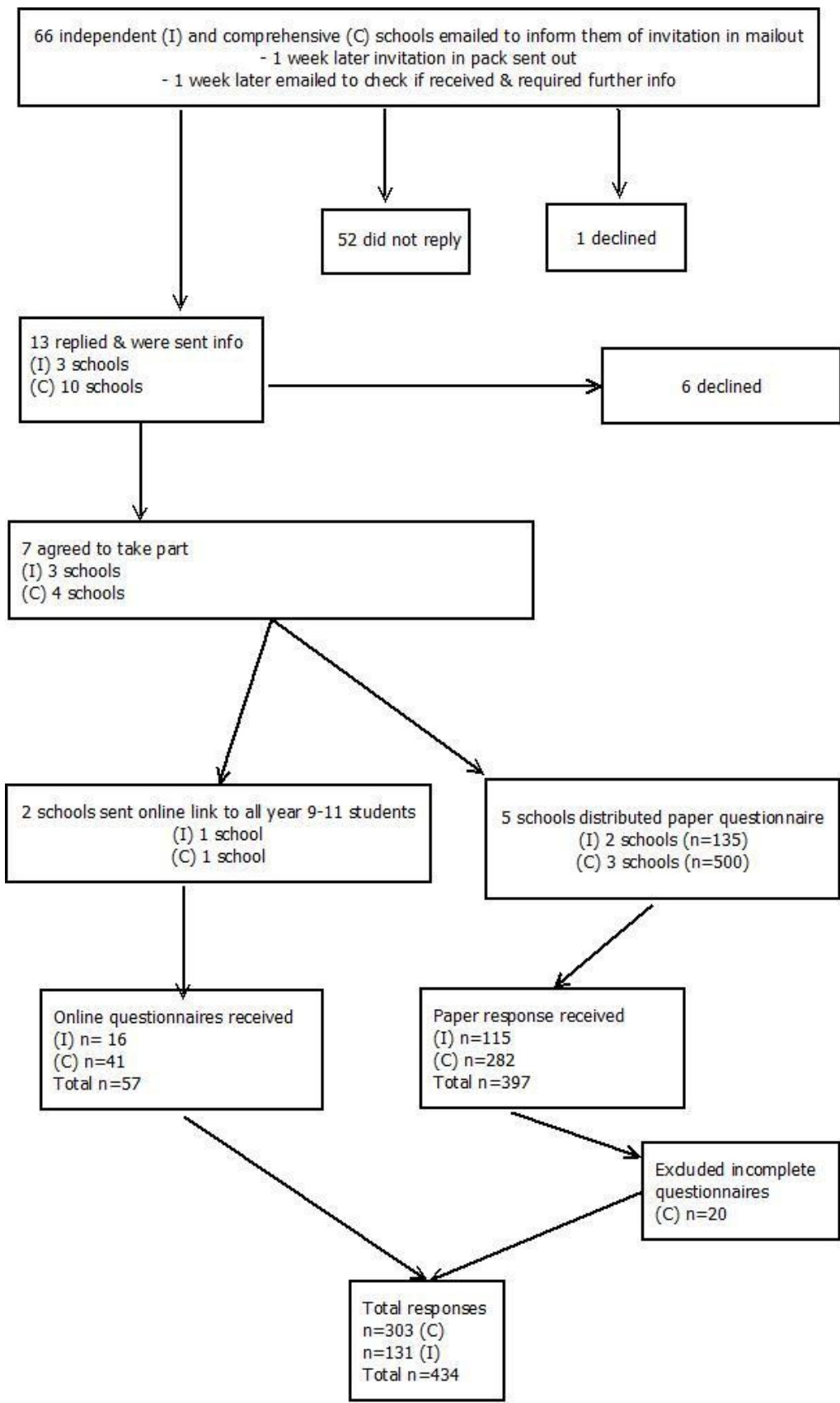


## 4.3 Methods

### 4.3.1 Participants and recruitment

All 66 secondary schools in Oxfordshire were invited by post and email to participate. Thirteen schools expressed interest and received detailed study information (Appendix 2a-2e). Six schools opted out due to time constraints, so questionnaires (Appendix 2f) were distributed to seven schools (four comprehensive, three independent). Students aged 13-17 years were eligible (encompassing mid-adolescence to completion of secondary school); participation was voluntary and anonymous, and parents were given the opportunity to opt their child out of the study if desired. Study procedures were approved by the Ethical Committee at OBU. Paper questionnaires ( $n=635$ ) were distributed to students via teachers, of which 397 were returned (Figure 12). Two schools opted to distribute the online link from which 57 responses were received. Questionnaires missing gender were excluded, along with obviously fictional responses, leaving a total of 434 completed questionnaires.

Figure 12. Flow of participants through questionnaire recruitment



### 4.3.2 Design and measures

Measures were based on previously developed and validated questionnaires (Ajzen, 2006; Kowalski, Crocker, & Kowalski, 1997; Tapper et al., 2008), and authors' permissions were obtained prior to use. SES was assessed by the highest level of academic achievement of either parent. Height and weight were self-reported. BMI ( $\text{kg/m}^2$ ) was calculated and converted to  $z$ -scores using online software (Pan & Cole, 2012) based on UK reference data (Cole et al., 1995). Breakfast was defined as the first meal before morning break during the week; or at the weekend, as the first meal before 11am. Response categories were selected based on a previously used questionnaire (Timlin et al., 2008) and recoded for analysis into 'infrequent' (0-2 days), 'frequent' (3-6 days) and 'daily' (7 days) breakfast eaters, representing similar cut points used previously to categorise the risk of developing metabolic conditions (Odegaard et al., 2013). PA levels were assessed by seven-day recall using the PA questionnaire for adolescents (PAQ-A) (Kowalski et al., 1997) which has shown satisfactory reliability and validity in this age group and correlates well with objective measures of PA (Kowalski et al., 1997).

TPB questions were developed in accordance with TPB guidelines (Ajzen, 2006) and items scored using a five-point Likert scale: *Attitudes* were assessed by agreement with 12 questions, e.g. 'eating breakfast is boring' (strongly disagree-strongly agree), based on a previously developed scale showing acceptable validity and reliability in 9-11 year olds (Tapper et al., 2008). The scale was modified for use with adolescents and piloted with students ( $n=20$ ) from a non-participating school. Following feedback, three questions with potentially ambiguous wording were modified. The new scale was checked using Cronbach's alpha ( $\alpha$ ) which resulted in the subsequent exclusion of two items pertaining to behaviours ('I usually eat a healthy breakfast' and 'teenagers do not need to be concerned about their eating habits'). The new 12-item scale showed high internal consistency ( $\alpha=.88$ ). A principal-components analysis (PCA) was performed and inspection of the correlation

matrix showed that all variables had at least one correlation coefficient greater than 0.3. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.92 with individual KMO measures all greater than 0.7 which represents classifications of 'middling' to 'meritorious' according to Kaiser (1974). Barlett's Test of Sphericity was statistically significant ( $p < 0.0001$ ), indicating that the data were likely factorisable. PCA revealed two components that had Eigenvalues greater than one and which explained 52.3% and 9.7% of the total variance, respectively. Visual inspection of the scree plot (Figure 13) indicated that three components should be retained (Cattell, 1966). In addition, a three-component solution met the interpretability criteria; as such three components were retained. The three-component solution explained 69.1% of the total variance. A varimax orthogonal rotation was employed to aid interpretability. The rotated solution exhibited 'simple structure' (Thurstone, 1947). The interpretation of the data was consistent with the key attitudes the scale was designed to measure, with strong loadings of affective attitudes on component 1, behavioural attitudes on component 2 and cognitive attitudes on component 3 (Table 15). Where items loaded on more than one factor, attitude groupings were decided based on comparisons with previously validated research (Tapper et al., 2008).

*SN* were assessed by agreement with 4 questions, e.g. 'people who are important to me think I should eat breakfast regularly' (strongly disagree-strongly agree) ( $\alpha = .84$ ). *PBC* was assessed by agreement with 2 questions, e.g. 'for me eating breakfast regularly would be' (very easy-very difficult) ( $\alpha = .81$ ). *Intention* to eat breakfast was assessed using 1 item: 'over the next week, I intend to eat breakfast on the following days'. *Behaviour* was assessed using 1 item: 'during the past 7 days, on how many days did you eat breakfast?'

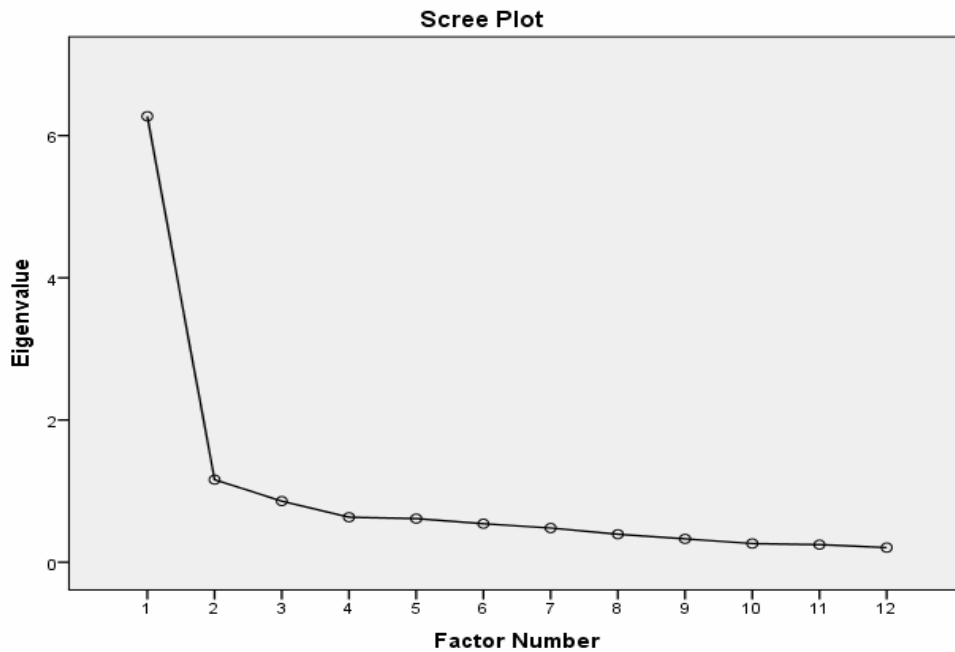


Figure 13. Scree plot of factors retained for PCA analysis

Table 15. Summary of factor analysis results for attitude scale. Major loadings for each item are in bold

Item	Rotated Factor Loadings		
	Affective Attitudes	Behavioural Attitudes	Cognitive Attitudes
I hate eating breakfast	<b>.67</b>	.28	.33
I'd rather have a snack at morning break than eat breakfast	<b>.66</b>	.40	.31
Eating breakfast is boring	<b>.61</b>	.17	.27
I hardly eat anything for breakfast	<b>.56</b>	<b>.46</b>	.37
I am too rushed in the morning to eat a healthy breakfast	.23	<b>.72</b>	.13
I am too rushed in the morning to eat breakfast	.18	<b>.69</b>	.20
I often miss breakfast	.43	<b>.61</b>	.39
I usually have a snack at morning break instead of breakfast	<b>.47</b>	<b>.49</b>	.21
I feel okay in the morning even if I haven't had breakfast	.38	.23	<b>.78</b>
I can concentrate in class even if I've missed breakfast	.24	.24	<b>.70</b>
It's okay to miss breakfast	<b>.46</b>	.28	<b>.48</b>
If I miss breakfast I feel more tired in the morning	.15	.10	<b>.48</b>
Eigenvalues	6.27	1.16	0.86
% of variance	52.26	9.68	7.17
Cronbach's $\alpha$	.85	.87	.80

Extraction methods: Principal axis factoring. Rotation Method: Varimax with Kaiser normalization.

### **4.3.3 Statistical analysis**

Data were analysed using IBM SPSS software V22. Spearman correlations, independent *t*-tests for continuous variables, and non-parametric tests (Mann Whitney, Kruskal Wallis) for ordinal variables were used to determine associations, or differences, in breakfast frequency, age, gender, BMI, SES, PA, ethnicity and school type. Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. PCA with Varimax rotation, and Kaiser normalisation were used to ensure the key attitude constructs were separate factors. Component scores representing the three attitude components of affective, behavioural and cognitive attitudes were retained for prediction analysis using multiple hierarchical regression analyses for intention to eat breakfast, and multinomial logistic regression for breakfast-eating frequency.

## 4.4 Results

In total 434 students were included in the analyses (263 girls and 171 boys, 13-17 years). Over half of the students (57%) consumed breakfast every day, whilst 22% ate breakfast on 0-2 days (Table 16). Boys were more likely to report eating breakfast every day ( $p < .001$ ) and were significantly older ( $p < .005$ ), heavier ( $p < .01$ ) and more physically active ( $p < .001$ ) than girls (small effect:  $r = .24$ ,  $r = .14$ ,  $r = .16$ ,  $r = .22$  respectively).

**Table 16.** Descriptive characteristics of sample as means ( $\pm$  SD) for BMI and age variables and percentages ( $n$ ) for all other variables †

	Total	Boys	Girls	MW or <i>t</i> -test <i>p</i> -value
Age (years)	14.0 (0.9)	14.1 (0.9)	13.9 (0.9)	.006* <sup>^</sup>
BMI ( <i>z</i> -score)	-0.31 (1.5)	-0.04 (1.4)	-0.53 (1.5)	.005* <sup>^</sup>
<i>Ethnicity</i>				.394
Arab/Asian/black	5.4% (23)	4.2% (7)	6.2% (16)	
Mixed/other	4.5% (19)	4.2% (7)	4.7% (12)	
White	90.1% (383)	91.6% (153)	89.1% (230)	
<i>SES</i>				.802
No formal education	1.2% (5)	1.8% (3)	0.8% (2)	
GCSE or equivalent	11.7% (50)	9.4% (16)	13.1% (34)	
A-level or university	54.5% (234)	58.2% (99)	52.1% (135)	
Don't know	32.6% (140)	30.6% (52)	34.0% (88)	
<i>PA levels</i>				< .001** <sup>^</sup>
Rarely active	32.2% (138)	23.7% (40)	37.8% (98)	
Moderately active	48.6% (208)	46.2% (78)	50.2% (130)	
Often active	17.8% (76)	27.2% (46)	11.6% (30)	
Very active	1.4% (6)	3.0% (5)	4.0% (1)	
<i>Breakfast</i>				< .001**
Breakfast: 0-2 days	22.4% (97)	11.7% (20)	29.3% (77)	
Breakfast: 3-6 days	20.7% (90)	17.5% (30)	22.8% (60)	
Breakfast: 7 days	56.9% (247)	70.8% (121)	47.9% (126)	

Abbreviations: BMI, body mass index; PA levels, physical activity levels (determined by PAQ-A questionnaire); SES, socio-economic status (determined by parental education). MW: Mann Whitney. <sup>^</sup> *p*-value independent *t*-test of scores (not categories); Significance \*\*  $p < .001$ , \*  $p < .05$  (2-tailed). † Sample *n* varies between questions (maximum  $n = 434$ ).

When analysed by breakfast frequency (Table 17) significant differences were observed between SES ( $H(3)=9.84, p=.020$ ), PA levels ( $F(2,425)=7.52, p<.001$ ) and school type ( $U=24,873, z=5.073, p<.001$ ). Pairwise analysis performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons (adjusted  $p$ -values are presented) revealed that median breakfast frequency score was significantly higher in students from the highest socio-economic group (median 3.0) compared to students reporting 'don't know' (median 2.0) to the question of parents' level of education ( $p=.028$ ). Furthermore, students from independent schools (median 3.0) were more likely to report eating breakfast everyday compared to students from comprehensive schools (median 2.0) ( $p<.001$ ). Students who ate breakfast every day were more active (mean PA score 1.98) than students who ate breakfast on 0-2 days (mean PA score 1.64) ( $p<.001$ ).



**Table 17.** Characteristics of participants ( $n=434$ ) stratified by frequency of breakfast consumption. Values are means ( $\pm$  SD) or percentages %

	Frequency of breakfast consumption			<i>p</i> value
	0-2 days	3-6 days	7 days	
	<i>n</i> = 97	<i>n</i> = 90	<i>n</i> = 247	
Age ( <i>yrs</i> )	13.9 (0.8)	14.0 (0.9)	14.0 (0.9)	0.925 <sup>^</sup>
BMI ( <i>z-score</i> )	-0.11 (1.6)	-0.14 (1.4)	-0.41 (1.5)	0.284 <sup>^</sup>
<i>Ethnicity</i>				0.117 <sup>†</sup>
Arab/asian/black	9.6%	5.7%	3.7%	
Mixed/other	5.3%	3.4%	4.5%	
White	85.1%	90.8%	91.8%	
<i>SES</i>				0.020 <sup>*</sup>
No formal education	3.1%	0.0%	0.8%	
GCSE or equivalent	13.5%	10.1%	11.4%	
A-level or University	40.6%	55.7%	59.6%	
Don't know	42.7%	34.1%	28.2%	
<i>PA levels</i>				< .001 <sup>**^</sup>
Rarely active	46.8%	36.4%	25.2%	
Moderately active	43.6%	42.0%	52.8%	
Often active	8.5%	19.3%	20.7%	
Very active	1.1%	2.3%	1.2%	
<i>School type</i>				< .001 <sup>**~</sup>
Independent	7.9%	18.1%	74.0%	
Comprehensive	28.3%	21.8%	49.8%	

Abbreviations: BMI, body mass index; PA Levels, physical activity levels (determined by PAQ-A questionnaire). SES, socioeconomic status (determined by parental education). †Kruskal Wallis test. ^ ANOVA test. ~Mann Whitney test. Significance \*\*  $p < .001$ , \*  $p < .05$  (2-tailed)

#### 4.4.1 Correlations

Significant positive correlations were found between breakfast consumption and all TPB variables (range  $r = .41$  to  $r = .78$ ;  $p < .001$ ). Intention was most strongly correlated with PBC, whereas breakfast consumption was most strongly correlated with behavioural attitudes, PBC and intention ( $r = > .7$ ;  $p < .001$ ).

#### 4.4.2 TPB measures

Boys and girls generally responded positively to eating breakfast with mean scores above the midpoint of the scale (Table 18, upper table), but boys' scores were significantly higher than girls' scores on all TPB measures: Aff\_Att ( $F(1,423)=17.243, p<.001, \text{partial } \eta^2=.039$ ); Beh\_Att ( $F(1,418)=28.301, p<.001, \text{partial } \eta^2=.063$ ); Cog\_Att ( $F(1,421)=9.643, p<.005, \text{partial } \eta^2=.022$ ); SN ( $F(1,431)=11.302, p<.001, \text{partial } \eta^2=.026$ ); PBC ( $F(1,430)=25.699, p<.001, \text{partial } \eta^2=.056$ ); Int ( $F(1,420)=17.373, p<.001, \text{partial } \eta^2=.040$ ). When split by breakfast frequency (Table 18, lower table) significant linear differences were observed such that eating breakfast more frequently was associated with having positive affective ( $F(2,422)=105.029, p<.001, \text{partial } \eta^2=.332$ ), behavioural ( $F(2,417)=242.097, p<.001, \text{partial } \eta^2=.537$ ) and cognitive attitudes ( $F(2,420)=106.732, p<.001, \text{partial } \eta^2=.337$ ) as well as greater SN ( $F(2,430)=106.276, p<.001, \text{partial } \eta^2=.331$ ), PBC ( $F(2,429)=343.255, p<.001, \text{partial } \eta^2=.615$ ) and intention to eat breakfast ( $F(2,419)=372.783, p<.001, \text{partial } \eta^2=.640$ ).

**Table 18.** Mean scores ( $\pm$  SD) for TPB variables by all sample and gender (upper table) and breakfast consumption frequency (lower table).

		Aff_Att	Beh_Att	Cog_Att	SN	PBC	Int
All	( $n = 425$ )	3.76 (1.1)	3.62 (1.2)	3.41 (1.1)	3.74 (0.8)	4.04 (1.3)	6.69 (2.2)
Boys	( $n = 168$ )	4.02 <sup>a</sup> (1.0)	3.98 <sup>a</sup> (1.0)	3.62 <sup>a</sup> (1.0)	3.90 <sup>a</sup> (0.7)	4.42 <sup>a</sup> (1.0)	7.23 <sup>a</sup> (1.8)
Girls	( $n = 257$ )	3.58 (1.2)	3.38 (1.2)	3.28 (1.1)	3.64 (0.8)	3.80 (1.4)	6.34 (2.4)
		Aff_Att	Beh_Att	Cog_Att	SN	PBC	Int
0-2 days	( $n = 96$ )	2.71 (0.9)	2.21 (0.8)	2.41 (0.9)	3.03 (0.7)	2.30 (1.0)	3.39 (2.0)
3-6 days	( $n = 90$ )	3.42 (1.0)	3.15 (1.0)	3.00 (1.0)	3.52 (0.7)	3.84 (1.1)	6.72 (1.6)
7 days	( $n = 247$ )	4.26 <sup>b</sup> (0.9)	4.30 <sup>b</sup> (0.7)	3.94 <sup>b</sup> (0.9)	4.10 <sup>b</sup> (0.6)	4.79 <sup>b</sup> (0.5)	7.85 <sup>b</sup> (0.7)

Abbreviations: Attitude measures: Aff\_Att: affective; Beh\_Att: behavioural; Cog\_Att: cognitive, SN: subjective norm; PBC: perceived behavioural control (maximum score 5); Int: intention to eat breakfast (maximum score 8).

<sup>a</sup>Significantly higher than girls, ( $p<.01$ , 2-tailed). <sup>b</sup>Significantly higher than 0-2 days & 3-6 days ( $p<.001$ , 2-tailed).

Sample  $n$  based on individuals answering all TPB questions.

#### 4.4.3 Predicting intention to eat breakfast

Hierarchical multiple regressions were performed to determine if the addition of the TPB variables improved the prediction of intention to eat breakfast over and above demographics and PA levels (Table 19). Demographics and PA were entered first (model 1) and explained a small (6.9%) but significant proportion of the variance ( $R^2=.069$ ,  $F(3,397)=9.76$ ,  $p<.001$ ). Significant beta weights were identified for gender and PA levels such that stronger intentions were associated with being male and being more active. The addition of the TPB variables (model 2) explained an additional 58.2% of the variance ( $\Delta R^2 =.582$ ,  $F(8,397)=90.61$ ,  $p<.001$ ). The beta weights indicated that all TPB variables, except affective attitudes, were significant positive predictors of intentions, such that stronger intentions were associated with having a positive attitude (behavioural, cognitive), stronger SN and in particular, greater PBC. Including the TPB variables in the model reduced the predictive power of gender and PA levels to non-significance. Adding the interactions between TPB variables and gender at an additional step did not add to the predictive power of the model, which indicated that gender did not moderate the relationship between TPB variables and intentions. However, to develop gender-specific interventions, the relative importance of the TPB variables for the prediction of intentions to eat breakfast were separately analysed for the sample of boys and the sample of girls.

**Table 19.** Hierarchical regression analyses for variables predicting intentions to eat breakfast in individuals answering every question ( $n=395$ ), girls ( $n=238$ ) and boys ( $n=157$ )

Variable	Model Number					
	1 (All)	2 (All)	1 (Girls)	2 (Girls)	1 (Boys)	2 (Boys)
	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$
Age	.13	.05	-.03	.03	.10	.09
PA levels	.17*	.03	.16*	.01	.20*	.08
Gender	.16*	-.03	-	-	-	-
PBC		.53**		.51**		.52**
Aff_Att		-.02		-.02*		-.03
Beh_Att		.16*		.22*		.04
Cog_Att		.13*		.110		.14
SN		.12*		.09		.18*
$R^2$	.069**	.651**	.029*	.652**	.048*	.581*
$\Delta R^2$		.582**		.634**		.532**

Abbreviations: SES: socio-economic status; PA: physical activity levels; PBC: perceived behavioural control; Attitude measures; Aff\_Att: affective; Beh\_Att: behavioural; Cog\_Att: cognitive; SN: subjective norm.  $\beta$ : standardised coefficient. \*  $p < .05$ , \*\*  $p < .001$ .

Demographics and PA levels explained 2.9% of the variation in girls ( $p < .05$ ) and 4.8% in boys ( $p < .05$ ) and significant beta weights indicated that stronger intentions were associated with being more active. The addition of the TPB variables (model 2) explained an additional 63.4% of the variance in girls ( $\Delta R^2 = .634$ ,  $F(7, 239) = 64.97$ ,  $p < .001$ ) and 53.2% in boys ( $\Delta R^2 = .532$ ,  $F(7, 157) = 29.66$ ,  $p < .001$ ). Significant beta weights indicated that for girls, stronger intentions towards breakfast were associated with all attitude components (except cognitive attitudes) ( $p < .05$ ) and PBC ( $p < .001$ ), whereas in boys stronger intentions were associated with SN ( $p < .05$ ) and PBC ( $p < .001$ ).

#### 4.4.4 Predicting breakfast behaviour

Multinomial logistic regression was conducted with demographic and TPB predictors to predict breakfast frequency category (0-2, 3-6, 7 days). For the whole sample, the model was significantly predictive of breakfast frequency ( $R^2 = .62$  (Cox & Snell),  $.73$  (Nagelkerke)  $\chi^2(18) = 377.75$ ,

$p < .001$ ) (Table 20), explaining 62% of the variation. Compared to those who ate breakfast 0-2 days, those who ate it 3-6 days had higher PBC (odds ratio (OR) =2.33), intentions (OR=1.56), and behavioural attitudes (OR=2.38). Compared to those who ate breakfast 0-2 days, those who ate it 7 days had higher PBC (OR=2.91), intentions (OR=1.97), SN (OR=2.44) and behavioural attitudes (OR=6.93). Interactions between gender and intentions were significant when comparing 0-2 days breakfast eaters to 3-6 days breakfast eaters ( $p=.004$ ) and when comparing 0-2 days breakfast eaters to 7-day breakfast eaters ( $p=.002$ ), demonstrating a stronger relationship between intentions and behaviours for females than males. No other significant interactions with gender were found.

Again, the predictive power of the model for the sample of boys and the sample of girls were analysed and were also significantly predictive of breakfast frequency explaining 69% of the variation in girls and 46% of the variation in boys. Compared to girls who ate breakfast 0-2 days, girls who ate it 3-6 days had higher PBC (OR=2.95) and intentions (OR=1.97). Compared to girls who ate breakfast 0-2 days, girls who ate it 7 days had higher PBC (OR=4.07), intentions (OR=2.52), SN (OR=4.94) and behavioural attitudes (OR=6.40). When compared to boys who ate breakfast 0-2 days, boys who ate breakfast 7 days had higher behavioural attitudes (OR=8.69). No other differences were observed.

**Table 20.** Multinomial logistic regression models predicting breakfast eating (0-2 days, 3-6 days, 7 days) from demographic and TPB variables for the whole sample and comparing models for girls and boys separately

	Whole sample		Girls		Boys	
	B	OR (95% CI)	B	OR (95% CI)	B	OR (95% CI)
<b>Breakfast 0-2 days vs 3-6 days</b>						
Intercept	-9.629*		-6.46		-20.08	
Gender	0.291	1.34 (0.44-4.03)	-	-	-	-
Age	0.17	1.21 (0.67-2.16)	-0.23	0.80(0.5-1.80)	0.99	2.70(0.76-9.58)
PA Mean	0.61	1.85 (0.86-3.96)	0.24	0.70(1.27-0.37)	1.59	4.90(0.98-24.40)
PBC Mean	0.84*	2.33 (1.34-4.04)	1.08*	2.95(1.40-6.22)	0.93	2.54(0.60-10.74)
Intentions	0.47**	1.56 (1.27-2.00)	0.68**	1.97(1.43-2.72)	0.08	1.08(0.66-1.76)
Subjective norms	0.06	1.06 (0.55-2.07)	0.76	2.13(0.82-5.51)	-0.16	0.54(0.18-4.11)
Affective att	-0.29	0.75 (0.39-1.45)	-0.43	0.65(0.27-1.56)	-0.19	0.83(0.18-3.79)
Behavioural att	0.87*	2.38 (1.19-4.83)	0.93	2.54(0.93-6.93)	0.73	2.07(0.64-6.67)
Cognitive att	-0.4	0.67 (0.35-1.29)	-0.37	0.70(0.28-1.69)	-0.64	0.53(0.16-1.74)
Gender x intentions	.659*	1.93(1.24-3.00)				
<b>Breakfast 0-2 days vs 7 days</b>						
Intercept	-19.16**		-17.58*		-27.51*	
Gender	-0.54	0.95(0.28-3.18)	-	-	-	-
Age	0.27	1.31(0.68-2.49)	-0.2	0.82(0.33-2.02)	1.13	3.11(0.87-11.03)
PA Mean	0.46	1.58(0.67-3.74)	0.52	1.68(0.41-6.76)	0.93	2.53(0.50-12.73)
PBC Mean	1.07*	2.91(1.49-5.68)	1.40*	4.07(1.65-10.05)	1.12	3.05(0.65-14.33)
Intentions	0.68**	1.97(1.40-2.79)	0.93*	2.52(1.46-4.37)	0.39	1.48(0.83-2.64)
Subjective norms	0.89*	2.44(1.09-5.44)	1.60*	4.94(1.57-15.54)	0.34	1.41(0.28-7.01)
Affective att	-0.66	0.52(0.24-1.10)	-0.4	0.67(0.25-1.83)	-1.48	0.23(0.05-1.14)
Behavioural att	1.94**	6.93(3.06-15.73)	1.86*	6.40(2.00-20.49)	2.16*	8.69(2.41-31.42)
Cognitive att	0.07	1.07(0.53-2.19)	-0.12	0.89(0.33-2.37)	0.24	1.28(0.37-4.41)
Gender x intentions	.800*	2.23 (1.11-4.46)				

Abbreviations: PA, physical activity; PBC, perceived behavioural control. Whole sample:  $R^2 = .62$  (Cox & Snell),  $.73$  (Nagelkerke)  $\chi^2 (18) = 377.75$ ,  $p < .001$ . Reference category for gender = male. Female:  $R^2 = .69$  (Cox & Snell),  $.78$  (Nagelkerke)  $\chi^2 (16) = 279.90$ ,  $p < .001$ , Male:  $R^2 = .46$  (Cox & Snell),  $.60$  (Nagelkerke)  $\chi^2 (18) = 97.83$ ,  $p < .001$

## 4.5 Discussion

The findings presented here confirm the hypothesis that adolescents do not eat a regular breakfast and this was more apparent in girls and those reporting less positive attitudes, SN and PBC towards breakfast. Previous research was extended by considering the components of attitudes, utilising a validated scale formerly used in children (Tapper et al., 2008). The TPB successfully predicted intention to eat breakfast and breakfast consumption in adolescents, the extent of which varied when comparing the samples of boys and girls.

### 4.5.1 Breakfast consumption

The current study found that breakfast was consumed every day by significantly more boys than girls, supporting the findings from a large UK survey where 61% of adolescent boys (11-15 years) consumed breakfast on every school day compared to 51% of girls (Vereecken et al., 2009), and 73% of adolescent boys (10-16 years) always ate breakfast compared to 61% of girls, both ( $p < .001$ ) (Sandercock et al., 2010). In contrast to other breakfast studies (Hoyland, McWilliams, Duff, & Walton, 2012; Utter, Scragg, Mhurchu, & Schaaf, 2007) there were no significant differences between breakfast frequency and ethnicity or SES, apart from between the highest socio-economic group who reported eating breakfast more frequently than those who did not know their parents' level of education. Because almost a third of students reported 'don't know' to the question of parental education, SES was excluded from further analyses; however, previous research suggests an association between SES and breakfast eating (Mullan & Singh, 2010), and there were significant differences in the current study where students from independent schools reported significantly higher rates of breakfast consumption compared to students from comprehensive schools, highlighting the importance of accounting for this when developing interventions. Significant positive associations between PA levels and breakfast consumption were observed in agreement with studies reporting higher PA levels in adolescents who regularly eat breakfast

(Sandercock et al., 2010). This may be linked to observations that breakfast eating could act as a marker for other health-promoting behaviours (Sandercock et al., 2010).

#### **4.5.2 Attitudes**

In the present study, boys and frequent breakfast eaters held more positive attitudes than girls and infrequent breakfast eaters, respectively. Positive attitudes towards breakfast are commonly associated with being more likely to eat breakfast regularly in adolescents (DeJong et al., 2009; Martens et al., 2005) and children (Moore et al., 2007; Unusan, Sanlier, & Danisik, 2006); therefore, targeting adolescents who consume breakfast infrequently by promoting positive attitudes represents a viable target for interventions. There is little evidence, however, to help determine which attitude components to target and how to address gender differences. Outside of the TPB, attitude-based interventions in young people are limited to children (Moore et al., 2007) and university students (Kennedy, Hajek, Morris, Linnell, & Gines, 2005), where increases in positive attitudes towards breakfast were coupled with increases in breakfast consumption (Kennedy et al., 2005), or improvements in the quality of breakfast consumed (Murphy, 2007). As breakfast quality also declines during adolescence (Hallstrom et al., 2012) targeting attitudes may potentially improve other aspects of breakfast consumption.

#### **4.5.3 Predicting intention to eat breakfast**

TPB measures predicted 58.2% of intention above the effects of age, gender and PA levels. This compares with a meta-analysis which found that 50% of the variation in intentions of dietary behaviours were explained by the TPB (McEachan et al., 2011) and is close to the values reported in adolescents ranging from 28% to 58% (Mullan, B. et al., 2013). Comparing boys and girls, the TPB explained an additional 63.4% of the variation in girls and 53.2% in boys, with PBC the strongest predictor for both. In girls behavioural attitudes made significant contributions (with



cognitive attitudes approaching significance  $p=.051$ ); however, in boys only cognitive attitudes were approaching significance ( $p=.056$ ), supporting previous research highlighting the importance of adolescents' attitudes in the prediction of intention to eat breakfast (Mullan, B. et al., 2013).

An additional challenge to consider in young people is that the attitudes most predictive of intention (or behaviour) are also more likely to be resistant to persuasion (Haddock & Maio, 2004). Affective attitudes did not contribute to intentions which was in contrast to suggestions that affective attitudes are a better predictor of intentions than cognitive attitudes (Haddock & Maio, 2004). This may suggest that adolescents' feelings towards breakfast are not important for this behaviour, but more research in this area is required.

SN were significant predictors of intention in boys (and behaviour in girls) supporting Martens *et al.* (Martens et al., 2005) who reported SNs and attitudes as significant predictors of adolescents' intention to eat breakfast. SN consists of two distinct dimensions: injunctive norms (linking influential roles of significant others) and descriptive norms (improving behaviours in significant others). Detailed examination of SN was beyond the scope of this study; however, findings suggest that SNs could be a viable focus for breakfast interventions in adolescents, particularly as studies in university students generally report a low predictive power of SN in regards to breakfast frequency (Kothe et al., 2011; Wong & Mullan, 2009). Interventions targeting the social influences and modelling of peers or family, as suggested by associations between the dietary intakes of parents and siblings with those of adolescents (van der Horst et al., 2007), particularly with regard to breakfast (Pearson, Biddle, & Gorely, 2009), may be successful in this age group.

#### **4.5.4 Predicting breakfast behaviour**

Demographics, PA and the TPB predicted 62% of the variation in breakfast behaviours. In contrast with previous studies considering gender (Berg et al., 2000; Conner et al., 2011), there was a significant interaction between gender and intentions, where girls were associated with having

stronger intentions than boys. Overall, intention most strongly predicted behaviour, consistent with other breakfast studies (Kothe et al., 2011; Mullan, B. et al., 2013; Wong & Mullan, 2009) and dietary behaviours (McEachan et al., 2011).

PBC was an important factor when comparing those who ate breakfast 0-2 days per week with the other two frequency groups in girls, but not in boys. PBC contributes less when volitional control is high; therefore, interventions might target increasing perceptions of control over breakfast consumption in girls. For example, access to healthy breakfast items in the home or at school may increase the perception of available resources and opportunities to consume a regular breakfast.

Behavioural attitudes most strongly predicted breakfast consumption, followed by PBC, when comparing those who ate breakfast 0-2 days with those who ate breakfast 7 days. Previous research used only a single construct for attitudes, but reported that adolescents' attitudes were the strongest predictor of breakfast consumption (Martens et al., 2005). Perceptions of time loaded strongly on the behavioural attitudes components which may account for the strong association with behaviour. Barriers towards regular breakfast consumption in adolescents are frequently reported to revolve around a lack of time as well as food availability, stress and weight control (Mullan & Singh, 2010). Understanding the differences in breakfast behaviours between boys and girls requires further research; however, the current study observed significant differences between BMI z-scores which may support suggestions that breakfast skipping is used as a method of weight control in girls (Neumark-Sztainer et al., 2002), perhaps explaining why the model predicted behaviour better in girls than in boys.

Taken together, the model suggests that targeting TPB variables in interventions might increase breakfast consumption frequency; however, when considering boys and girls separately, TPB variables might predict behaviour better in girls (69% of the variation in girls and 46% of the variation in boys); suggesting factors outside of the model exert greater influence over boys'

breakfast behaviours. To increase breakfast consumption in girls, interventions should aim to change PBC, intentions, SN and behavioural attitudes, but for boys a better focus may be SN and PBC.

#### **4.5.5 Limitations**

A criticism of the TPB is the notable proportion of behaviour left unaccounted for (Sniehotta, Pesseau, & Araújo-Soares, 2013) as well as the potential for additional variables, such as past behaviour, to improve the predictive power of the model (Wong & Mullan, 2009). When compared to the 'health action process' approach the TPB was superior in predicting breakfast consumption (Mullan, B. A. et al., 2013); however, it is yet to be compared to other theories, specifically those that include additional variables. In the current study intention was more strongly predicted than behaviour. For 'inclined abstainers' good intentions will not always translate into behaviour (Abraham et al., 1999) and TPB variables are known to consistently predict intention better than behaviour, commonly referred to as the 'intention-behaviour' gap (Sniehotta, Scholz, & Schwarzer, 2005). Techniques to promote planning, maintenance, self-efficacy and action control may help to bridge the intention-behaviour gap (Sniehotta et al., 2005), and TPB interventions that include techniques, for example implementation intentions, (Adriaanse, Vinkers, De Ridder, Hox, & De Wit, 2011) may help maximise outcome potential in especially difficult to study groups.

Adolescents are difficult to recruit and gaining access to schools is a challenging aspect of research in this area. Interventions to increase school breakfast consumption in adolescents were reported as difficult to implement and maintain control over, creating problems of contamination between control and intervention conditions (Shemilt et al., 2004).

The cross-sectional nature of this study which measured intention and behaviour simultaneously is likely to inflate the intention-behaviour relationship due to consistency bias, where individuals

report intentions consistent with their current behaviour; however, this remains an issue even in prospective studies where a short time interval is used (Ogden, 2008). Furthermore, this study cannot infer conclusions about causality, so interventions to increase breakfast frequency based on these findings should be carefully evaluated.

## **4.6 Conclusion**

These findings provide good support for considering an extended TPB to strengthen the prediction of intention to eat breakfast and breakfast behaviours in adolescents. Given the evidence for differences in the predictive power of the TPB between gender, and the limited number of effective breakfast interventions in adolescents, it is vital to target interventions appropriately.

Collecting information on the composition of breakfast identified a popular RTEC consumed by adolescents, which would be used as a comparison breakfast to the FB and CB (chapter 6).

Furthermore, this study provided valuable experience in terms of recruitment and the practical and ethical issues involved when working with schools. It facilitated the opportunity to form relationships with local school teachers and gauge interest in taking part in the school breakfast study (chapter 7).

## Chapter 5: The development of a functional food breakfast

### 5.1 Summary

Consuming functional foods rich in polyphenols and fibre can benefit individuals by improving markers of metabolic health, including GR and IR. To contribute to the development of a FB which would provide a rich source of polyphenols, and which would reduce the amount of sugars released, compared to a control breakfast, the aims of this study were to:

1. Compare antioxidant and polyphenol levels of baobab extracts.
2. Bake the baobab extract into a breakfast muffin with other functional food ingredients (the FB) and compare antioxidant and polyphenol levels to the CB.
3. Compare the effects of freezing on starch breakdown and antioxidant levels.

There were significant differences in antioxidant and polyphenol levels between extracts and between the FB and CB. Additionally, the CB released significantly more sugars than the FB during the later stages of digestion. Freezing the muffins appeared to have a protective effect on polyphenols without significantly impacting the effect on rapidly digested starch (RDS). The addition of polyphenol-rich ingredients to a breakfast muffin significantly increased the total antioxidant and polyphenol content and decreased starch breakdown during the later stages of digestion.

## 5.2 Introduction

The regular consumption of antioxidant-rich foods high in polyphenols is strongly associated with the prevention of disease, particularly CVD and cancers (Scalbert, A. et al., 2005). Polyphenol intake is estimated to be much higher than intakes of other classes of known antioxidants and phytochemicals (Scalbert, Augustin et al., 2005).

Some of the benefits associated with polyphenols are due to their effects on the breakdown of sugar and starch which can be altered when they are added to foods (McDougall et al., 2005). Previous *in-vitro* research from our laboratory investigated the potential of polyphenol-rich food sources to influence the amount of sugars released from starch-rich foods (Coe & Ryan, 2015; Coe et al., 2013). The addition of baobab to white bread significantly reduced the amount of sugars released at 20 minutes and 60 minutes into the duodenal digestion phase, attributed to the polyphenols and potentially the high soluble and insoluble fibre content (Coe et al., 2013). The beneficial effects of oats have mostly been attributed to their  $\beta$ -glucan content (Braaten et al., 1994); however, *in-vitro* results suggested a possible interaction between the polyphenols in oat-based porridges and a reduction in starch digestibility (Thondre et al., 2011). Observing starch digestion *in-vitro* can provide some insight into the effects on GR expected *in-vivo* (Englyst, Englyst, Hudson, Cole, & Cummings, 1999).

Polyphenols are extremely sensitive to oxidation by air, heat or light, and the processing or preparation of polyphenol-rich ingredients may rapidly degrade the polyphenol content (Rodrigues, Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2009). The polyphenol profile may also vary depending on country of origin, season and variety. Previous research from our laboratory identified significant differences between the total antioxidant and polyphenol content of commercially available brands of green tea (Ryan & Carolan, 2011), oat-based breakfast cereals (Ryan et al., 2011) and baobab extracts (Coe et al., 2013); however, the latter study included just one

commercial extract, the only one available to purchase at that time. Additionally, exposing oat-based products to freezing may reduce  $\beta$ -glucan solubility and potentially attenuate the blood glucose lowering benefits (Lan-Pidhainy, Brummer, Tosh, Wolever, & Wood, 2007).

### **5.2.1 Aims and hypothesis**

The aims of the current study were:

1. To identify and compare the antioxidant and polyphenol levels of two commercially available baobab extracts.
2. To compare the effect of baking and freezing on antioxidant and polyphenol profiles of a FB and CB, to inform preparation methods for the next study (chapter 5.3.4).
3. To compare the effect of freezing vs. not freezing on sugar release (i.e. CB frozen vs. CB fresh; FB frozen vs. FB fresh) to inform storage methods for the next study (chapter 5.3.4).
4. To compare differences between the frozen CB and the frozen FB on sugar release.

#### Hypothesis:

It was hypothesised that *in-vitro*, the FB would contain a significantly higher amount of polyphenols and would be superior in its potential to reduce the amount of sugars released, when compared to the CB.

## 5.3 Materials

### 5.3.1 Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, UK). The extracts were two commercially purchased baobab fruit powdered extracts (sample 1: Baobab super fruit powder, Min Vita, London, UK; sample 2: Baobab super fruit powder, Aduna, London, UK). These extracts were selected to compare variability in total antioxidant and polyphenol content. Ingredients were identified from the manufacturer's labelling (Table 21).

**Table 21.** Comparison of nutritional composition from product packaging (per 100g). MinVita (extract 1) and Aduna (extract 2) Super Fruit Baobab Powder

Nutritional component/100g	Baobab extract	
	1	2
Kilocalories (kcal)	170	256
Total fat	<1 g	<1g
Carbohydrate	78g	39g
of which sugars	25g	29.5g
Protein	3g	2.4g
Fibre	60g	45g
of which soluble	30g	22.5g
Vitamin B1 (Thiamin)	0.6mg	0.4mg
Vitamin C	300mg	225mg
Potassium	3000mg	2500mg
Calcium	350mg	300mg
Iron	2mg	ns
Sodium	0.23mg	2.3mg

Abbreviations: ns, not specified on website or packaging.

### 5.3.2 Study protocol

Antioxidant and polyphenol analysis of powdered extracts was based on a 200mg sample. Analysis of muffin samples was based on a 1g sample. Both samples were extracted with solvent in preparation for polyphenol analysis. For *in-vitro* digestion muffin samples were weighed out at



2.5g. All tests were carried out on a minimum of three separate occasions and samples were analysed in triplicate for each test.

### **5.3.3 Ferric-ion reducing antioxidant power**

The antioxidant power of powdered extract samples and muffin samples were analysed using the ferric-ion reducing antioxidant power (FRAP) method, adapted from Benzie and Strain (Benzie & Strain, 1996). FRAP uses antioxidants as reductants in a reduction-oxidation reaction. To briefly summarise, at low pH, ferric is reduced to ferrous ion which causes a coloured ferrous-tripyridyltriazine complex to form. FRAP values can be determined by measuring the change in absorbance at 593 nm in test solutions compared with known concentrations of ferrous ion solutions.

The FRAP reagent was prepared from acetate buffer 300 mM, pH 3.6 (3.1g sodium acetate trihydrate and 16 mL glacial acetic acid made up to 1L dH<sub>2</sub>O), TPTZ 10 mM in 40mM HCl (3.4 mL HCl made to final volume 1 L dH<sub>2</sub>O), 20 mM Ferric chloride (FeCl<sub>3</sub>.6H<sub>2</sub>O) (5.406 g iron chloride made to final volume 1L dH<sub>2</sub>O). The FRAP reagent was made up to a 10:1:1 ratio.

The FRAP assay was performed by warming 1 mL of dH<sub>2</sub>O to 37 °C before adding 25 µL of sample and 1 mL of reagent. The tubes were then placed in a water bath at 37 °C for exactly 4 minutes after which absorbance was measured at 593 nm, with each standard and sample measured against the blank. The FRAP value was then calculated in µmol/ L against a standard of ferrous sulphate (1000 µM).

### **5.3.4 Folin-Ciocalteu**

Total polyphenols were analysed from powdered extract samples and muffin samples using the folin-ciocalteu (FCR) method (Sharma & Gujral, 2010). FCR is a colorimetric assay which

measures the total phenolic content of a sample by measuring the reducing capacity of the metal oxides in the reagent (Prior, Wu, & Schaich, 2005).

Polyphenols were extracted from powdered extracts and muffin samples. 4mL of 70% acetone were added to powdered extract or blended muffin in amber vials and secured using parafilm to prevent oxidation. Incubation occurred at room temperature in a shaking bath for 2 hours, following which solvent and samples were transferred to tubes and centrifuged at 3000xg for 10 minutes.

Supernatant was removed and analysed immediately or covered and frozen at -20°C.

To analyse polyphenol content, 200µl of the extracted sample was added to 1.5 mL of freshly prepared FCR reagent (1:10 v/v with dH<sub>2</sub>O). The mixture equilibrated for 5 min and was mixed with 1.5 mL of 60 g/L dH<sub>2</sub>O sodium carbonate solution. After incubation in a dark air-tight space at room temperature for 90 min, the absorbance of the mixture was read at 725 nm using the respective solvent as blank. The results were expressed as µg of gallic acid equivalent (GAE) per mL of sample.

### **5.3.5 Muffin preparation**

Muffins were prepared in the afternoon following a recipe developed based on quantities which would deliver 50g available CHO for the CB and FB (Table 22). Previous *in-vitro* research identified an optimal baobab dose of 1.98g/50g available CHO for reducing sugar release in white bread (Coe et al., 2013).

Muffins were prepared by separately mixing the dry and the wet ingredients. These were then combined and briefly mixed before quickly measuring into greased muffin cases and baking in a pre-heated oven. The CB was baked for 10 minutes at 200°C and then a further 8 minutes at 180°C. The FB was baked for 10 minutes at 200°C and then a further 28 minutes at 180°C. Muffins were allowed to cool for 10 minutes before being removed from the baking tray and left for 30 minutes to

cool completely on a wire rack. Muffins were stored overnight in a dark, airtight container and analysed the following morning (fresh samples), or frozen immediately for 24 hours and thoroughly defrosted at room temperature before analysis (frozen samples).

**Table 22.** Ingredients for CB and FB

	Unit	CB	FB
Ground porridge oats	g	-	53.0
Baking powder	g	5.0	5.0
Olive oil	g	-	2.6
Whole milk	mL	50.0	40.0
Bananas	g	-	30.0
Ground cinnamon	g	-	1.0
Blueberries	g	-	30.0
Baobab superfruit powder	g	-	1.98
Clear honey	g	-	11.0
Olive oil spray	g	0.7	0.7
Egg whites	g	32.0	30.0
Strong white bread flour	g	57.0	-
Unsalted butter	g	8.4	-
Granulated white sugar	g	3.0	-
Fructose powder	g	6.81	-
Total (wet product)	g	162.91	205.28

### 5.3.6 Nutrient profiles

To determine nutrient profiles which would inform for *in-vivo* serving size in the forthcoming studies, and to establish any effect of freezing on the nutrient composition of the breakfasts, a frozen and a fresh muffin were analysed by Eurofins Food Testing UK Ltd. (Wolverhampton, UK) (Table 23). Four samples were sent for analysis: Sample 1, 500g of frozen and then thawed CB, Sample 2, 500g of a frozen and then thawed FB; Sample 3, 500g of a freshly baked CB; Sample 4, 500g of a freshly baked FB.

**Table 23.** Nutrient profiles as determined by Eurofins

	FB fresh/100g	FB fresh/serving	FB frozen/100g	FB frozen/serving	Difference: frozen - fresh/serving	CB fresh/100g	CB fresh/serving	CB frozen/100g	CB frozen/serving	Difference: frozen - fresh/serving
<b>g</b>		<b>164.5</b>		<b>161.8</b>			<b>141.2</b>		<b>140</b>	
Moisture (g)	49.7	81.8	49.4	79.9	-1.8	46.4	65.5	44.4	62.2	-3.4
Crude protein (g)	6.8	11.2	6.9	11.2	0.0	7.8	11.0	8.1	11.3	0.3
Ash (g)	2.6	4.3	2.6	4.2	-0.1	2.5	3.5	2.5	3.5	0.0
CHO (avail) (g)	30.4	50.0	30.9	50.0	0.0	35.4	50.0	35.7	50.0	0.0
Fructose (g)	2.3	3.8	2.7	4.4	0.6	3.8	5.4	3.2	4.5	-0.9
Galactose (g)	0.1	0.2	0.1	0.2	0.0	0.1	0.1	0.1	0.1	0.0
Glucose (g)	2.1	3.5	2.5	4.0	0.6	0.2	0.3	0.4	0.6	0.3
Lactose (g)	0.7	1.2	0.8	1.3	0.1	1.5	2.1	1.5	2.1	0.0
Maltose (g)	0.1	0.2	0.2	0.3	0.2	0.4	0.6	0.4	0.6	0.0
Sucrose (g)	2.3	3.8	2.5	4.0	0.3	2.5	3.5	2.1	2.9	-0.6
Total sugars (g)	7.5	12.3	8.7	14.1	1.7	8.4	11.9	7.6	10.6	-1.2
Total fat (g)	5.0	8.2	5.0	8.1	-0.1	6.1	8.6	7.5	10.5	1.9
Total fibre (AOAC) (g)	5.5	9.0	5.2	8.4	-0.6	1.8	2.5	1.8	2.5	0.0
Energy (kcal)	205.0	337.2	207.0	334.9	-2.3	231.0	326.2	246.0	344.4	18.2
Energy (kJ)	861.0	1416.3	869.0	1406.0	-10.3	975.0	1376.7	1040.0	1456.0	79.3
MUFAs	2.5	4.1	2.5	4.1	-0.1	1.6	2.2	1.9	2.7	0.5
PUFAs	1.0	1.7	1.0	1.6	-0.1	0.4	0.6	0.5	0.6	0.0
SFA	1.2	2.0	1.2	2.0	0.0	3.6	5.1	4.5	6.3	1.2
TFA	0.1	0.2	0.1	0.2	0.0	0.2	0.2	0.2	0.3	0.1
Sodium	0.6	1.0	0.6	0.9	0.0	0.7	1.0	0.8	1.1	0.1

Abbreviations: FB, functional breakfast; CB, control breakfast; CHO, carbohydrate; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids. Matching of breakfasts was based on differences between items highlighted in green.

### 5.3.7 *In-vitro* digestion

Fresh and frozen muffin samples were compared using an *in-vitro* digestion procedure consisting of a simulated gastric digestion phase followed by an ileal digestion phase, with timed sampling at the end of the gastric phase and during the ileal phase (Mishra, Monro, & Hedderley, 2008). Samples of the muffins were prepared by weighing 2.5g samples into 60mL specimen pots. Samples were finely crumbed into small uniform pieces (millimetres in diameter) to reflect the chewing process. The pots were inserted into an aluminium heating block and covered with an insulating sheet in readiness for testing.

30 mL of dH<sub>2</sub>O was added to each muffin sample. A 250µL baseline sample was extracted for each sample at 0 min and added to a test tube in a ratio of 1:4 in ethanol. This was followed by the addition to each sample of: 0.1mL 10% porcine  $\alpha$ -amylase (Type VI-B,  $\geq 10$  units/mg solid), 0.8mL 1M HCl and 1mL 10% porcine pepsin (800-2500 U/mL) protein solution in 0.05M HCl. The resultant mixture was stirred slowly at 130 rpm every 15 sec for 30 min at 37°C to complete the gastric digestion phase. A 250µL gastric sample was extracted. The ileal phase was initiated by the addition of 2mL 1M Sodium hydrocarbonate NaHCO<sub>3</sub> and 5mL 0.2M Na maleate buffer (pH 6) to each sample, and the volume was increased to 55mL with dH<sub>2</sub>O. In quick succession, 0.1mL of amyloglucosidase ( $\geq 300$  U/mL, aqueous solution) and 1mL of 2% porcine pancreatin solution (8 x USP; in maleate buffer, pH 6), were added to each sample. Samples were incubated for a further 120 min with constant slow mixing, and aliquots taken at 20, 60 and 120 min during ileal digestion. The tubes were centrifuged (1000 x g, 2 min) in a Biofuge Primo Centrifuge (Heraeus Instruments, Kendro Laboratory Products, Germany) and an aliquot of the supernatant was removed for analysis of reducing sugars.

### 5.3.8 Analysis of reducing sugars released during digestion

Sugar released from the muffins during digestion was measured by a colorimetric method adapted from Englyst and Hudson (Englyst & Hudson, 1987), designed to measure monosaccharides after an amyloglucosidase secondary digestion to complete depolymerisation of starch fragments.

A total of 0.05mL of 10mg/mL glucose standard, or sample from the *in-vitro* digestion, was added to a mixture of 0.25mL of enzyme solution A (1% amyloglucosidase, Megazyme, 3260 U/mL) in 0.1M acetate buffer (pH 5.2) and 0.25mL of invertase (1% *S. cerevisiae*). Each sample was incubated for 10 min at room temperature and then 0.75mL of 3,5-Dinitrosalicylic acid (DNS) mixture (0.5 mg/mL glucose: 4M NaOH: DNS reagent mixed in ratio 1:1:5) was added. The resultant sample was heated for 15 min at 95°C in a water bath and then placed in a cold water bath to terminate the reaction. Each sample was thoroughly mixed with 4mL of dH<sub>2</sub>O and absorbance was measured at 530nm on a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Australia). Sugar release was measured in mg per g of muffin sample.

### 5.3.9 Statistical analysis

Statistical analyses were performed using SPSS V.22. Powdered extracts were compared using paired samples *t*-test. Muffin samples were compared using one-way ANOVA for FCR and FRAP analysis where pairwise comparisons were performed using Games-Howell adjustment (if assumption of homogeneity of variances violated) or Tukey HSD (if assumption of homogeneity of variances is not violated). Muffin samples were compared using two-way repeated measures ANOVA for *in-vitro* analysis where multiple comparisons were performed using a Bonferroni adjustment. The strength of relationships was assessed using Pearson's correlation. Results were expressed as the mean  $\pm$  SD and significance set at  $p < .05$ .

## 5.4 Results

### 5.4.1 Antioxidant and polyphenol content of powdered extracts

Extract 1 (MinVita) had a significantly ( $p<.005$ ) greater antioxidant and polyphenol content than Extract 2 (Aduna) as measured by both FRAP and FCR respectively (Table 24) and was therefore selected for use in subsequent muffin analysis. There were significant correlations between FRAP and FCR values for extract 2 suggesting that polyphenols were the predominant antioxidant contributing to the overall antioxidant power.

**Table 24.** FCR and FRAP analysis of baobab extracts 1 (MinVita) and 2 (Aduna).

Values represent means  $\pm$  SD of three independent experiments

Baobab Extract	FRAP ( $\mu\text{mol/L}$ )	FCR (mg GAE/g)	<i>r</i>
1	1250.78 $\pm$ 213.73**	2.29 $\pm$ 0.09**	0.34
2	910.11 $\pm$ 139.26	1.98 $\pm$ 0.12	0.93**

Abbreviations: FRAP, Ferric reducing antioxidant power assay; FCR, Folin-Ciocalteu assay;  $\mu\text{mol/L}$  relative to ferrous sulphate (1000  $\mu\text{M}$ ), GAE/g, gallic acid equivalents (GAE)/g sample. *r*, Pearson's correlation coefficient. Statistically significant \*\*  $p<.001$  (paired samples *t*-test).

### 5.4.2 Antioxidant and polyphenol content of muffin samples

Total antioxidants analysed by FRAP were significantly different between muffins ( $F(3,32)=65.09$ ,  $p<.001$ ,  $\eta^2=0.86$ ). Post hoc analysis using Tukey HSD revealed that antioxidants were higher in the FB frozen muffin compared to the FB fresh ( $p=.013$ ) (Table 25). Furthermore, total antioxidant power was significantly higher in the FB compared to the CB, regardless of whether they were fresh or frozen ( $p<.001$ ).

Total polyphenols analysed by FCR were significantly different between muffins (Welch's  $F(3,15.7)=67.53$ ,  $p<.001$ ,  $\eta^2=0.88$ ). Post hoc analysis using Games-Howell adjustment for multiple comparisons revealed that polyphenols were higher in the frozen muffins compared to the fresh muffins ( $p=.005$ ) and again, were significantly higher in the FB compared to the CB regardless of

whether they were fresh or frozen ( $p < .001$ ). There were strong positive correlations between FRAP and polyphenol values for the FB regardless of whether it was fresh or frozen ( $p < .001$ ).

**Table 25.** FRAP and FCR analysis of fresh vs. frozen muffins. Values represent means  $\pm$  SD of three independent experiments

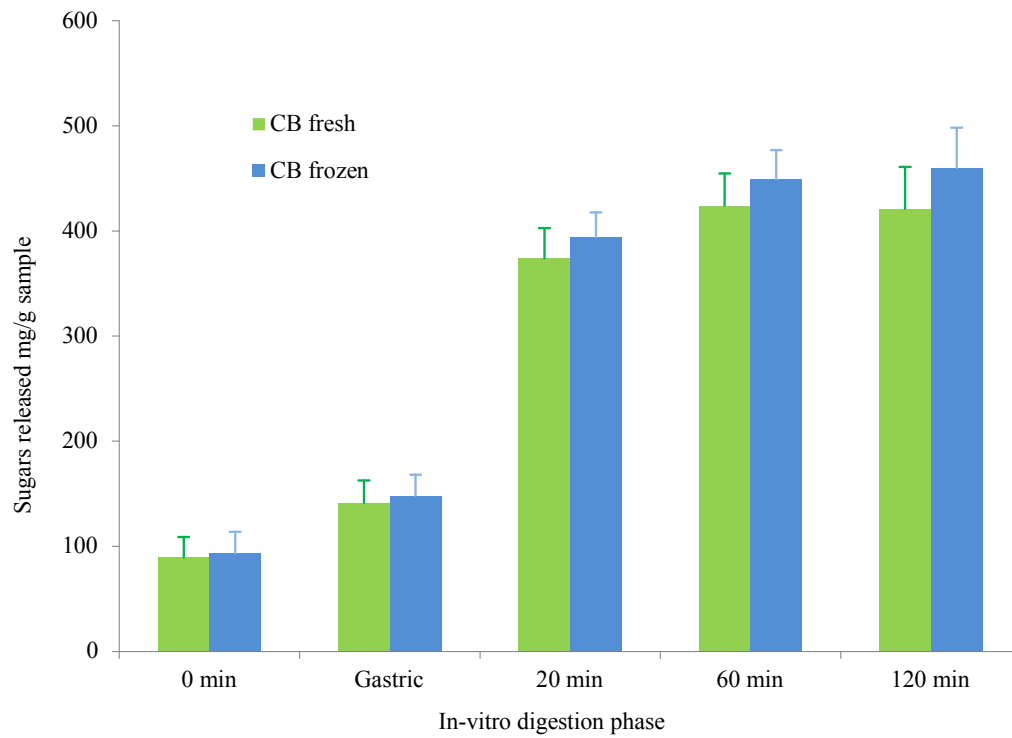
Muffin	FRAP ( $\mu\text{mol/L}$ )	FCR (mg GAE/g)	<i>r</i>
FB fresh	2908.21 $\pm$ 428.77 <sup>b</sup>	1.26 $\pm$ 0.06 <sup>b</sup>	0.83**
FB frozen	3518.20 $\pm$ 553.56 <sup>ab</sup>	1.42 $\pm$ 0.10 <sup>ab</sup>	0.97**
CB fresh	1273.43 $\pm$ 280.61	1.02 $\pm$ 0.05	0.36
CB frozen	1595.49 $\pm$ 241.22	1.05 $\pm$ 0.02	0.38

Abbreviations: FRAP, Ferric reducing antioxidant power assay; FCR, Folin-Ciocalteu assay;  $\mu\text{mol/L}$  relative to ferrous sulphate (1000  $\mu\text{M}$ ), GAE/g, gallic acid equivalents (GAE)/g sample. <sup>a</sup> significantly higher than FB fresh, <sup>b</sup> significantly higher than CB fresh and CB frozen \*\*  $p < .001$  (one-way ANOVA).

#### 5.4.3 Sugars released from muffin (fresh vs. frozen)

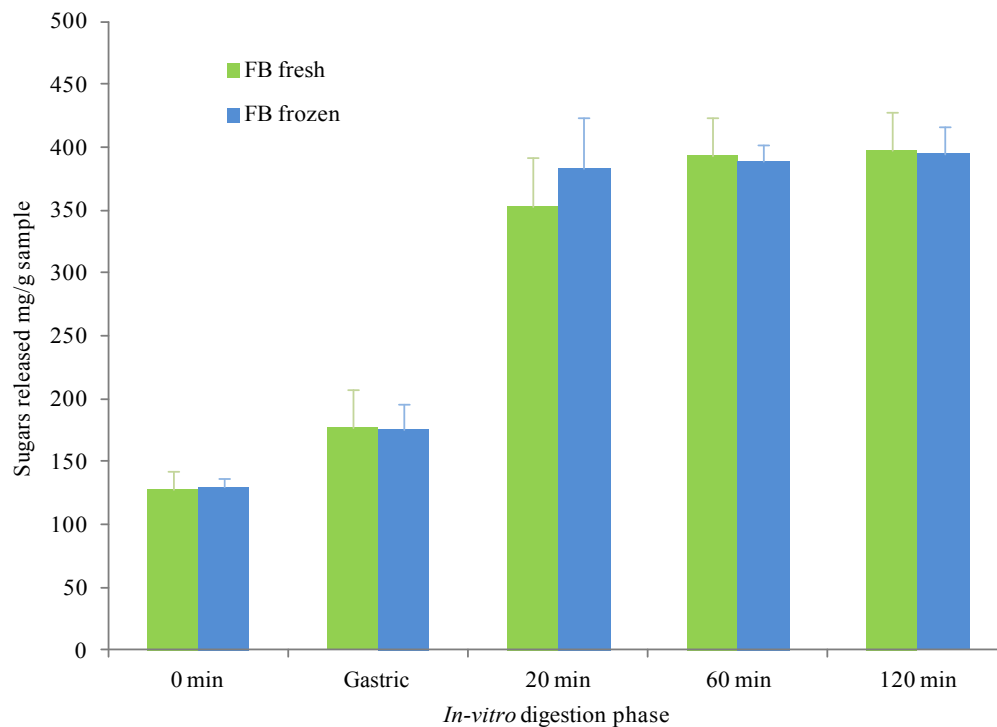
There were no significant interactions between time and muffin sample ( $p = .255$ ), but there was a main effect between the CB fresh and CB frozen muffins ( $F(1,5) = 9.25$ ,  $p = .029$ ). Post hoc analysis using a Bonferroni adjustment revealed that significantly more sugar was released from the CB frozen muffin (mean  $289.9 \pm 161.6$  mg/g) compared to the CB fresh muffin (mean  $308.8 \text{ mg/g} \pm 174.7$ ) ( $p = .029$ ) (Figure 14).





**Figure 14.** Sugars released between the CB fresh and the CB frozen muffin

There were no significant interactions between time and muffin, or main effects between sugars released from FB frozen and the FB fresh at any phase of *in-vitro* digestion process ( $p > .05$ ) (Figure 15).



**Figure 15.** Sugars released between the FB fresh and FB frozen muffins.

Comparisons between the CB and FB frozen muffin saw the FB muffin release significantly more sugar than the CB muffin at baseline ( $F(1,5)=11.813, p=.018$ ) and during the gastric phase ( $F(1,5)=9.294, p=.028$ ). Post hoc analyses performed using a Bonferroni adjustment revealed there were no significant differences during the first 20 minutes, representing rapidly digested starch (RDS); however, in the intestinal phase of digestion the FB released less sugars compared to the CB at 60 minutes ( $p=.002$ ) and 120 minutes ( $p=.004$ ), suggesting differences between the amount of slowly digestible starch (SDS) (Figure 16).

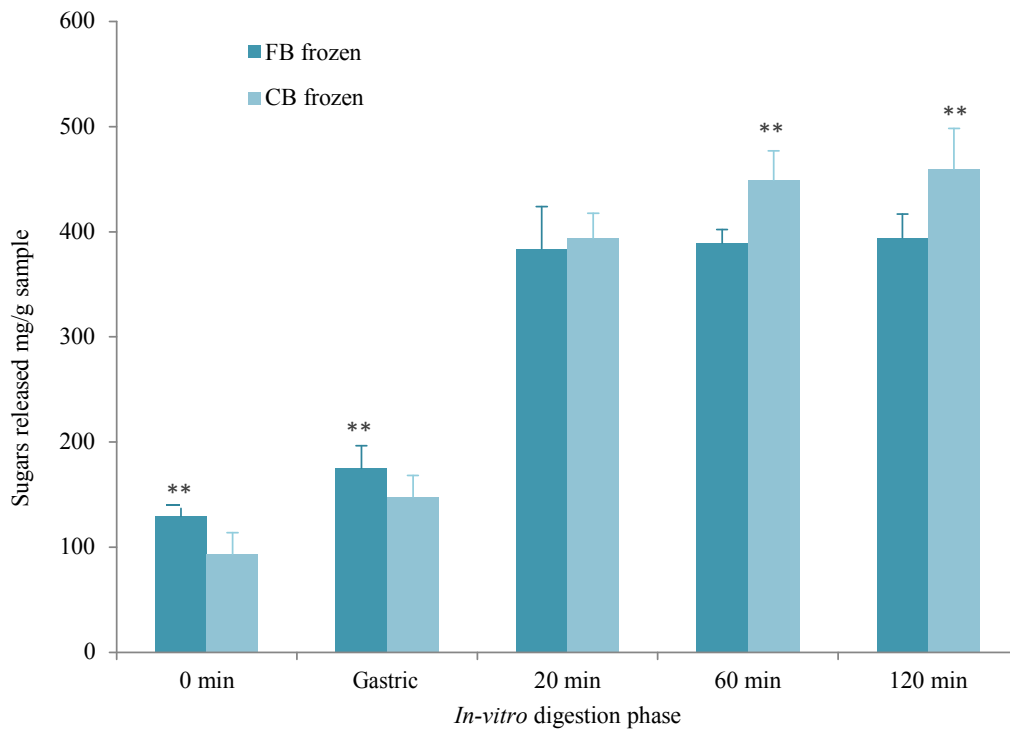


Figure 16. Sugars released between the CB frozen and the FB frozen

## 5.5 Discussion

### 5.5.1 Antioxidant and polyphenol content of extracts

Both commercially available baobab fruit powdered extracts were sources of antioxidants and polyphenols, but there were significant differences between products. In line with a study comparing the same commercial baobab extract used in the current study (Minvita) to baobab extracts sourced from different regions of Africa, the commercial extract was the most concentrated source of polyphenols (Coe et al., 2013). Absolute values, however, were notably higher than found in the current study (FRAP  $2168 \pm 53$   $\mu\text{mol/L}$ , polyphenols  $28.9 \pm 0.5$  mg GAE/g) highlighting the potential variation in total antioxidants and polyphenols even within the same brands.

### 5.5.2 Antioxidant and polyphenol content of muffins

Both the CB and the FB were a source of antioxidants and polyphenols, although as expected the FB was a significantly higher source. Polyphenols can be easily degraded by heat (Rodrigues et al., 2009) so some decline between polyphenol values obtained from the powdered extract and the cooked FB muffin was expected. However, the combining of polyphenol-rich ingredients into the FB may have promoted synergism helping to minimise complete degradation (Rios et al., 2002). Additionally, employing a protocol that involved quickly freezing and thawing the muffins, shown to have a protective effect on  $\beta$ -glucans (Lan-Pidhainy et al., 2007), appeared also to have a protective effect on total antioxidants and polyphenols.

### 5.5.3 Sugar release and starch digestion

Muffin samples were digested under simulated gastrointestinal conditions. At 20 minutes into the intestinal phase of digestion, when RDS is at its peak, there were no significant differences between the amount of sugars released within the CB fresh and frozen or the FB fresh and frozen. For practical reasons, during the *in-vivo* study (chapter 6 and 7), the muffins would be baked and,

immediately after cooling, quickly frozen (by allowing space around the muffins), then stored for a maximum of five days, which is recommended for preserving  $\beta$ -glucan solubility (Regand, Tosh, Wolever, & Wood, 2009). When subjected to repeated bouts of freezing,  $\beta$ -glucan solubility has been shown to reduce, which can negatively affect the GR (Lan-Pidhainy et al., 2007); however, results from this *in-vitro* study provide support that this freezing procedure should not significantly impact GR results *in-vivo*.

The present study found that the frozen FB released significantly less sugars during the later stages of intestinal digestion compared to the frozen CB, which suggests that the FB contained more SDS. The rate of starch digestion is the predominant factor determining its glycaemic impact, as it controls how quickly glucose is released into the gut for absorption into the blood (Jenkins et al., 1982) and preventing the large spikes in blood glucose 30-45 minutes after a meal may be more important than lowering the overall AUC (Ceriello, Colagiuri, Gerich, & Tuomilehto, 2008). SDS releases energy at a slower rate than RDS and therefore should produce a lower GR *in-vivo* (Mishra & Monro, 2009). Additionally, polyphenol sources may have contributed to the prevention of starch breakdown due to their inhibiting effects on digestive enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) (McDougall & Stewart, 2005).

There was a significant increase in sugars released during the gastric phase of digestion in the FB muffin compared to the CB muffin, although this effect was absent at 20 minutes. Although polyphenols are generally associated with reducing sugar release from starch-rich foods, they have also been shown to increase starch digestibility (Wu, Chen, Li, & Li, 2009). Nevertheless, even if polyphenols do not reduce RDS, the current study's findings suggest they may still have the potential to produce an overall reduction in the amount of sugar released.

## 5.6 Conclusion

This series of studies informed the development of the FB and CB and demonstrated the potential of baobab extracts and the combination of polyphenol-rich functional food ingredients to reduce the amount of sugars released from a FB muffin. Additionally, findings demonstrated that freezing the muffins would not negatively impact the polyphenol or antioxidant content, or the amount of sugars released.

## **Chapter 6: The effects of a functional food breakfast on gluco-regulation, cognitive performance, mood and satiety in healthy adults**

### **6.1 Summary**

The regular consumption of functional food ingredients, which are naturally rich in antioxidants, polyphenols, flavonoids and fibre, has been associated with improvements in risk factors for metabolic disorders and neurodegenerative diseases (chapter 1.10.2). Individually, polyphenol-rich functional foods have been identified as improving the GR and IR to CHO meals (chapter 1.10.3), as well as enhancing cognitive performance, particularly memory, in healthy and at-risk individuals (chapter 1.10.4; 1.11). Combining functional food ingredients into the diet over a period of weeks has shown potential for improvements in metabolic and cognitive measures (chapter 1.10.2), but the acute effects delivered as a breakfast have not been considered in either adolescents or adults. As preparation for assessing these differences in adolescents in a school setting (chapter 7), a study was first implemented in adults in a laboratory setting. This aimed to:

- 1) Assess the impact of a FB combining multiple functional food ingredients on GR and IR in healthy adults, compared with a CB and a RTEC.
- 2) Assess differences between breakfasts on cognitive function, satiety, mood and palatability.

In a crossover study, sixteen healthy adults consumed a FB, a CB and a RTEC. GR and IR were measured at baseline and at 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Additional measures were collected at timed intervals including cognitive tests, appetite, mood and palatability. Consumption of the FB lowered the insulin AUC at 60 minutes compared to the RTEC, and at 120 and 180 minutes compared to the RTEC and CB ( $p < .05$ ). Peak GR was lower compared to the RTEC ( $p < .05$ ). There were no differences between cognitive measures, mood or appetite ( $p > .05$ ). Findings suggest the composition of breakfast has potential to affect gluco-regulation in adults, and the need for further consideration of the effects on cognitive function, mood and satiety.

## 6.2 Introduction

### 6.2.1 Gluco-regulation

The regular consumption of breakfast promotes enhanced gluco-regulation compared to breakfast omission, and the composition of breakfast influences the GR and IR to the breakfast meal, and subsequent meals (chapter 1.6.1-3). Dietary interventions based on functional food ingredients, which are naturally rich in antioxidants, polyphenols, flavonoids and fibre, have shown potential for improving risk factors for metabolic disease in healthy adults, who are overweight or obese, when consumed over 4-8 weeks (chapter 2.0.2).

Associations between the composition of breakfast and improvements in the GR and IR of adolescents are generally attributed to the consumption of low-GI, fibre-rich, wholegrain-based breakfasts (chapter 1.6.3.2), which is also supported by findings from the adult literature (Liljeberg et al., 1999; Nestler, Barlascini, Clore, & Blackard, 1988; Nilsson, Östman, Granfeldt, & Björck, 2008; Pereira et al., 2011). However, there are no studies to date in healthy-weight adolescents or adults considering intakes of multiple functional foods at a single meal, namely breakfast, and the effects on gluco-regulation. In preparation for studying the effects of a functional food breakfast in adolescents in a school environment (chapter 7), the study was first considered in healthy adults in a laboratory-setting. This enabled the collection of measures in a controlled environment, reducing the risk of confounders, and contributed to the existing limited adult literature thus informing the power calculation for the adolescent study (chapter 7.3.12).

There are a number of experimental studies considering the role of individual functional foods (blueberries, baobab, oats, and cinnamon) on GR/IR in adults, which support overall benefits to gluco-regulation, attributed to their polyphenol and  $\beta$ -glucan properties (chapter 1.11), although to date there are no studies in adolescents. Polyphenols can help to regulate GR and IR through their ability to inhibit activities of key enzymes and transporters involved in starch degradation



(McDougall & Stewart, 2005) (chapter 1.10.3). In addition to their polyphenol properties, oats and baobab provide a rich source of fibre, in particular  $\beta$ -glucans, which can improve GR/IR and satiety potentially due to increasing viscosity and solubility (Slavin & Green, 2007) (chapter 1.11.2-3).

### **6.2.2 Cognitive performance, mood & satiety**

One study to date reports on the inclusion of multiple functional foods into the diet over 4 weeks and improvements in cognitive function in adults who were overweight, but free from disease (Nilsson et al., 2013). There is wider support from the literature for the benefits of individual functional food ingredients, particularly blueberries, and improvements in cognitive performance (chapter 1.11.1). However, methodological inadequacies from frequently cited studies (Krikorian et al., 2010a; Krikorian et al., 2010b) and the inclusion of older individuals showing signs of cognitive impairment, limit inferences to healthy adults with no signs of cognitive decline. Two recent studies in younger adults (18-35 years) reported improvements in memory and attention following the consumption of berries (including blueberries) (Dodd, 2012; Watson et al., 2015), suggesting that specific cognitive domains can benefit even in healthy individuals. A review of studies including children and older adults suggests that effects on specific cognitive domains may be more pronounced at certain times points over the life course, potentially due to developmental differences in the brain (Bell et al., 2015), although more studies are required to determine definitive conclusions.

Cognitive benefits are attributed to the high polyphenol content of berries, particularly anthocyanins (chapter 1.10.1.2). A review of polyphenol-rich sources identified berries (as well as cocoa and soy-isoflavones) as being beneficial to cognitive performance in adults, particularly immediate verbal memory and spatial memory (Lamport et al., 2012). There is emerging evidence around the benefits

of polyphenol-rich cinnamon and oats on cognitive performance (chapter 1.11.3-4); although studies are currently limited by small sample sizes (oats) and effects in pre-diabetics (cinnamon).

There is support from the literature that memory (verbal and spatial), attention and executive function, are the most sensitive domains to breakfast consumption (compared to breakfast omission) in young people (chapter 1.7.2), although specific to adolescents there was less support for benefits within the attention domain. In adults, verbal memory and selective attention were improved following the consumption of a functional food diet (Nilsson et al., 2013). A measure of verbal memory includes delayed recall which can be measured using word lists (chapter 1.7.3.1) and which has shown sensitivity to interventions assessing GI in adolescents (Smith & Foster, 2008), functional foods in adults (Nilsson et al., 2013) and breakfast interventions in young people (Defeyter & Russo, 2013; Ingwersen, Defeyter, Kennedy, Wesnes, & Scholey, 2007).

Spatial memory is a broad term encompassing domains of memory and attention (chapter 1.7.3.2). In previous studies with young children and adolescents (6-11 years) and university students (18-22 years), Mahoney and colleagues (2005; 2007) developed two map tasks which were both reported by the authors to measure spatial memory (Busch, Taylor, Kanarek, & Holcomb, 2002; Mahoney, Taylor, & Kanarek, 2007; Mahoney et al., 2005). However, the map task in younger children (6-8 years) was designed using pictures and stickers of previously learned objects, whereas the map task in older children (9-11 years) and university students was designed using words. Most neuropsychological studies on spatial memory function use object-location memory tasks (Kessels, Nys, Brands, van den Berg, & Van Zandvoort, 2006) and spatial memory tasks are generally developed based on this methodology (de Jager et al., 2014). The use of objects minimises the confounding effects of verbal mediation on spatial memory measures, which are inherently difficult to determine due to the interactions between other cognitive aspects including motor skills and visual processing (de Jager et al., 2014). In a systematic review of the effects of breakfast on

cognitive performance in young people (Hoyland et al., 2009), and a review guiding the selection of cognitive tests for use in nutrition interventions (de Jager et al., 2014), the Mahoney study (2005) is referenced as measuring spatial memory, and, as being a sensitive test to the effects of a breakfast intervention; however, the use of pictures and words uses different cognitive processes and therefore, reference to the tests as a true measure of spatial memory is equivocal. Furthermore, spatial memory is a complex domain which includes aspects of memory and attention suggesting that if effects are observed it is difficult to attribute these to specific domains. Although the current study initially selected the word-based map task as a measure of spatial memory, it is on reflection a cross-domain task; therefore, from here on it will no longer be referred to as measuring spatial memory, it will simply be referred to as a map task.

### **6.2.3 Breakfast development**

During the development of the FB (chapter 5), specific functional food ingredients were selected based on their ability to: exert favourable effects on GR and IR, promote enhanced cognitive function and positively influence mood and satiety. Additionally, other ingredients were selected (olive oil, honey and banana) as they are often promoted as healthier alternatives to butter (olive oil) and sugar (honey and banana) (Public Health England (PHE), 2016). Laboratory comparisons between the CB and the FB confirmed that the FB was a richer source of antioxidants (chapter 5.4.1), and polyphenols, and released sugar at a slower rate than the CB (chapter 5.4.3), suggesting potential for these results to be replicated *in-vivo*. The CB was devoid of the functional food ingredients, representing what a typically consumed breakfast might consist of. To test this hypothesis a third condition was introduced to the study which consisted of a commonly consumed commercial RTEC amongst Oxfordshire adolescents, identified from the findings of the questionnaire study (chapter 4).

#### **6.2.4 Aims and hypothesis**

The current study was developed to investigate three aims.

- 1) Assess the impact of a FB combining multiple functional food ingredients on GR and IR in healthy adults, compared with a CB and a RTEC (primary outcome).
- 2) Assess differences between breakfasts on cognitive function (map task and delayed word recall), satiety, mood and palatability (secondary outcomes).
- 3) Inform the implementation of the study protocol before being taken into schools (chapter 7).

#### Hypothesis:

Based on the inclusion of the functional food ingredients, it was hypothesised that the FB would promote enhanced gluco-regulation compared to the CB and the RTEC. Furthermore, the synergistic effects of the ingredients served as breakfast would enhance cognitive performance, mood and satiety. It was further hypothesised that there would be no difference between the CB and the RTEC as ingredients were matched and both breakfasts were devoid of the selected functional food ingredients.

## 6.3 Methods

### 6.3.1 Study design

A repeated measures crossover study design was used with each participant serving as his/her own control. Breakfast order was randomised using OBU randomisation generator (Oxford Brookes University, 2011). In the preceding 24 hours, unusual vigorous exercise, alcohol, nicotine and caffeine were avoided and after 21:00 hours only water was consumed. Test days were on the same day of the week where possible, separated by a minimum of two days and a maximum of eight days. All measures were collected within three weeks.

### 6.3.2 Participants

Twenty participants were assessed for eligibility. One participant was excluded due to allergies and three participants withdrew from the study for personal reasons. Prior to measurements, participants attended a screening session, either before or on the morning of the study, during which an information sheet and health questionnaire were completed (Appendix 3a-3b). Fasting blood glucose, anthropometric measures and blood pressure were measured, confirming that participants were in good health. Height was recorded to the nearest centimetre using a Stadiometer (Seca Ltd, UK) with subjects standing erect and without shoes. Body weight was recorded using the Tanita BC- 418 MA (Tanita UK Ltd) with subjects wearing light clothing and no shoes. BMI ( $\text{kg}/\text{m}^2$ ) was calculated with the standard formula  $\text{weight}/\text{height}^2$ . Blood pressure was measured using a digital blood pressure monitor (A&D UA-767, Oxfordshire, UK). All measures were collected in the laboratory at OBU. Study procedures were approved by the Ethical Advisory Committee at OBU according to the guidelines laid down in the Declaration of Helsinki. Exclusion criteria included:

- (i) diagnosis of medical condition or medication interfering with metabolism including diabetes (type 1 or type 2) or neurological illnesses
- (ii) BMI  $<18.5\text{kg}/\text{m}^2$  or  $>30\text{kg}/\text{m}^2$

- (iii) three consecutive fasting blood glucose readings  $>6.1$  mmol/L
- (iv) post-menopausal
- (v) very high polyphenol consumer as identified through food frequency questionnaire (Appendix 3c)
- (vi) food intolerance or allergy to breakfast ingredients
- (vii) blood pressure outside of normal range 110-120/75-85 mmHg

Participants were recruited from advertisements within the university and local press, and received a £25 voucher for taking part. Written informed consent was received from each participant prior to study measures (Appendix 3d).

### **6.3.3 Testing schedule**

The schedule for each testing day was identical (Figure 15). Upon arrival, and before the consumption of breakfast, baseline (T0) blood glucose, insulin, appetite, mood and performance on the map task were measured. Further blood measures and satiety scales were taken at 15, 30, 45, 60, 90, 120, 150 and 180 minutes after the start of breakfast, which was either the RTEC, the CB or the FB. The map task was completed at 0, 60 and 120 minutes and the delayed word recall task at 60, 120 and 180 minutes. Mood questionnaires were completed at 0, 60, 120 and 180 minutes. During testing, the previous evening's dinner was recorded and participants were asked to replicate this prior to subsequent sessions. A menu reminder was emailed at least 24 hours before subsequent test days, and during baseline measures participants recorded their evening meal to check compliance. Between measures, participants read quietly or watched films on a laptop. A light snack was provided at the end of each testing session.

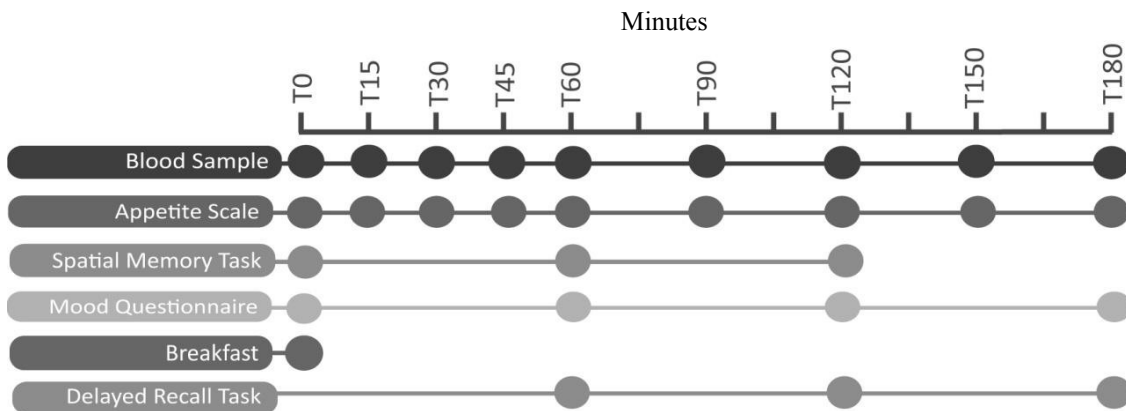


Figure 17. Testing day schedule

### 6.3.4 Test meals

The RTEC was chocolate Weetos served with milk, selected based on previous findings (chapter 4) which identified it as a commonly consumed breakfast cereal. CB and FB were prepared by the same researcher two days prior to testing, in the university kitchen, using a standardised recipe (Table 19) and protocol (chapter 5.3.5). Muffins were cooled rapidly and frozen for 24-72 hours in a -20°C freezer. The day prior to testing they were defrosted overnight in a dark container at room temperature to preserve antioxidants. Previous *in-vitro* analyses comparing frozen and freshly baked muffins had confirmed that the freezing procedure was not detrimental to their polyphenol profile or sugar-releasing potential (chapter 5.4.2 and 5.4.3). Nutrient profiles were verified by Eurofins Food Testing UK Ltd on which serving sizes were based (Table 23). Breakfasts were matched to contain 50g available CHO and similar amounts of energy, protein and fat (Table 26).

**Table 26.** Nutrient contents of all breakfast meals (per serving)

	Unit	RTEC <sup>a</sup>	CB <sup>b</sup>	FB <sup>b</sup>
Serving size; cereal or muffin	g	60.0	140.0	161.8
Serving size; milk	mL	180.5		
Energy				
	kJ	1475	1456	1406
	kcal	353	344	335
Protein	g	10.8	11.3	11.2
Fat	g	9.4	10.5	8.1
Total carbohydrate	g	53.7	52.5	58.4
Total sugars	g	22.6	10.6	14.1
Total fibre (AOAC)	g	3.7	2.5	8.4
Available carbohydrate	g	50.0	50.0	50.0

Abbreviations: AOAC, Enzymatic-Gravimetric Method.

<sup>a</sup> Nutrients as per manufacturer labels, <sup>b</sup> Nutrient profiles obtained from Eurofins UK Ltd

Breakfasts were coded as breakfast one (RTEC), two (CB) or three (FB). Participants were blinded from breakfast condition; however, there were clear differences between the ingredients which may have made the FB easily identifiable. Participants were supervised during eating and finished within 15 minutes. 200mL of water was consumed with breakfast and 100mL of water was consumed after 90 minutes.

### 6.3.5 Blood samples

Glucose and insulin were measured from finger-prick capillary blood samples obtained using a single-use BD Microtainer® contact-activated safety lancet (high flow, 1.5mm blade, 2.0mm depth; BUNZL Healthcare, Enfield, UK) at -5 min and 0 (baseline) and post breakfast at 15, 30, 45, 60, 90, 120, 150 and 180 minutes.

*Blood glucose:* After discarding the first drop, 5µL was drawn into a Hemocue Glucose 201 microcuvette and immediately analysed using HemoCue® 201<sup>+</sup> analyser (Radiometer Ltd, Crawley, UK). Baseline samples taken in duplicate or triplicate were checked to be within a CV of 3%.



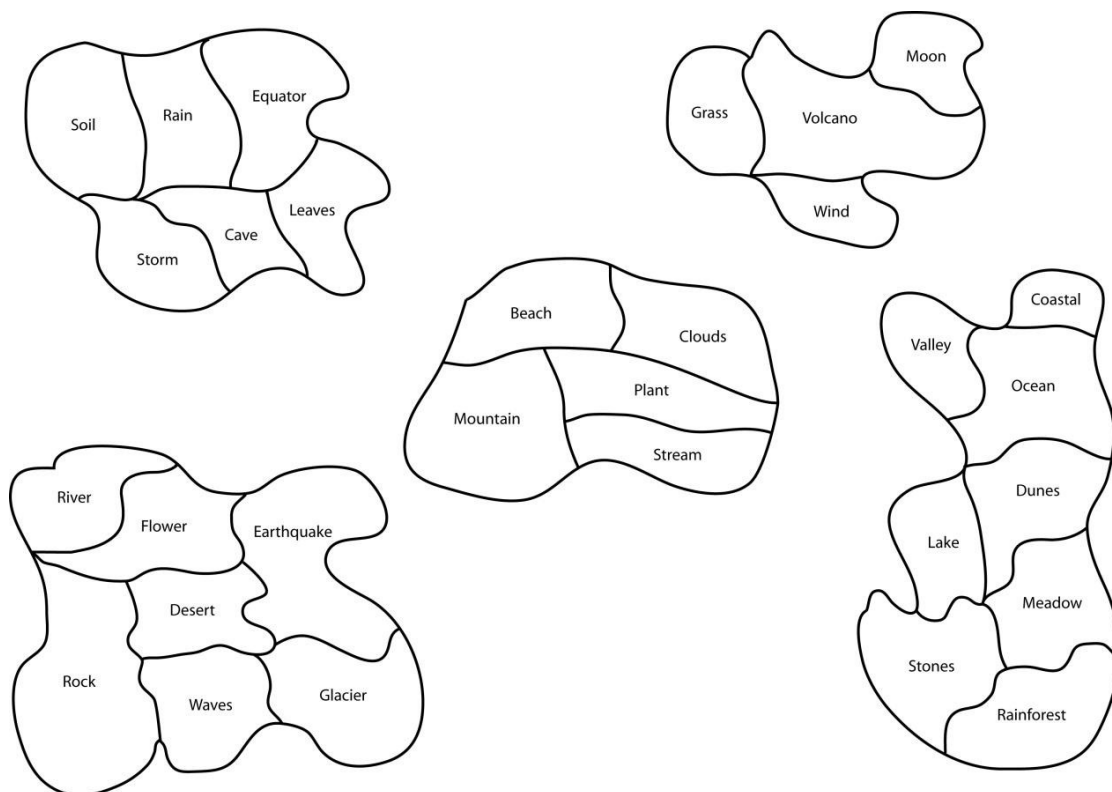
*Insulin:* 300µL of capillary blood was collected into EDTA-coated microtainers (Bunzl Healthcare, Enfield, UK) and placed immediately on ice. Samples were centrifuged at 4000rpm for 10 minutes and 200µl of the supernatant plasma removed and placed into 1.5mL Eppendorphs, frozen at -40°C until analysis. Insulin concentrations (µU/ml) in the plasma samples were determined by electrochemiluminescence immunoassay using an automated analyzer (Cobas® E411; Roche diagnostics). The Cobas® system has been found to be a reliable method of plasma insulin determination (Siahanidou, Margeli, Kappis, Papassotiriou, & Mandyla, 2011).

### **6.3.6 Map task**

A map task based on previous work from Mahoney and colleagues (2005) was developed (Mahoney et al., 2005). Permission for use was granted from the author and the task was piloted with non-participating staff and students ( $n=6$ ). The task consists of three maps which contain 25 fictitious countries within four continents based on nature, colours and animals. Due to ceiling effects during pilot work the difficulty of the task was intensified by increasing the number of words (countries) from 25 to 30 and time to memorise the map was reduced by one minute (Figure 18). Furthermore, more categories were added as nine separate tests (over three breakfast conditions) were required. The additional categories were included based on internet searches identifying commonly used lists of words. The selected categories ranged in perceived difficulty in an attempt to make it more relevant for use in university students and staff, as factors including age of acquisition of words and intelligence can influence performance (chapter 1.7.3). In a randomised order on three separate test days, participants were presented with each of the nine different categories (e.g. nature, sports, and vegetables on T1; animals, colours and transport on T2, fruit, household and anatomy on T3). Each category used a different map layout to reduce the memory interference observed with a within-participant design. To control for performance at baseline in the analysis, a baseline measure was

completed before breakfast on each test day. Performance was measured at T60 and T120 to correlate with blood measures which were also collected at these time points.

The final protocol required each map to be studied for seven minutes on a laptop following which a blank paper map and pencil were provided and two minutes were given to recreate the map from memory.



**Figure 18.** Example of ‘nature’ spatial memory map task

### **6.3.7 Delayed word recall**

To attempt to further control for influences on memory tasks (chapter 1.7.3) the list of words to be recalled was based on the map task, resulting in a long list of words which would potentially increase the difficulty (Benton et al., 2005). Pilot work in non-participating university students and staff confirmed that there were no ceiling effects, as is often seen in memory tests used in this population (Uttl, 2005).

In the final protocol participants were asked to write down as many words as they could remember from the previous map task in one minute (maximum score 30) (Appendix 3e). For example, if they had completed the ‘nature’ map at baseline then at T60 they would be asked to recall words, in any order, from the ‘nature’ map. As it was the previous map task that was being recalled, a baseline measure of verbal memory was not collected. Verbal memory was measured at T60, T120 and T180 to coincide with blood measures, which were also collected at these time points. The recall task was performed immediately before the next map task was given.

### **6.3.8 Mood**

A 20-item questionnaire was based on the ‘Activation–Deactivation Check List’ (ADACL) short form (Thayer, 1989) (Appendix 3f), which is theoretically derived and reported to have content and construct validity (Hammersley et al., 2014). Items were grouped to measure four components of mood: energy, tiredness, calmness and tension. Participants were asked to rate on a scale of 1 to 4 their subjective feelings of mood (1 = definitely do not feel, to 4 = definitely feel). The mood questionnaire was completed immediately after the cognitive tests at T0, 60, 120 and 180 minutes.

### **6.3.9 Satiety and palatability**

Visual analogue scales (VAS) consisting of 100mm lines were used to measure four components of participants' subjective feelings of satiety: hunger, fullness, desire and amount (Appendix 3f). The use of VAS scales in nutrition studies is widespread and they have been found to be a valid, sensitive and unbiased measure of satiety (Blundell et al., 2010). VAS scales were completed immediately after blood collection. Palatability, namely the pleasantness of the taste and appearance of the breakfast, was recorded using 100mm VAS scales 15 minutes after the breakfast.

### **6.3.10 Statistical analysis**

Data were analysed using Microsoft Excel 2010 and SPSS V.22 (Chicago, IL, USA). Values are mean  $\pm$  SD unless otherwise specified. The GR and IR were calculated geometrically as the incremental AUC for each test food, using the trapezoidal rule (FAO, 1998), and included the area above the fasting level only. Normality of the data was tested using the Shapiro-Wilk test. Blood glucose data were analysed using a two-way repeated measures ANOVA (breakfast x time point) to compare AUC means at time point 60, 120 and 180, and peak differences. Pairwise comparisons were performed using a Bonferroni correction. Insulin data were analysed using non-parametric Friedman tests based on abnormal distributions. Map tasks were analysed using a repeated measures ANCOVA with baseline map score entered as a covariate, and with analysis performed on the absolute scores for correctly named and located items, items left blank, and wrongly answered items. The recall task was analysed using a two-way repeated measures ANOVA (breakfast x time point) on correctly recalled items and blank items. Satiety and mood time points were analysed using a two-way repeated measures ANCOVA using baseline scores as a covariate. This is recommended practice to correct for baseline differences when analysing appetite scales (Blundell et al., 2010). Palatability data were analysed using a one-way repeated measures ANOVA comparing taste and appearance between breakfasts. There was a non-normal distribution for taste, but using studentised residuals ( $>\pm 3SD$ ) one participant was identified as an extreme outlier and removal of this participant normalised the data.

### **6.3.11 Power calculation**

The sample size was estimated based on the following assumptions:

- Power  $\geq 0.95$  was considered acceptable
- Significance under the null hypothesis was set at level of  $\alpha = 0.05$
- The primary outcome was GR and IR AUC

The method for glycaemic response and insulin testing used is in line with procedures recommended by the Food and Agricultural Organisation / WHO (FAO/WHO) (1998) and the International Organization for Standardization (ISO) 26642 (2010) guidelines (ISO, 2010; FAO, 1998). These state that to determine the GR of a food tests should be repeated in a minimum of 10 volunteers. The secondary outcome of the study was cognitive performance. In a previous meta-analysis of the glucose facilitation effect on cognitive performance a medium overall effect size of  $d=0.56$  was found (Riby, 2004). Using G\*power (Version 3.1.2) (Faul, Erdfelder, Lang, & Buchner, 2007) this revealed that for a medium effect size, with  $\alpha=0.05$  (two-tailed) and 0.95 power, a sample size of 16 was required.

## 6.4 Results

Measures were collected from sixteen adults (9 male, 7 female) (Table 27).

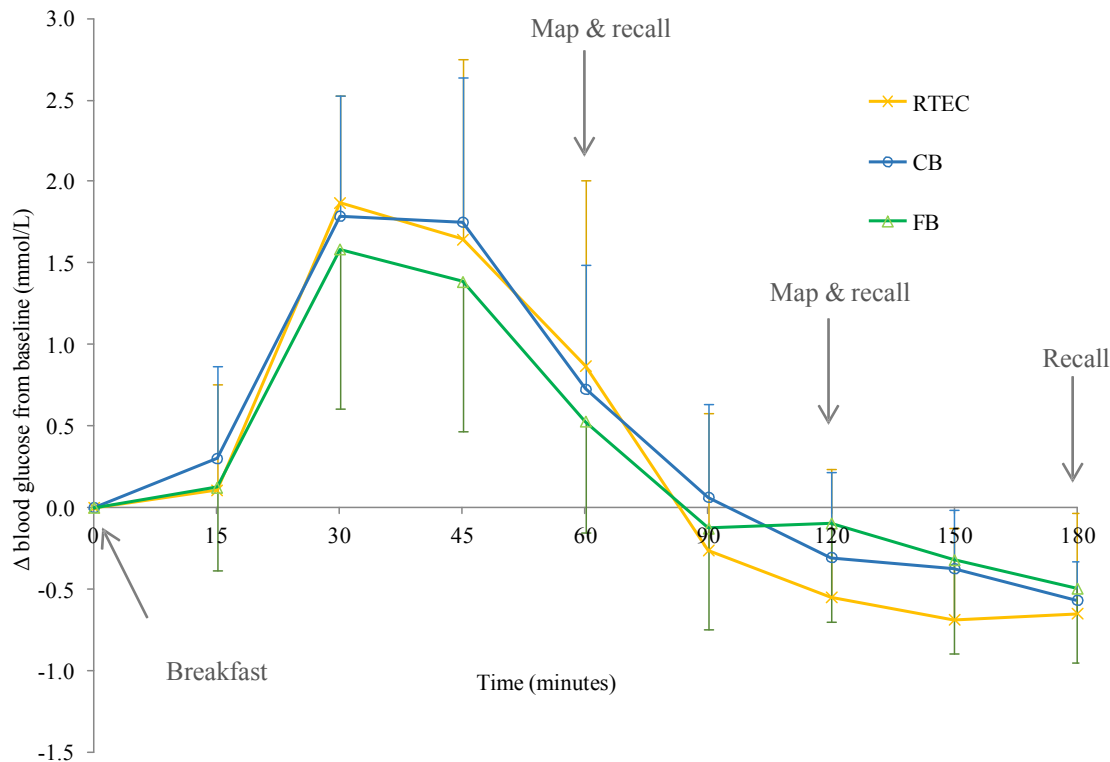
**Table 27.** Baseline characteristics of sample ( $n=16$ )

		All ( $n=16$ )	Males ( $n = 9$ )	Females ( $n = 7$ )	$p$ -value
	Unit	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Age	years	32 $\pm$ 10	33 $\pm$ 9	30 $\pm$ 10	>.05
Weight	kg	71.9 $\pm$ 8.5	74.9 $\pm$ 8.5	68.0 $\pm$ 7.5	>.05
Height	m	1.7 $\pm$ 0.1	1.8 $\pm$ 0.1	1.7 $\pm$ 0.1	>.05
BMI	kg/m <sup>2</sup>	23.8 $\pm$ 2.7	23.8 $\pm$ 2.0	23.9 $\pm$ 3.6	>.05
Fasting glucose	mmol/L	4.4 $\pm$ 0.3	4.4 $\pm$ 0.3	4.4 $\pm$ 0.4	>.05

Abbreviations: *BMI*, body mass index

### 6.4.1 Glucose response

There were no significant differences between breakfast AUC values at T60 (RTEC 63.34  $\pm$  33.74; CB 63.94  $\pm$  29.02; FB 52.56  $\pm$  33.66 mmol/L/min), T120 (RTEC 84.38  $\pm$  54.95; CB 81.81  $\pm$  42.53; FB 69.08  $\pm$  44.50 mmol/L/min) or T180 (RTEC 86.63  $\pm$  56.43; CB 83.04  $\pm$  42.66; FB 75.13  $\pm$  51.16 mmol/L/min) (all  $p > .05$ ) (Figure 19).

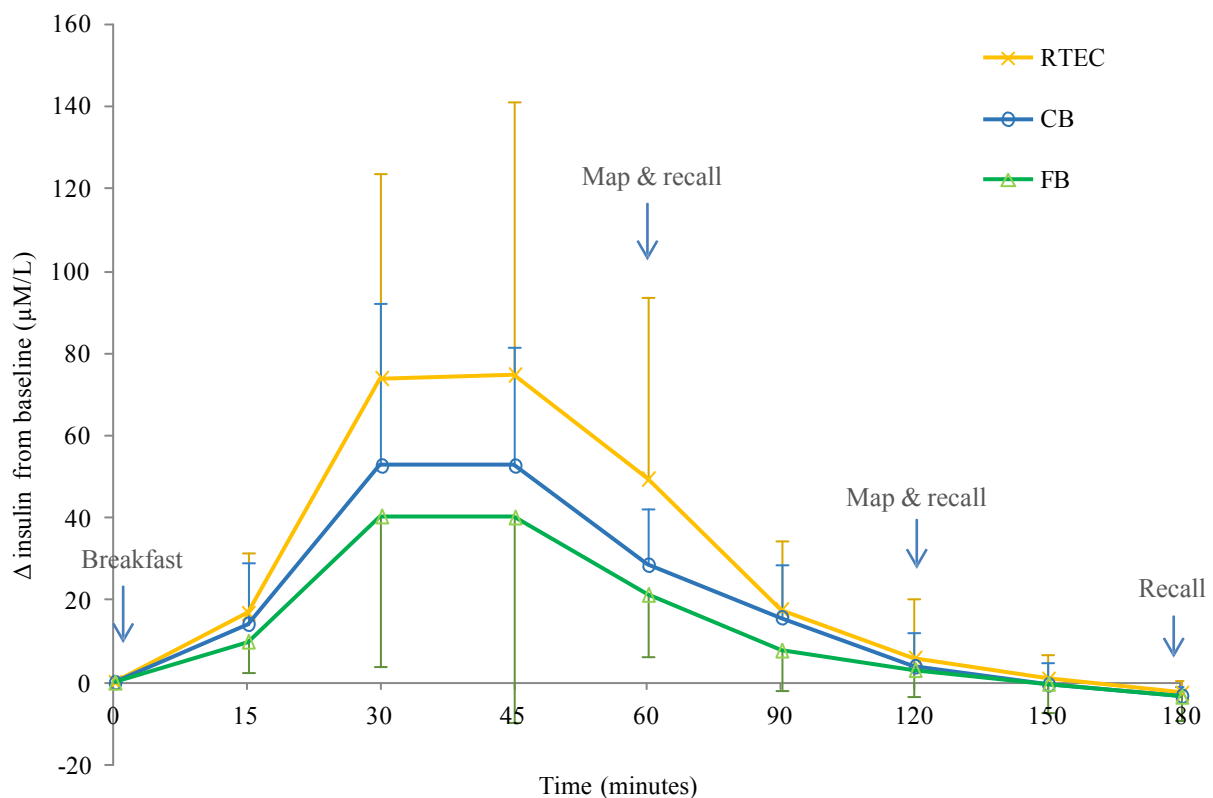


**Figure 19.** Change from baseline in glucose response (GR) ( $n = 16$ ). Values are mean difference  $\pm$  SD.

However, there was a significant difference between breakfasts in peak glucose concentration ( $F(2, 30)=4.16, p=.025$ ). Post hoc analysis performed using a Bonferroni adjustment revealed a higher peak GR following the consumption of the RTEC ( $6.55 \pm 0.82$  mmol/L) compared to the FB ( $5.99 \pm 0.90$  mmol/L), a significant difference of 0.56 (95% CI 0.02 to 1.09) mmol/L ( $p=.042$ ).

#### 6.4.2 Insulin response

Insulin AUC concentrations were significantly different between breakfasts at T60 ( $\chi^2(2) = 18.00, p<.001$ ), T120 ( $\chi^2(2) = 19.63, p<.001$ ) and T180 ( $\chi^2(2) = 19.63, p<.001$ ) (Figure 20).



**Figure 20.** Change from baseline in insulin response (IR) ( $n = 16$ ). Values are means  $\pm$  SD.

Post hoc analysis performed using a Bonferroni adjustment (median scores reported) revealed significant differences in absolute insulin concentration at T60 between the FB ( $1034.7\mu\text{M/L}$ ) and the RTEC ( $2158.0\mu\text{M/L}$ ;  $p<.001$ ). At T120 the FB ( $1329.9\mu\text{M/L}$ ) was significantly lower than the

RTEC (2893.9 $\mu$ M/L;  $p < .001$ ) and the CB (2559.6 $\mu$ M/L;  $p = .040$ ). Again, at T180 the FB (1457.7 $\mu$ M/L) was significantly lower than the RTEC (2931.10 $\mu$ M/L;  $p < .001$ ) and the CB (2612.4 $\mu$ M/L;  $p = .040$ ).

Additionally, peak insulin concentration was significantly different between breakfasts ( $\chi^2(2) = 19.63$ ,  $p < .001$ ) where the FB (36.35 $\mu$ M/L) was significantly lower than the RTEC (75.15 $\mu$ M/L,  $p < .001$ ) and the CB (60.60 $\mu$ M/L,  $p = .040$ ).

### 6.4.3 Cognitive tests: map task and delayed recall

There were no effects of breakfast on memory performance for either the map task (Table 28) or the delayed word recall task (Table 29). Furthermore, GR did not significantly correlate with any of the cognitive measures.

**Table 28.** Mean scores  $\pm$  SD for map task at T0, T60 & T120 in RTEC, CB and FB. Baseline score for the map task was used as covariate in map task analysis.

	T0			T60			T120			<i>p</i> -value
	RTEC	CB	FB	RTEC	CB	FB	RTEC	CB	FB	
Correct items	18 $\pm$ 8	19 $\pm$ 8	17 $\pm$ 8	20 $\pm$ 7	20 $\pm$ 8	18 $\pm$ 8	20 $\pm$ 8	20 $\pm$ 9	19 $\pm$ 8	>.05
Wrong items	2 $\pm$ 2	2 $\pm$ 2	3 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2	>.05
Blank items	10 $\pm$ 6	8 $\pm$ 6	10 $\pm$ 7	9 $\pm$ 6	7 $\pm$ 6	10 $\pm$ 6	8 $\pm$ 6	8 $\pm$ 7	9 $\pm$ 6	>.05

Abbreviations: RTEC, Weetos breakfast cereal; CB, control breakfast; FB, functional food breakfast

**Table 29.** Mean scores  $\pm$  SD for delayed recall task measured at T60, T120 & T180 in RTEC, CB and FB.

	T60			T120			T180			<i>p</i> -value
	RTEC	CB	FB	RTEC	CB	FB	RTEC	CB	FB	
Correct items	13 $\pm$ 3	13 $\pm$ 5	13 $\pm$ 5	12 $\pm$ 5	13 $\pm$ 5	12 $\pm$ 6	16 $\pm$ 5	15 $\pm$ 5	15 $\pm$ 4	>.05
Wrong items	0 $\pm$ 0	0 $\pm$ 1	0 $\pm$ 1	0 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 1	0 $\pm$ 1	>.05
Blank items	17 $\pm$ 4	16 $\pm$ 6	18 $\pm$ 5	18 $\pm$ 4	15 $\pm$ 6	18 $\pm$ 6	14 $\pm$ 5	14 $\pm$ 6	15 $\pm$ 4	>.05

Abbreviations: RTEC, Weetos breakfast cereal; CB, control breakfast; FB, functional food breakfast

For delayed word recall there were no significant interactions between breakfast and time for the number of items correctly recalled ( $F(4,60)=0.47$ ,  $p=.761$ ). Furthermore, breakfast had no effect on the number of items correctly recalled ( $F,(2,30)=0.25$ ,  $p=.784$ ); however, there was a main effect of

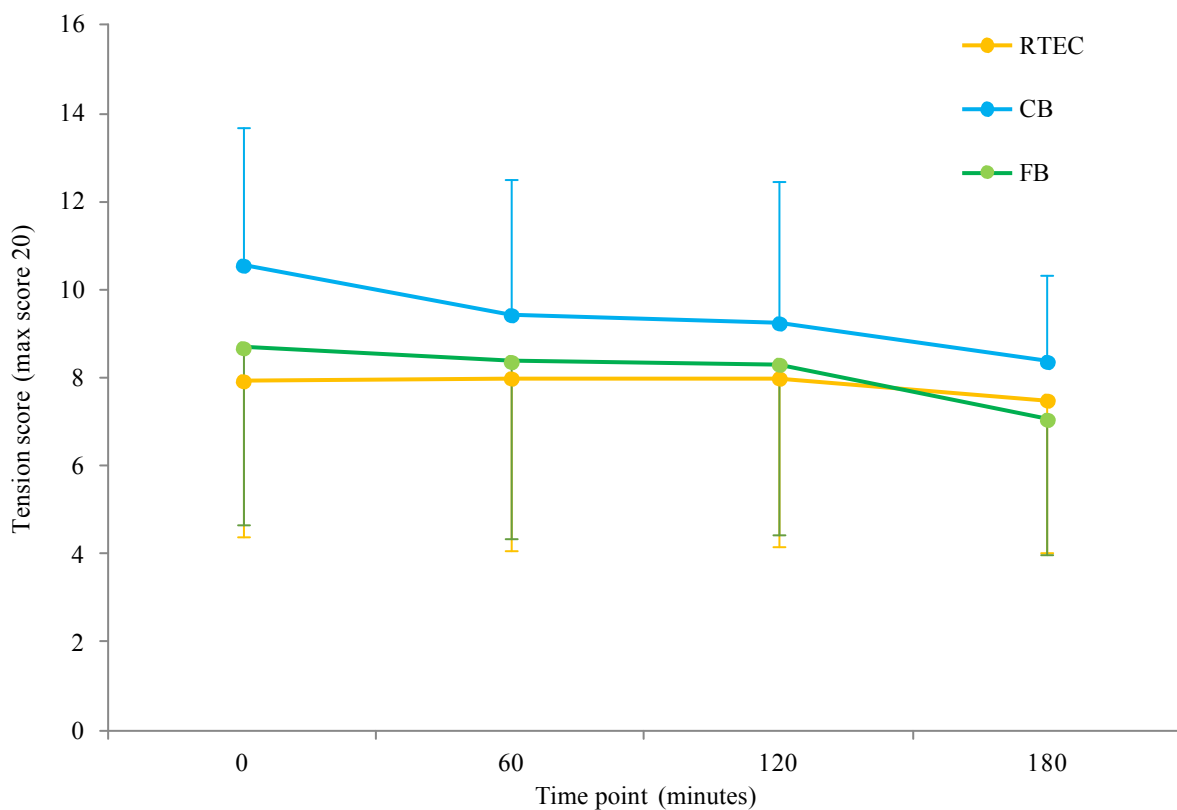


time ( $F(2,30)=8.73, p<.001$ ), post hoc analysis (Bonferroni adjustment) revealed that performance improved and at the end of the test (T180) participants recalled significantly more words ( $15.17 \pm 4.35$ ) compared to T120 ( $12.29 \pm 5.01, p=.002$ ) and T60 ( $12.67 \pm 4.28, p=.002$ ).

#### 6.4.4 Mood, satiety and palatability

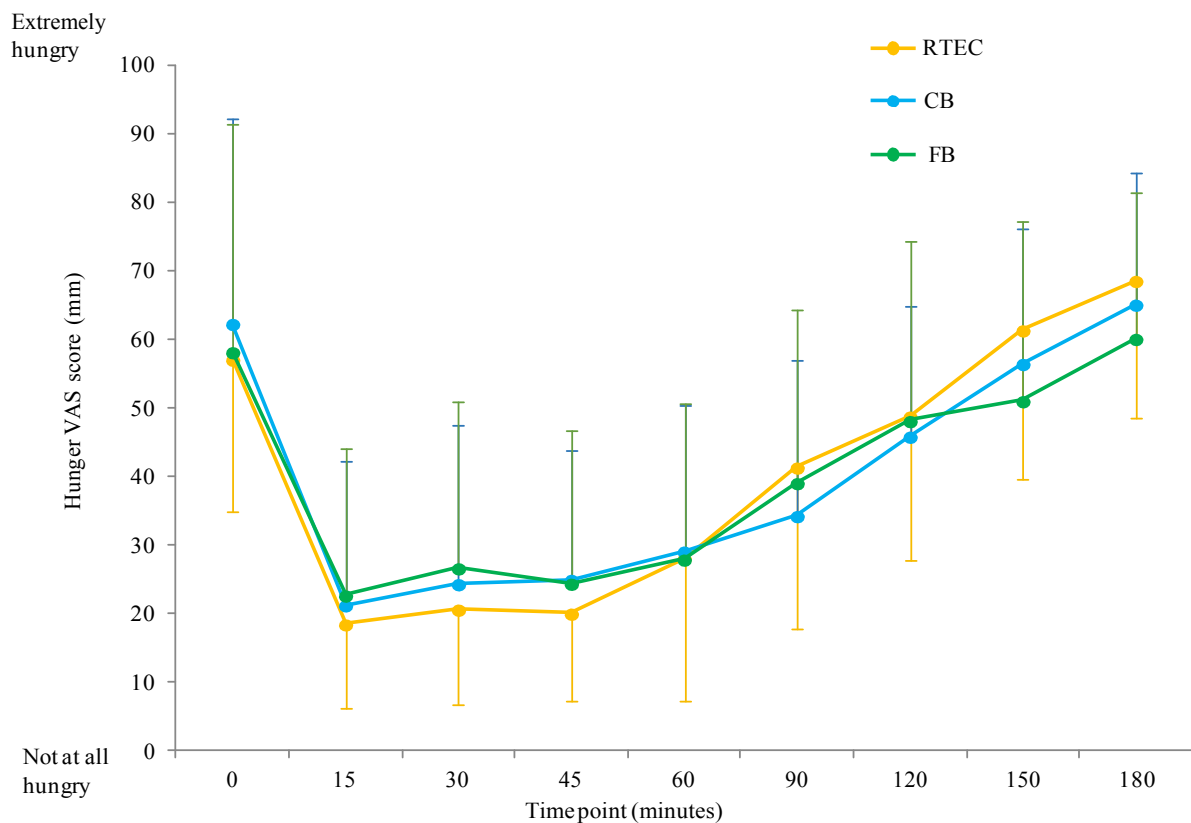
Mood was not affected by consumption of any of the breakfasts ( $p>.05$ ). There were no significant interactions between breakfast and time on energy ( $F(4,86)=0.775, p=.545$ ), tiredness ( $F(3,74)=1.148, p=.338$ ), calmness ( $F(3,75)=0.211, p=.912$ ) or tension ( $F(4,86)=0.523, p=.719$ ).

There was an overall main effect of time on tension ( $F(1,43)=11.568, p<.001$ ). Post hoc analysis revealed that tension was scored higher at baseline ( $9.06 \pm 3.55$ ) but then steadily decreased over the testing session by 1.42 (CI, 0.34 to 2.49,  $p=.007$ ) points to  $7.65 \pm 2.84$  (Figure 21).



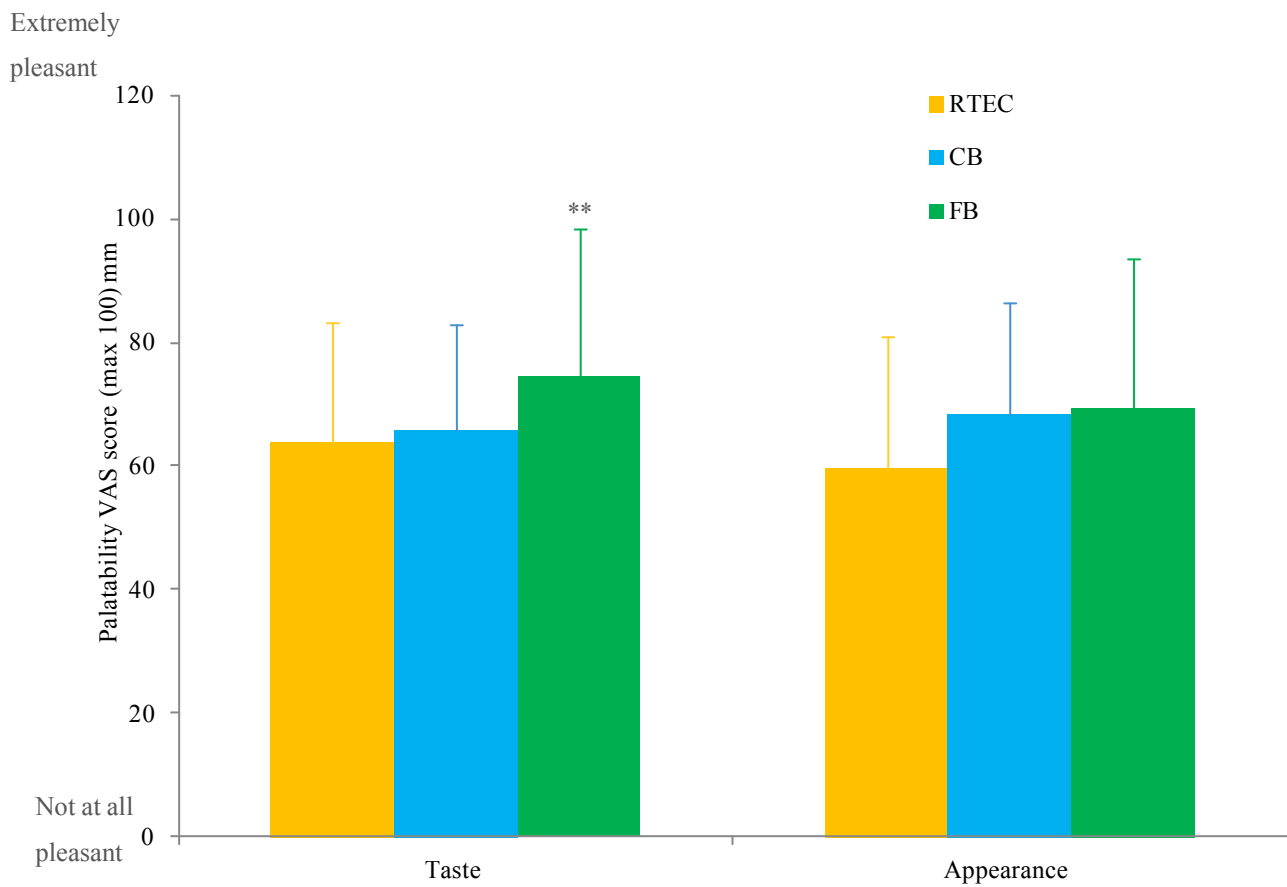
**Figure 21.** Tension score (means  $\pm$  SD) recorded at T0, T60, T120 and T180 minutes in all ( $n=16$ ) participants

Satiety was not affected by consumption of any of the breakfasts ( $p > .05$ ). There were no significant interactions between breakfast and time on hunger ( $F(4,86)=1.061, p=.362$ ), fullness ( $F(4,86)=.448, p=.662$ ), desire to eat breakfast ( $F(4,86)=.476, p=.643$ ) or amount of food that could be eaten ( $F(4,86)=.362, p=.721$ ). As expected, there was a main effect of time on hunger ( $F(2,86)=5.908, p=.015$ ). Post hoc analysis using Bonferroni adjustment revealed that hunger ratings significantly increased over the testing period ( $p < .001$ ) (Figure 22).



**Figure 22.** Hunger scores (mean  $\pm$  SD) as measured by VAS scale in all participants ( $n=16$ ).

There were no significant differences in rating the appearance of the breakfasts ( $F(2,30)=1.235, p=.305$ ); however, there were significant differences in rating taste ( $F(1.41,19.75)=5.343, p=.022$ ). Post hoc analysis (Bonferroni adjustment) revealed a significantly higher rating given to the FB ( $78.80 \pm 14.0$ ) compared to the CB ( $63.10 \pm 18.8, p=.010$ ) and the RTEC ( $64.53 \pm 19.2, p=.015$ ) (Figure 23).



**Figure 23.** Palatability scores (mean ± SD). Participants were asked to rate ‘How pleasant is the taste of the breakfast?’ ( $n=15$ ) and ‘How pleasant is the appearance of the breakfast?’ ( $n=16$ ). \*\* $p<.05$  compared to CB and RTEC.

## 6.5 Discussion

The primary outcome of this study was to measure the effects of a breakfast based on functional food ingredients with health-promoting properties, on gluco-regulation in healthy adults.

### 6.5.1 Gluco-regulation

Consumption of the FB resulted in a significantly lower glucose peak compared with the RTEC, and a significantly lower insulin peak compared with the RTEC and the CB. Additionally, insulin AUC for the FB breakfast was significantly lower than the RTEC at T60 and significantly lower than both breakfasts after T120 and T180.

All three breakfasts were matched to provide 50g of available CHO and contained similar levels of energy, fat and protein (Table 23). The FB included functional food ingredients (blueberries, oats, cinnamon, baobab) selected for their potential to improve gluco-regulation (chapter 1.11), as well as ingredients which are advocated as healthier alternatives (honey, bananas, olive oil) (Public-Health-England, 2016).

The combination of these ingredients naturally increased the fibre content of the FB (8.4g) compared to the CB (2.5g) and the RTEC (3.7g). *In-vitro* analysis revealed that the FB released sugar at a slower rate during the later stages of digestion, which could be attributed to the additional fibre, or to the polyphenol levels, which were also significantly higher than the CB (chapter 5.4). Based on *in-vitro* findings the FB was expected to produce a lower overall GR *in-vivo*, but the current study findings did not support this. Including laboratory analysis of the CB and FB using *in-vitro* digestion and antioxidant analysis methods, gives strength to the current study's findings, but highlights the difficulties replicating laboratory findings to *in-vivo* studies. Nilsson and colleagues (2008) found that increasing the fibre content of breakfast through the inclusion of low-GI, whole-grain cereal products improved GR over 120 minutes in healthy adults ( $n=12$ ), at breakfast and at

subsequent meals, compared to white bread (Nilsson et al., 2008). However, when whole-grains were ground to flour the effect was attenuated. Oats and baobab were the predominant sources of fibre in the FB, but both were provided in a powdered form which may have rendered the starch more susceptible to digestive enzymes, thus reducing potential effects on GR and satiety.

The FB contained significantly higher polyphenol levels (229.7mg) compared to the CB (147.1mg). The addition of polyphenol-rich foods to starch- or sugar-rich CHO meals has been found to improve GR and IR in healthy adults, although to varying extents. A mixed berry puree sweetened with sucrose was beneficial to GR during the early phases of testing and at 150 minutes, compared to a matched sucrose control (Törrönen et al., 2010), but there was no difference in GR total AUC. The berry puree delivered an estimated 800mg of polyphenols, which was considerably higher than polyphenols delivered by the FB; however, polyphenol content was not measured as part of the study, and their analysis of individual time points may have increased the likelihood for type 2 errors. In another study from the same group, early phase benefits to GR (at individual time points) and effects on GR total AUC were reported when 150g of berry nectar or puree was added to sucrose, compared to a control, which, compared to the berry conditions, was not matched for available CHO (Törrönen et al., 2012b). It is preferable for meals to be matched on all nutrients and compounds to reduce the potential for confounding factors that may influence metabolism, and in the current study, available CHO was matched between breakfasts; however, total sugars between the muffins and the RTEC varied (14.1g FB, 10.6g CB, 22.6g RTEC). Despite these CHOs being potentially 'unavailable' for digestion, there is potential for metabolism to be affected through fermentation (FAO, 1998), or indeed through other unknown mechanisms, which should be considered in the interpretation of the study's findings.

Results have been more varied when polyphenol-rich berries were incorporated into starch-rich foods. In three acute studies (range  $n=13$  to  $n=20$ ), Törrönen and colleagues (2013) reported that the

addition of 150g of berry puree to a low-fibre white bread and a high-fibre rye bread significantly lowered the IR total AUC by relatively similar amounts, with a concurrent early phase reduction on GR AUC (Törrönen et al., 2013). The amount of berries used (150g) was considerably higher than the current study (30g), and although the total polyphenol content of the meals was not measured, findings suggest that the addition of polyphenol-rich berries may improve gluco-regulation, in addition to the GR and IR benefits from consumption of a high-fibre, low-GI meal. Conversely, Clegg and colleagues (2011) reported no glucose-lowering effects of blueberries or raspberries when added to pancakes, and no differences in reported satiety ( $n=12$ ) (Clegg et al., 2011). However, only 100g of berries were used suggesting a potential dose-response for polyphenol effects on GR. Furthermore, insulin was not measured and the effects for this may have been more pronounced. A systematic review by Coe and colleagues (2016) suggests that the addition of polyphenol-rich sources may reduce peak IR and sustain the IR (chapter 1.10.3), particularly when added to bread (Coe & Ryan, 2016).

In the current study peak glucose was significantly lower in the FB compared to the RTEC, whereas a smaller reduction was observed between the FB and CB (0.53mmol/L), which was approaching significance ( $p=.099$ ). It has been suggested that a reduction in peak glucose may be more important than lowering overall GR for the development of cardiovascular complications (Ceriello, 2005), which, based on current findings has implications for deciding the composition of breakfast to consume. Törrönen and colleagues (2010) reported that the berry puree significantly reduced peak glucose by 1.0mmol/L compared to the sucrose control, which they attributed to the anthocyanin content of the blueberries and blackberries (Törrönen et al., 2010). The polyphenol contents of berries, or other food sources, as well as the physical forms of delivery, will inhibit starch-degrading digestive enzymes to differing extents (Johnson et al., 2011). In the current study the CB

also contained polyphenols (although to a lesser extent than the FB), and this may in part explain the lack of a significant difference between CB and FB peak glucose values.

Generally, effects on peak insulin appear to be more consistent when polyphenol-rich berries and baobab are added to starch-rich (Coe & Ryan, 2015; Törrönen et al., 2013) or sugar-rich (Törrönen et al., 2012b; Törrönen et al., 2012a) CHO foods. In contrast, effects on total insulin AUC have only been reported when berries (Törrönen et al., 2013) or baobab (Coe & Ryan, 2015) were added to starch-rich CHO sources. Coe and colleagues (2015) reported a significant improvement in IR AUC during later phase digestion, and insulin peak, following the consumption of white bread enriched with baobab, compared to green-tea enriched and matched control bread. The brand and percentage of baobab extract used was the same as in the current study and delivered 61.24mg of added polyphenols, although total polyphenol content after baking was not measured. Benefits were potentially attributed to the polyphenol and/or fibre content of baobab. Baobab is high in tannins and flavonoids, which have been associated with improved insulin sensitivity *in-vitro* and in mice, but not yet in humans. Baobab is also high in soluble fibre, but the fibre content of the bread was low (1.2g) and was therefore less likely to be driving the effect. The findings from the current study suggest that the acute benefits from individual foods may also work synergistically when functional foods are combined and delivered as a breakfast meal. This adds to the currently limited literature which reported the potential for the inclusion of multiple functional ingredients into the diet over eight weeks, and improvements in insulin sensitivity (Tovar et al., 2015).

### **6.5.2 Cognitive performance, satiety and mood**

The secondary outcome of the current study was to investigate the effect of the FB on cognitive performance, mood and satiety, and identify associations between cognitive performance and blood measures. There were no significant differences between cognitive scores on the map task or the

recall task, or no differences between satiety and mood, regardless of which breakfast had been consumed. Furthermore, there were no significant associations between cognitive performance and GR or IR.

Functional food ingredients selected for the FB have been studied for their cognitive-enhancing properties, mostly relating to their polyphenol content, particularly the anthocyanins, which predominate in blueberries and other dark red/blue/purple foods. The studies by Krikorian and colleagues (chapter 1.11.4.2) delivered large amounts of polyphenols (>1000mg) via a blueberry or grape juice drink over 12 weeks, and reported significant improvements in memory in adults with MCI (Krikorian et al., 2010a; Krikorian et al., 2010b). Hendrickson and colleagues also considered the effects of grape juice in young adult smokers ( $n=36$ ) (Hendrickson & Mattes, 2008), but they reported no improvements to performance on an implicit memory task, or to mood, despite serving a large amount of polyphenols (>1260mg).

Similar to the current study, a few recent studies have considered young, healthy populations. A RCT in adults ( $n=36$ ; 18-35 years) comparing blackcurrant extracts delivering 525mg polyphenols per 60kg bodyweight, observed improvements to performance on two attention tasks compared to a sugar-matched control, with no effect on mood (Watson et al., 2015). Although performance on both tasks was enhanced, this was specific to whether the juiced or powdered extract was consumed. Furthermore, blood glucose levels were significantly different between the juice and the control, but not between the powder and control. The anthocyanin content varied slightly between extracts, but findings suggest that the way an extract is prepared can influence physiological and cognitive outcomes. The current study provided polyphenols via the whole fruit, ground flour and a powdered extract; therefore, it is unclear what influence this may have had, if any, on cognitive performance.



It is suggested that cognitive benefits in healthy adults may only present during the later stages of testing (Bell et al., 2015). A RCT in young adults ( $n=19$ ) reported that letter-memory performance was improved following consumption of freeze-dried berries (200g fresh equivalent) compared to a sugar-matched control; however, effects were only observed after 5 hours, and there were no reported effects on other measures of executive function, memory or mood (Dodd, 2012). In the same group of studies, some effects in older adults ( $n=18$ ) were observed at 2h and 5h, but only on immediate word recall. Total polyphenol levels were not reported, but the serving delivered 631mg of anthocyanidins, which suggests effects at high intakes of anthocyanidins specific to age-related cognitive domains and potentially longer testing periods.

In theory, the relatively high-fibre content of the FB could have slowed gastro-intestinal transit, which is associated with improvements in GR/IR and satiety, and which may potentially improve cognitive performance and mood in the later stages of testing (chapter 1.11.6.1); however, the current study's findings did not support this. Nilsson and colleagues (2012) reported improvements in selected attention following the consumption of a low-GI bread enriched with guar-gum, compared to a high-GI control bread, in healthy adults ( $n=40$ ; 49-71 years) (Nilsson et al., 2012). Cognitive benefits were observed in the later phases of testing (75-225 minutes) and were associated with improved gluco-regulation, emphasising that slowing the rate of glucose release and the duration of elevated blood glucose levels, can independently benefit cognitive performance (Benton et al., 2003; Pollitt & Mathews, 1998). This suggests that it may be effects of the GI of the food, and not the fibre content, that elicits improvements to cognitive performance.

Improvements in memory were observed where the consumption of an active diet (AD), based on multiple functional food ingredients, significantly enhanced aspects of attention and working memory in healthy adults ( $n=44$ , 50-73 years), compared to a control diet (Nilsson et al., 2013). Benefits were attributed to the inclusion of polyphenol-rich blueberries, which were provided in

greater quantities (74.5-94.5g) than in the current study (30g), as well as to the AD containing low-GI foods, and omega-3 fatty acids. Cinnamon was also included at higher quantities (3g) than the current study (1g). The polyphenol content was not measured, but the AD was consumed over six weeks, suggesting that measurable cognitive improvements may be associated with the regular consumption of functional foods, potentially using larger quantities; therefore, effects from a single meal over a sustained period warrants further investigation.

### **6.5.3 Satiety and mood**

The fibre content of the FB may not have been enough to elicit a measurable effect on satiety. The amount of  $\beta$ -glucan in the FB was estimated to be at least 1.5g, plus an additional contribution from baobab, although the exact value was not measured. Humans lack the enzyme to hydrolyse  $\beta$ -glucan which increases viscosity during digestion, and a significant inverse relationship between  $\beta$ -glucan dose and glucose peak or glucose AUC has been observed at servings of  $\sim$ 5g  $\beta$ -glucan (Tappy et al., 1996). However, some effects on IR and satiety have also been observed with breakfasts varying in  $\beta$ -glucan, ranging from 2.2g to 5.7g per serving (Beck et al., 2009a).

Mood can be influenced by a number of factors including: the alleviation of hunger, the composition and palatability of the meal, performance on a cognitive test (Hammersley et al., 2014) and the GR to the meal (Benton and Owens, 1993). In the current study there were no significant differences between mood scores regardless of which breakfast was consumed, but palatability of the FB was significantly higher than the other breakfasts. It is possible that the overall effect of eating was stronger than effects related to the composition of the breakfasts.

The inclusion of banana in the FB may have mediated positive effects on mood by increasing levels of tryptophan, which enhances serotonin levels (Fernstrom & Wurtman, 1971) and reduces low mood (chapter 1.9.2.3). However, protein consumed at the same meal, in amounts smaller (<5%)

than were delivered from the CB or FB, can inhibit this effect (Teff et al., 1989), making it unlikely to be a contributing factor in the current study

#### **6.5.4 Map task and word recall task design**

The cognitive tests used in the current study were carefully selected based on cognitive domains which have shown sensitivity to the effects of an oat-based breakfast compared to no breakfast (Mahoney et al., 2005) and reliability from their use in previous studies (Busch et al., 2002; Mahoney et al., 2007; Mahoney et al., 2005). In the current study, the map word task was extended from the original version used in children and young adolescents (9-11 years) (Mahoney et al., 2005) to increase the difficulty relative to adults, and to account for the increased frequency of delivery. The map object task, which was used previously in younger children (6-8 years) by Mahoney and colleagues (2005) was considered too easy by the authors to use in the older children, therefore, this was not considered for use in adults in the current study. The current study observed no difference between performance on the word map task regardless of breakfast composition, which may be a true effect, but which may also suggest that the sensitivity of the word map task to detect differences between the compositions of breakfasts should be considered further. When used previously with university students to compare the effects of a snack (Mahoney et al., 2007), an additional test ran concurrently thus increasing the task complexity. If future studies consider using the map task, this could form part of study designs.

Map scores indicated a trend for performance to increase over time suggesting a potential practice effect, despite the use of different map layouts and random order allocation of the tasks which was expected to have accounted for some of this. As part of the development process the cognitive tests were piloted in an adult population where no ceiling effects were observed; however, during the study a ceiling effect was observed where three participants obtained maximum marks across six of

the map tasks, subsequently reducing the overall power of the study's cognitive findings. This may have been due to differences in age of acquisition of verbal material, educational attainment between participants (Benton et al., 2005), or differences in implicit learning strategies, which was not measured as part of the study (chapter 1.7.3). The map tasks may also have varied in terms of their concreteness and imagery which should be considered in future work using either of these tests. Although most participants were recruited from the university, some external participants were recruited through local media; therefore, levels of education could have varied considerably between participants. Completing the test three times on each visit and the length of each map task (nine minutes), may have induced boredom and reduced motivation of participants, although a decrease in the number of blank items was observed over the testing period, and there was no indication of this from the mood questionnaire. However, the mood questionnaire was completed immediately after the cognitive tests which may have represented perceived performance on the cognitive task. Furthermore, habitual breakfast frequency was not measured, which may have potentially affected performance on cognitive tasks (Dye & Blundell, 2002) and mood (Lloyd, Rogers, Hedderley, & Walker, 1996).

During the word recall task participants recalled significantly more items at T180 than at earlier time points. This may have been due to a reduction in the overall cognitive load at T180, as at this time point only the recall task (without the map task) was completed, and participants reported feeling significantly less tense during the later stage of testing. The number of non-responses in the recall task was generally greater than the number of responses, which could indicate reduced motivation, but which may also highlight the difficulty of the task in the given time frame.

## **6.6 Conclusions**

The findings from this study highlight the potential of combining selected functional food ingredients into a breakfast (FB) to work synergistically to improve gluco-regulation, particularly

peak glucose and insulin levels, and IR AUC in healthy adults. The FB provided a rich source of polyphenols and fibre, and it is likely that benefits were attributed to either, or both, of these properties. The breakfasts were matched closely for macronutrients and available CHO, but not for total sugars or fibre; therefore, benefits attributed to specific ingredients or compounds should be considered in the context of these differences. However, the purpose of this study was to consider differences between breakfasts as they would be consumed in everyday settings, where foods are consumed in combination. The findings indicated no difference between the RTEC and CB suggesting that these breakfasts represented similar concepts. This resulted in the removal of this condition for the school study in order to reduce participant burden for blood and cognitive measures. Based on results from the current study, the school study (chapter 7) was designed to recruit a larger sample size to potentially address this confounding issue.

## **Chapter 7: The effects of a functional food breakfast on gluco-regulation, cognitive performance, mood and satiety in adolescents**

### **7.1 Summary**

Adolescent breakfast studies have considered breakfast composition and its effects on gluco-regulation and cognitive performance, mood and satiety in the context of GI and/or GL only.

Previous research performed as part of this PhD (chapter 6) supports the consumption of a FB, rich in antioxidants, polyphenols, flavonoids and fibre, and improvements in gluco-regulation in adults.

The aim of the current study was to continue with the assessment of the FB and investigate its potential to enhance GR and IR, cognitive performance, mood and satiety, compared to a CB, in adolescents in a school environment.

In a crossover study 22 healthy adolescents (13-15 years) consumed a FB and CB matched for available CHO, energy, fat and protein. GR and IR were measured at baseline and at 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Additional measures were collected at timed intervals including cognitive performance, appetite, mood and palatability. Peak glucose and peak insulin were higher after consumption of the CB compared to the FB ( $p<.05$ ). Furthermore, insulin AUC values were significantly lower after consumption of the FB at T60 ( $p=.046$ ) and T120 ( $p=.041$ ). There were no effects of breakfast on cognitive performance, mood or satiety. These findings provide further support for the benefits to acute gluco-regulation of including multiple functional food ingredients at breakfast. The composition of breakfast should be considered when promoting the regular consumption of breakfast to adolescents.

## **7.2 Introduction**

### **7.2.1 Gluco-regulation**

A large amount of research reports associations between the regular consumption of breakfast in children and adolescents and benefits to weight status and gluco-regulation, compared to no breakfast (chapter 1.5.1). The literature also supports that the type of breakfast food consumed differentially affects the metabolic response to a meal in children and adolescents (chapter 1.5.2), with most benefits to gluco-regulation attributed to the consumption of a low-GI meal which elicits a sustained GR and IR and increases satiety (Tolfrey & Zakrzewski, 2012) (chapter 1.6.3). This is supported in the adult literature although more recent studies have also considered the GR and IR lowering effects of functional food ingredients (chapter 6.5.1). Adolescent breakfast studies measuring GR and IR are limited to studying the effects of GI on individuals who are overweight or obese (Zakrzewski et al., 2012), or reporting the prevalence of impaired glucose tolerance (Sinha et al., 2002). The findings show that the weight status of individuals is associated with the GR to a meal (Zakrzewski et al., 2012), and adolescents who are overweight or obese show signs of impaired gluco-regulation when compared to their normal-weight counterparts (Sinha et al., 2002). This suggests that health campaigns promoting the consumption of breakfast to adolescents, particularly as a means of weight control, should also consider the composition of breakfast consumed.

### **7.2.2 Cognitive performance, mood & satiety**

Improvements in cognitive and academic performance in children and adolescents have been attributed to the consumption of a breakfast which elicits a sustained postprandial GR (Adolphus et al., 2016; Edefonti et al., 2014; Philippou & Constantinou, 2014). Specific to adolescents, breakfast studies are limited (Adolphus et al., 2016) and consider the composition of breakfast relative to its GI and/or GL only (Brindal et al., 2012; Cooper et al., 2012; Cooper et al., 2015; Mahoney et al.,

2005; Micha et al., 2010; Micha et al., 2011; Smith & Foster, 2008) (Table 5). Studies performed in a school environment (Cooper et al., 2012; Cooper et al., 2015; Mahoney et al., 2005; Micha et al., 2010; Micha et al., 2011) tend to report positive effects of a low-GI or low-GI/high-GL breakfast on aspects of memory, attention and executive function, potentially explained by alterations to blood glucose levels (Cooper et al., 2012; Cooper et al., 2015; Micha et al., 2010; Micha et al., 2011). However, when measures were collected in a laboratory environment there were no effects of a low-GI/GL breakfast on adolescents' cognitive performance (Brindal et al., 2012; Smith & Foster, 2008), which highlights the potential importance of studying adolescents in naturalistic settings. Inconsistencies between the cognitive tests selected and the cognitive domains targeted is a limitation of research in this area (Adolphus et al., 2016). Studies comparing breakfast composition often use a range of food items, which are not always matched for energy and macronutrients; however, much of the evidence guiding the selection of cognitive tests and domains is based around the breakfast versus no breakfast literature (Adolphus et al., 2016), or individual ingredients or food groups (de Jager et al., 2014). This makes comparisons between studies difficult, particularly where a variety of cognitive tests are reported.

Functional food ingredients which are high in polyphenols and antioxidants are abundant in the diet, and their consumption often goes unnoticed within foods consumed on an everyday basis (for example; fruit, vegetables and whole-grains). Functional foods (blueberries and oats) were reported to improve cognitive performance, particularly memory and attention in children (Mahoney et al., 2005; Whyte et al., 2015; Whyte & Williams, 2015). The consumption of an oat cereal improved childrens' (6-8 years and 9-11 years) performance on a map task, auditory attention and short-term memory (in girls), compared with a RTEC (and no breakfast) (Mahoney et al., 2005); however, post-hoc analysis revealed map task performance was significant between the oat cereal and no-breakfast group only. In children (7-10 years), consuming blueberries was associated with



improvements in verbal memory (Whyte et al., 2015) and auditory memory (Whyte & Williams, 2015), but blood measures were not collected thus limiting the understanding of potential mechanisms. Improvements in acute performance on cognitive tasks would be especially desirable for adolescents, particularly during intense academic periods (Bell et al., 2015), but as yet there are no studies in adolescents.

Mood should also be considered in the context of cognitive function as it can influence, or be influenced by cognitive performance (Dye & Blundell, 2002). In adolescents, positive effects on mood were associated with cognitive performance in one study only (Micha et al., 2011), although not all adolescent studies include mood as a measurable outcome (Cooper et al., 2012).

Where measured, no effect of breakfast composition on satiety has been reported in adolescents (Brindal et al., 2012; Smith & Foster, 2008). In children and young adolescents (9-11 years), self-reported hunger was lower following the consumption of an oat-based breakfast compared to a RTEC at one hour, but not two hours (Mahoney et al., 2005).

### **7.2.3 Breakfast development**

Previous research performed as part of this PhD developed a breakfast based on functional food ingredients (chapter 5) and studied the effects in adults (chapter 6). Consumption of the FB significantly improved overall IR and peak insulin, compared to a CB and a RTEC; however, there were no effects on cognitive performance, mood or satiety regardless of which breakfast was consumed. Potential limitations from the adult study were considered and addressed in the current study by implementing the following changes:

- Increased sample size
  - The current study based the power calculation on primary *and secondary* outcome measures

- Study performed in a school
  - The current study was performed in a naturalistic setting (a school) compared to the adult study (performed in a laboratory)
- Crossover study design comparing 2 breakfasts
  - The adult study compared the FB, CB and RTEC; however, previous research performed as part of this PhD (chapter 4) highlighted the tight time constraints and ethical considerations involved with gaining access to schools. Based on this, and no effect on GR/IR measures between the CB and RTEC (chapter 6.4), the RTEC condition was removed.
- Further pilot work in adolescents testing the difficulty of the map task
  - In the adult study there was a ceiling effect observed which would have compromised findings. To avoid this reoccurring, the map tasks were extensively piloted with adolescents.

#### **7.2.4 Aims and hypothesis**

The current study had two aims:

- 1) To measure the effects of a FB on GR and IR in adolescents compared to a CB (primary outcome).
- 2) To assess the effects of the breakfasts on cognitive performance, as well as mood, satiety and palatability (secondary outcomes).

#### Hypothesis:

It was hypothesised that inclusion of the functional food ingredients in the FB would promote enhanced gluco-regulation compared to the CB. Furthermore, it was hypothesised that the ingredients would enhance cognitive performance, mood and satiety.

## 7.3 Methods

### 7.3.1 Study design

An order-balanced repeated-measures crossover study design was employed, with each participant serving as his/her own reference. Breakfast order was randomised among consenting participants using the OBU randomisation generator (Oxford Brookes University, 2011). In the preceding 24 hours to screening and testing unusual vigorous exercise, alcohol, nicotine and caffeine were avoided, and after 21:00 hours only water was consumed. Test days were on the same day of the week separated by seven days.

### 7.3.2 Participants

Seven schools which had participated in the questionnaire study (chapter 4), and had expressed interest in participating in future studies, were approached. All seven schools responded, but five declined due to time restraints. School-level consent (Appendix 4a) was obtained from the head teachers of two comprehensive schools, and the study information was delivered by the teachers to year nine students (aged 13-15 years). Written parental consent, and student assent, was obtained prior to screening and a health questionnaire was completed to confirm that all participants were in good health (Appendix 4b-4e). Eligible participants were between 13 and 15 years old. Exclusion criteria included:

- (i) diagnosis of medical conditions or medication interfering with metabolism, including diabetes (type 1 or type 2) or neurological illnesses
- (ii) three consecutive fasting blood glucose readings  $>6.1$  mmol/L
- (iii) food intolerance or allergy to breakfast ingredients
- (iv) diagnosis of learning difficulties

Thirty adolescents (16 girls, 14 boys) were recruited and all were eligible to take part. All students attended a screening session and two study sessions. Students who completed both study days received a £20 high street voucher. Study procedures were approved by the Ethical Advisory Committee at OBU according to the guidelines laid down in the Declaration of Helsinki.

### **7.3.3 Screening schedule**

A screening session preceded testing by seven days during which fasting blood glucose and anthropometric measures were measured. Height was recorded to the nearest centimetre using a Stadiometer (Seca Ltd, UK) with the subjects standing erect and without shoes. Body weight was recorded using the Tanita BC- 418 MA (Tanita UK Ltd) with the subjects wearing light clothing and no shoes. BMI ( $\text{kg/m}^2$ ) was calculated with the standard formula  $\text{weight}/\text{height}^2$  and converted to *z*-scores using online software (Pan & Cole, 2012) based on UK reference data (Cole et al., 1995). All measures were collected on the school grounds by trained researchers. At the end of the screening session participants were provided with a light breakfast.

### **7.3.4 Testing schedule**

The schedules for both testing days were identical (Figure 24). The protocol was the same as the adult study (chapter 6.3.3), but over two study days (CB and FB only). Upon arrival baseline measures were taken. Following the consumption of breakfast, measures were taken at the timed intervals (see chapter 6.3.3). The previous evening's meal was recorded and participants were reminded to replicate this prior to the next session. Compliance was checked during testing. Participants were supervised throughout the entire testing period and between measures, took part in educational, nutrition-based workshops which included quizzes and other non-strenuous tasks.

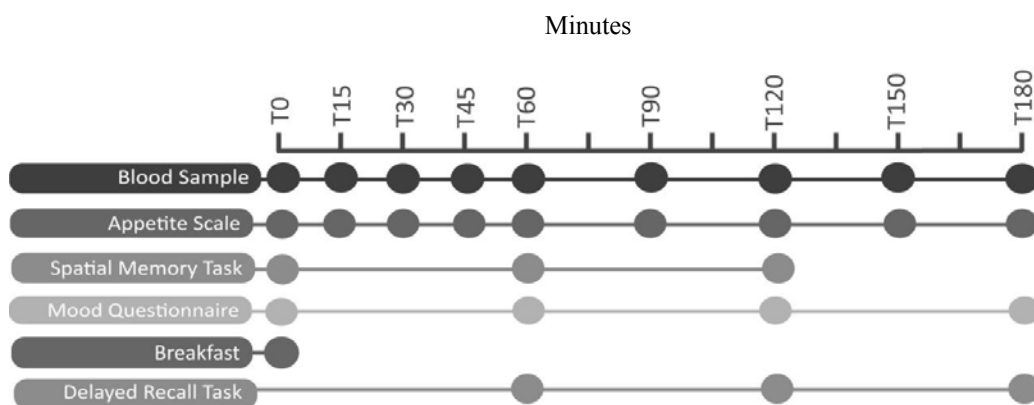


Figure 24. School testing day schedule

### 7.3.5 Test meals

The CB and FB were prepared in the university kitchen to the same recipe and protocol as previously described (chapter 6.3.4), at least two days prior to testing. Participants were blinded to breakfast condition until consumption and were separated into small groups whilst eating; however, differences between the ingredients may have made the FB easily identifiable. Participants were supervised while eating and were required to consume the entire breakfast within 15 minutes of starting, although some participants ( $n=8$ ) were unable to comply with this. Where a breakfast was not finished on test day 1 the amount consumed was matched on test day 2. Participants consumed 200mL of water with each breakfast and 100mL water after 90 minutes.

### 7.3.6 Blood samples

Glucose and insulin levels were collected, measured and processed from finger-prick capillary blood samples as previously described (chapter 6.3.5). To improve blood flow, participants' hands were submerged in warm water prior to each measure.

### 7.3.7 Map task

The same map task was used as described previously (chapter 6.3.6). The number of maps was reduced to six maps over two study days (household, sport, anatomy, vegetables, nature and transport). This included a mix of maps consisting of words which, as with adults, would differ in

their frequency of use and age of acquisition, but which the random order allocation and adequately powered sample should control for. Pilot work in non-participating adolescents ( $n=9$ ) found the test to be acceptable and no ceiling effects were observed. Participants were assigned an individual laptop and ear defenders (to aid concentration). Before the start of the test, an example, supported by a detailed explanation of the cognitive task, was given. Each map was studied for seven minutes, a blank paper map was then provided and two minutes were given to recreate the map from memory. To control for performance at baseline in the analysis, a baseline measure was completed before breakfast on each test day. Performance was measured at T60 and T120 to correlate with blood measures, which were also collected at these time points.

### **7.3.8 Delayed word recall**

Delayed memory was measured using the same recall task described previously (chapter 6.3.7). Briefly, participants wrote down as many words as they could remember from the previous map task in one minute. This was assessed at T60, T120 and T180 to coincide with blood measures and was completed immediately before the next map task was given.

### **7.3.9 Mood**

Mood was measured using the same mood questionnaire as previously described (chapter 6.3.8) at the same time points. Following feedback from the pilot study the scale was modified to include a midpoint option. Participants were asked to rate on a scale of 1 to 5 their subjective feelings of mood (1 = definitely do not feel, to 5 = definitely feel) (Appendix 4f).

### **7.3.10 Satiety and palatability**

Satiety and palatability were measured using the same 100mm VAS as previously described (chapter 6.3.9) at the same time points.

### **7.3.11 Statistical analysis**

Data were analysed using Microsoft Excel 2010 and SPSS V.22 (Chicago, IL, USA). Values are mean  $\pm$  SD unless otherwise specified. The GR and IR were calculated geometrically as the incremental AUC for each test food using the trapezoidal rule (FAO, 1998), and included the area above the fasting level only. Normality of the data was tested using the Shapiro-Wilks test. Blood glucose and insulin data were analysed using paired samples *t*-tests to compare AUC means at time points 60, 120 and 180. Map tasks were analysed using repeated measures ANCOVA with baseline map score entered as a covariate, and analysis performed on the absolute scores for correctly named and located items, items left blank, and wrongly answered items. The recall task was analysed as a two-way repeated measures ANOVA (breakfast x time point) on correctly recalled items and blank items. Pairwise comparisons were performed using a Bonferroni correction. Satiety and mood time points were analysed using a two-way repeated measures ANCOVA (time point x breakfast), with baseline values used as a covariate to correct for baseline differences (Blundell et al., 2010). Palatability VAS scores were analysed using a paired samples *t*-test comparing taste and appearance between breakfasts.

### **7.3.12 Power calculation**

In the previous study in adults investigating the effects of a FB on GR and IR power was calculated based on the primary outcome (chapter 6.3.11) where a significant effect of the FB was observed ( $n=16$ ). The primary outcome of the current study was the same (GR/IR); however, to reduce the chance of reporting a type 2 error on the cognitive data, the power calculation was based on the secondary outcome, cognitive performance. A previous oat-based study using the same map task in children ( $n=30$ ), observed a significant effect of breakfast type  $F(2,44)=3.98$ ,  $p<.05$ ,  $MSe=12.33$ , between the oat breakfast and no-breakfast group only (Mahoney et al., 2005). This was used due to the lack of research showing an effect between breakfast compositions. The mean of scores between

groups presented by Mahoney and colleagues (2005) were used in an *a priori* power calculation using G\*-power (Faul et al., 2007). This revealed that for a large effect size, with  $\alpha=0.05$  (two-tailed) and 0.95 power, a sample size of 27 would be needed. To account for an expected 20% attrition, the current study aimed to recruit 33 participants.



## 7.4 Results

Participants who consumed a similar volume of breakfast (providing at least 43g of available carbohydrate) on both test days were included in the analysis ( $n=22$ ) (Table 29). The remaining participants who were unable to consume the breakfasts were excluded from further analysis.

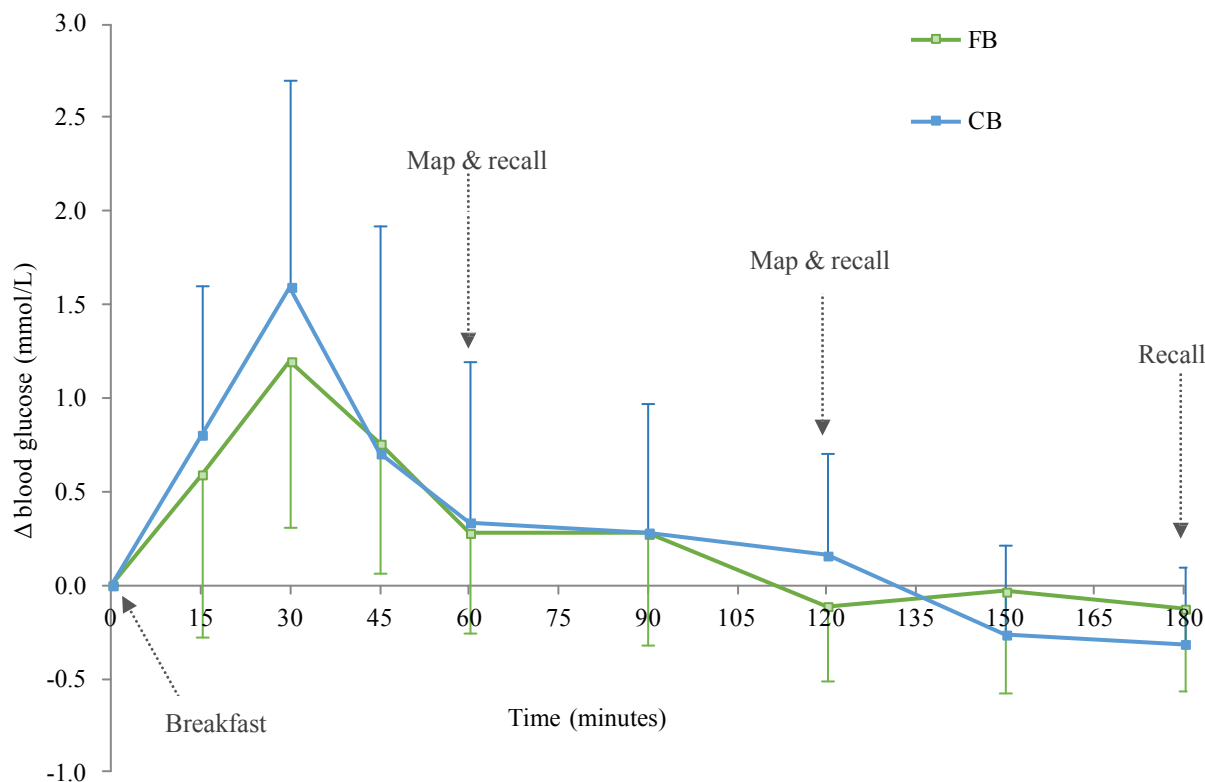
**Table 29.** Baseline characteristics of participants consuming >43g available CHO ( $n=22$ )

		All (n = 22)	Boys (n = 9)	Girls (n = 13)	<i>p</i> -value
	Unit	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	
Age	years	13.7 ± 0.5	13.7 ± 0.5	13.7 ± 0.5	.948
Weight	kg	55.6 ± 9.8	60.7 ± 12.9	52.1 ± 4.9	.096
Height	m	1.6 ± 0.1	1.7 ± 0.1	1.57 ± 0.04	.001**
BMI z- score		0.63 ± 0.77	0.66 ± 1.04	0.60 ± 0.56	.883
<i>Weight status<sup>a</sup></i>		n (%)	n (%)	n (%)	.126
Thinness Grade 2		1 (5)	1 (11)	0 (0)	
Thinness Grade 1		2 (9)	2 (22)	0 (0)	
Normal weight		14 (64)	5 (56)	9 (69)	
Overweight		4 (18)	0 (0)	4 (31)	
Obese		1 (5)	1 (11)	0 (0)	
Fasting glucose	mmol/L	4.7 ± 0.4	4.8 ± 0.4	4.7 ± 0.3	.471
Fasting insulin	μU/mL	14.3 ± 5.6	15.4 ± 6.0	13.5 ± 5.4	.324
Eats breakfast ≥ 5days	%	91	89	92	.697

Abbreviations: BMI, body mass index. \*\*  $p < .001$ , (2-tailed): Mann Whitney U tests. <sup>a</sup> Calculated using International Obesity Task Force grade.

### 7.4.1 Glucose response

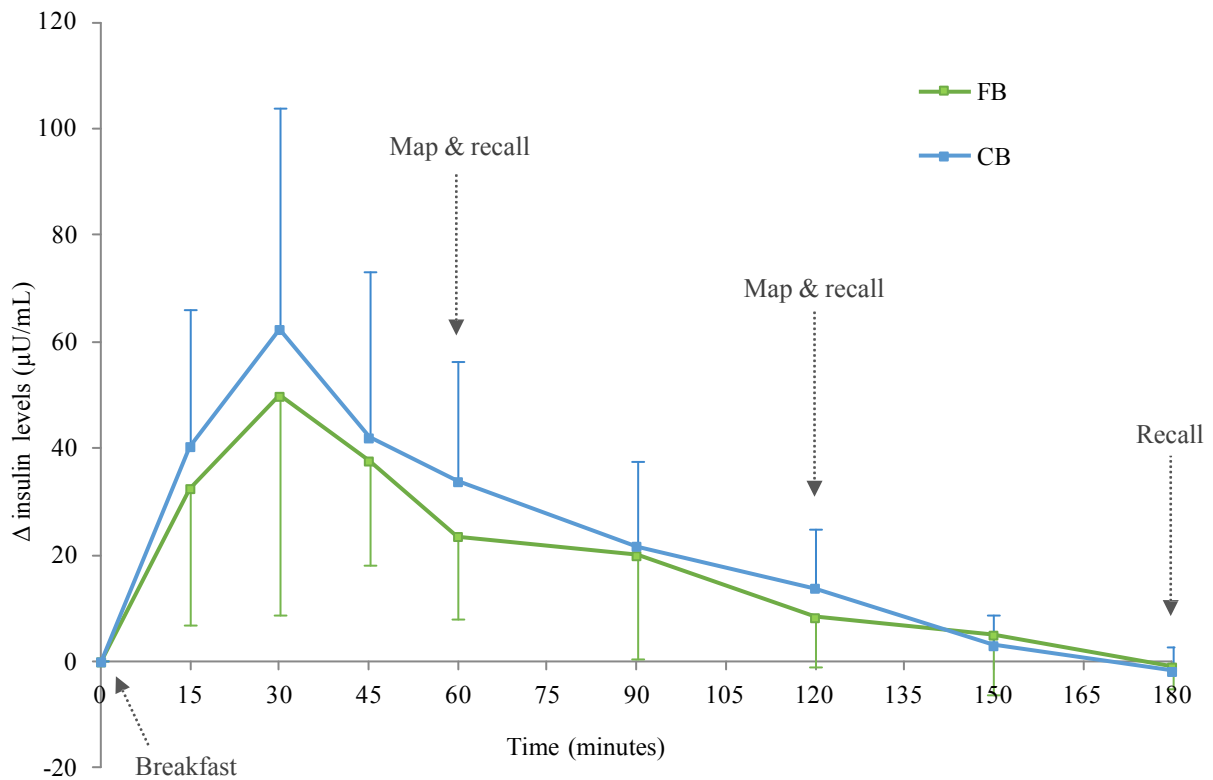
There were no significant differences between breakfasts on glucose AUC at T60 (CB  $53.2 \pm 35.1$  vs. FB  $42.2 \pm 23.1$  mmol/L/m,  $p = .125$ ), T120 (CB  $77.0 \pm 53.9$  vs. FB  $59.3 \pm 31.5$  mmol/L/m,  $p = .100$ ) or T180 (CB  $83.3 \pm 57.4$  vs. FB  $67.1 \pm 35.6$  mmol/L/m,  $p = .180$ ) (Figure 25). However, there was a higher glucose peak after consumption of the CB ( $6.8 \pm 0.5$  mmol/L) compared to the FB ( $6.3 \pm 0.7$  mmol/L) ( $t(21) = 2.88, p = .009, d = .61$ ).



**Figure 25.** Mean ( $\pm$ SD) change in glucose response (GR) from baseline to the functional breakfast (FB) and control breakfast (CB) ( $n=22$ )

### 7.4.2 Insulin response

There were significant differences between insulin AUC values at T60 (CB  $2426.3 \pm 1098.6$  vs. FB  $1981.1 \pm 1156.7 \mu\text{U}/\text{mL}/\text{m}$ ) ( $t(21)=2.12, p=.046, d=.45$ ) and T120 (CB  $3792.3 \pm 1751.3$  vs. FB  $3070.8 \pm 1879.1 \mu\text{U}/\text{mL}/\text{m}$ ) ( $t(21)=2.18, p=.041, d=.46$ ) (Figure 26). Post hoc analysis (Bonferroni adjustment) revealed that at T180 there was a trend for higher insulin AUC in the CB compared to the FB, but this was not significant ( $p=.061$ ). After consumption of the CB there was a significantly higher insulin peak ( $90.90 \pm 40.26 \mu\text{U}/\text{mL}$ ) compared to the FB ( $72.68 \pm 37.57 \mu\text{U}/\text{mL}$ ), ( $t(21)=2.71, p=.013, d=.58$ ).



**Figure 26.** Mean change from baseline in insulin response (IR) to the functional breakfast (FB) and control breakfast (CB) ( $n=22$ ).

### 7.4.3 Cognitive tests: map task and delayed recall

There were no significant effects of breakfast on cognitive performance for either the map task (Table 30) or the delayed word recall task (Table 31). There was a trend during the map task for the number of correctly answered items to decrease over the testing period and subsequently the number of wrong items to increase, but this was not significant ( $p=.067$ ,  $p=.096$  respectively). Furthermore, GR did not significantly correlate with any of the cognitive measures ( $p>.05$ ).

**Table 30.** Map task scores ( $n=22$ ).

	T0		T60		T120		<i>p</i> -value
	CB	FB	CB	FB	CB	FB	
Correct items	10 ± 6	11 ± 7	10 ± 7	11 ± 8	8 ± 5	10 ± 8	>.05
Wrong items	2 ± 2	1 ± 1	1 ± 1	2 ± 2	2 ± 2	2 ± 2	>.05
Blank items	18 ± 6	18 ± 6	18 ± 7	17 ± 8	19 ± 5	18 ± 8	>.05

Abbreviations: CB, control breakfast; FB, functional food breakfast.

There was a trend for the number of correctly answered items on the delayed recall task to decrease over time, despite the decreased cognitive load at T180 (where the map task was not completed), but this was not significant.

**Table 31.** Delayed recall scores ( $n=22$ )

	T60		T120		T180		<i>p</i> -value
	CB	FB	CB	FB	CB	FB	
Correct items	10 ± 3	10 ± 4	9 ± 5	9 ± 4	9 ± 4	9 ± 5	>.05
Wrong items	0 ± 1	0 ± 1	0 ± 1	0 ± 1	1 ± 1	1 ± 1	>.05
Blank items	20 ± 3	20 ± 4	21 ± 5	21 ± 4	19 ± 6	21 ± 5	>.05

Abbreviations: CB, control breakfast; FB, functional food breakfast

#### 7.4.4 Mood, satiety and palatability

There were no significant interactions between breakfast and time on energy

( $F(2,80)=0.55, p=.579$ ), tiredness ( $F(2,80)=0.86, p=.429$ ) and calmness ( $F(2,80)=1.57, p=.214$ ).

However, there was a significant interaction between breakfast and time on tension

( $F(2,80)=4.27, p=.017$ ). Post-hoc analysis (using Bonferroni adjustment) revealed that at T180,

tension scores were higher after the FB ( $7.7 \pm 4.3$ ) compared to the CB ( $6.1 \pm 2.1$ ),

( $F(1,21)=5.712, p=.026$ ) (Figure 27). Furthermore, tension scores decreased significantly between

baseline ( $7.2 \pm 0.6$ ) and T180 ( $6.0 \pm 0.5$ ) following consumption of the CB (CI: -2.17 to -0.19,

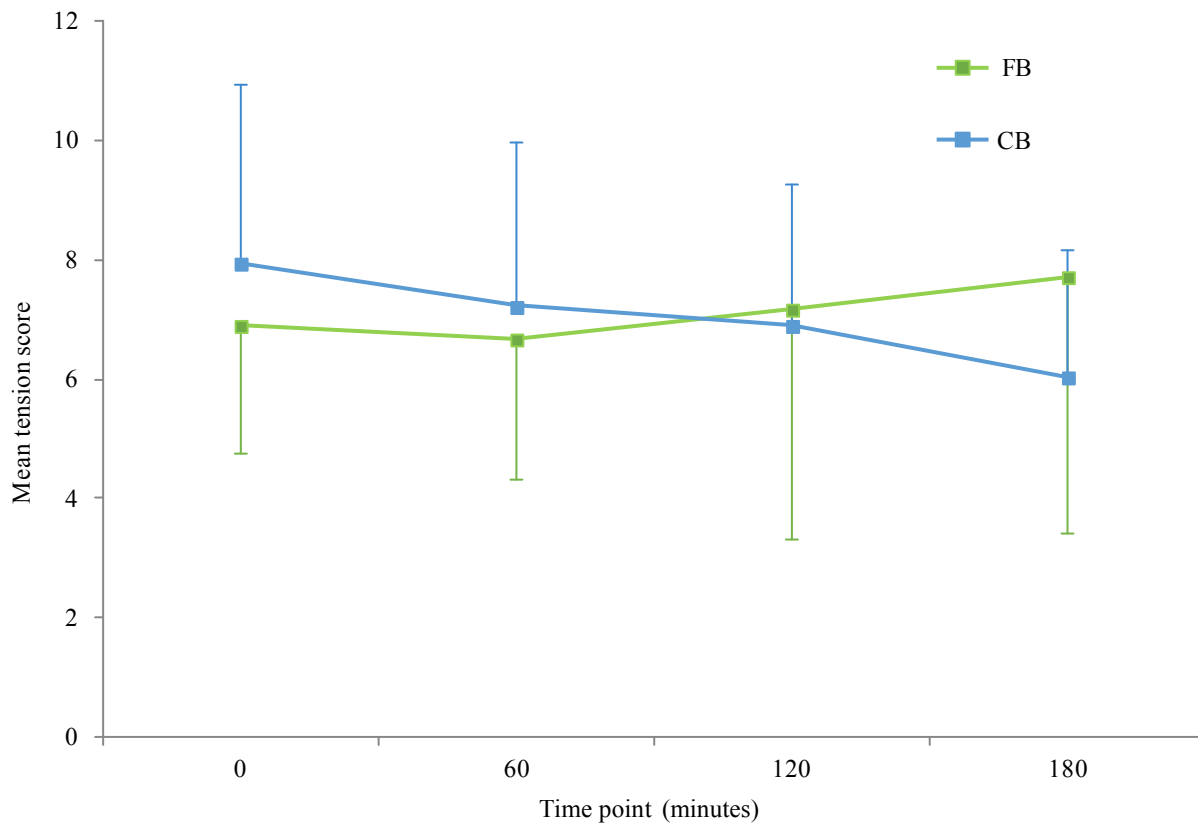
$p=.016$ ).

There were main effects of time on energy levels ( $F(1,40)=88.382, p<.001$ ), tiredness

( $F(1,40)=178.989, p<.001$ ) and calmness ( $F(1,40)=54.088, p<.001$ ). Post-hoc analysis revealed that

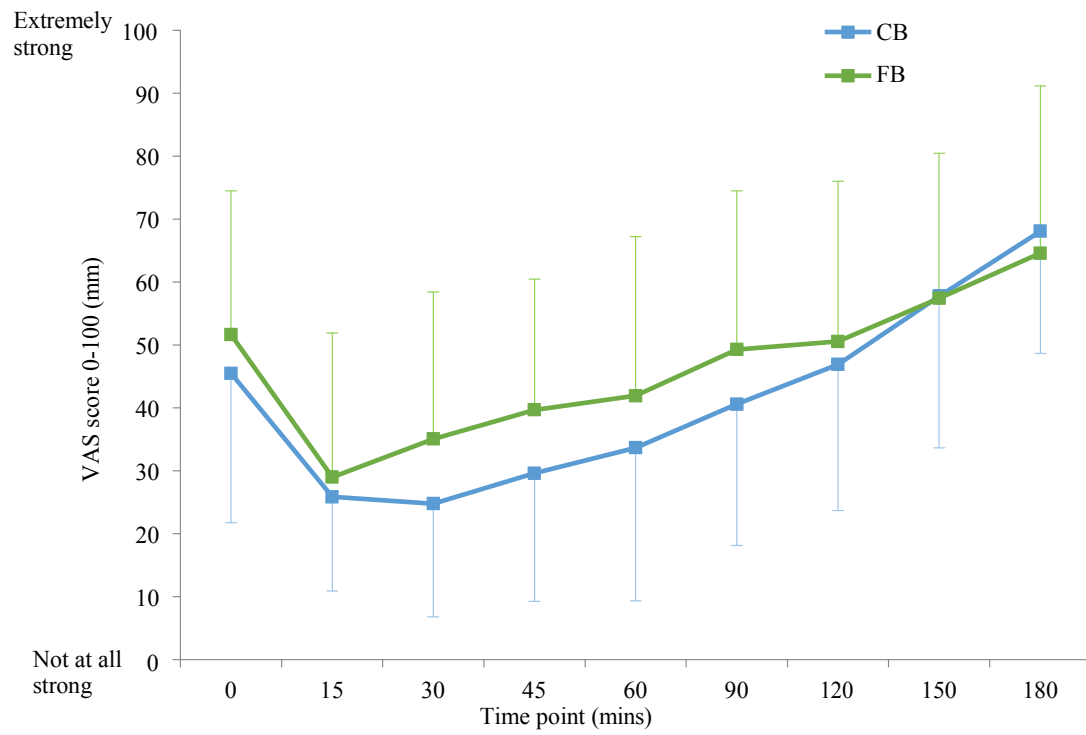
energy levels increased over the testing period ( $p=.010$ ), whereas tiredness ( $p=.018$ ) and calmness

decreased ( $p=.005$ ).



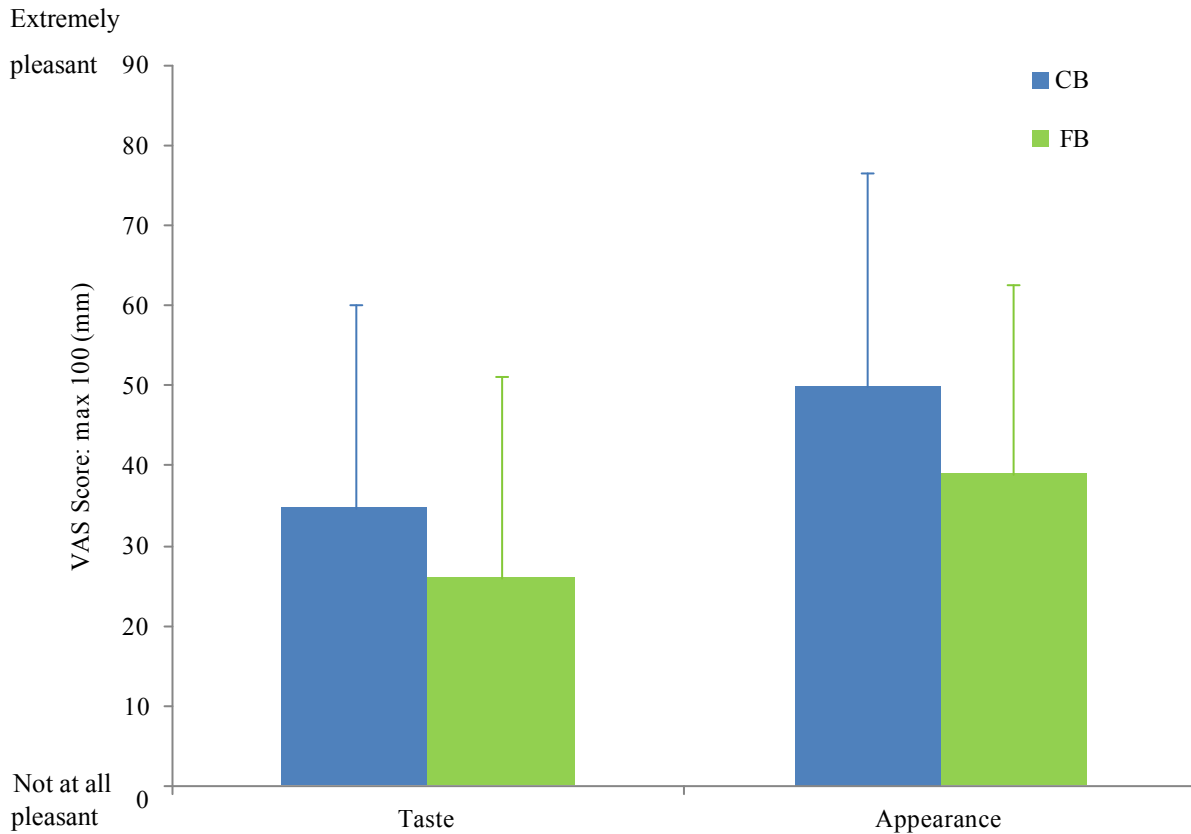
**Figure 27.** Tension score (means  $\pm$  SD) recorded at T0, T60, T120 and T180 minutes in all ( $n=22$ ) participants

Satiety was not affected by consumption of either of the breakfasts. There were no significant interactions between breakfast and time on: hunger ( $F,(3.78,147.45)=0.110,p=.357$ ), fullness ( $F,(5.39, 204.97)=0.61,p=.705$ ), desire to eat breakfast ( $F,(5.04,191.47)=1.29,p=.271$ ) or amount of food that could be eaten ( $F,(4.13,148.77)=1.70,p=.151$ ). As expected there was a main effect of time on: hunger ( $F(1,39)=220.473,p<.001$ ), desire to eat ( $F(1,38)=6.504,p=.015$ ), and perceived amount ( $F(4.132,148.765)=4.682,p<.001$ ). Post-hoc analysis (Bonferroni adjustment) revealed that hunger, desire to eat, and perceived amount which could be eaten significantly increased over the testing period ( $p<.001$ ) and fullness significantly decreased ( $p<.001$ ). There was also a trend for a greater desire to eat after consuming the FB ( $p=.071$ ) (Figure 28).



**Figure 28.** Mean  $\pm$  SD scores for  $n=22$  participants asked ‘How strong is your desire to eat?’

There were no significant differences in rating the appearance of the breakfasts ( $t, (19)=1.71, p=.103$ ) or in rating the taste ( $t, (20)=1.53, p=.142$ ); however, mean scores were generally quite low (Figure 29).



**Figure 29.** Participants ( $n=22$ ) rated the CB and FB by answering ‘How pleasant was the taste of the breakfast?’ and ‘How pleasant was the appearance of the breakfast?’

## 7.5 Discussion

The primary outcome of this study was to determine the effects of a FB compared to a CB on gluco-regulation in adolescents.

### 7.5.1 Gluco-regulation

Consumption of the FB resulted in a lower peak glucose, lower peak insulin and lower IR after 60 and 120 minutes, compared to the CB. There was a trend for a lower IR after the FB at the end of testing (T180), but this was not significant.

This is the first study to consider the effects of a FB on GR and IR in adolescents. In a previous study performed as part of this PhD (chapter 6.4.1), there were no significant differences in peak glucose values between the FB and CB in adults. The metabolic response to food between adolescents and adults (and younger children) would be expected to differ due to puberty and growth and their effects on metabolism (Goran & Gower, 2001). Furthermore, the prior gluco-regulation status of an individual can impact the GR and IR to a meal (WHO, 1999), and although the current study did not measure impaired glucose tolerance (IGT), the recruitment criteria excluded participants with fasting values outside of normal ranges based on established WHO cut-offs; therefore, it was unlikely to have had an effect in the current study. Additionally, differences may be partially explained by the habitual breakfast patterns of individuals, which as well as influencing energy intake throughout the day (chapter 1.5.3.1), may also affect individuals' GR. Adults ( $n=33$ ) who regularly skipped breakfast over six weeks were more likely to have a variable GR to meals throughout the day compared to those who did not (Betts et al., 2014). Irregular eating patterns are most likely to develop during adolescence (Höglund et al., 1998; Samuelson, 2000); however, most adolescents in the current study (91%) reported eating breakfast regularly ( $\geq 5$  days per week). This may have been more regular than the adult participants, although habitual consumption data from adults were not collected.



In both studies (adults and adolescents), the FB was developed using ingredients selected for their potential to improve gluco-regulation (oats, blueberries, baobab, cinnamon), and ingredients which are encouraged and promoted as healthier alternatives in the diet (honey, bananas, olive oil) (Public-Health-England, 2016). Breakfasts were matched for energy, available CHO, protein and fat, but contained different ingredients resulting in varying amounts of fibre (FB: 8.4g; CB: 2.5g) and total sugars (FB: 14.1g; CB: 10.6g). *In-vitro* laboratory analysis (chapter 5.2-3) showed that the FB released sugar at a slower rate during the later stages of digestion, despite a higher total sugar content, which may have been attributed to the additional fibre (Slavin & Green, 2007) (chapter 1.5.2.1), or to the polyphenol levels (McDougall & Stewart, 2005) (chapter 1.10.3), which were also higher in the FB (FB: 229.7mg; CB: 147.1mg). Based on the *in-vitro* findings, the inclusion of the functional food ingredients was expected to produce a lower overall GR *in-vivo* and subsequently a lower IR. However, in the current study, there were no significant differences in GR AUC between breakfasts, but significantly less insulin was produced in response to the FB suggesting that benefits to IR outside of the maintenance of glucose control could be attributed to the inclusion of functional food ingredients.

The addition of functional food ingredients to CHO meals and beneficial effects on postprandial glycaemia has been reviewed in adults (Coe & Ryan, 2016); however, in adolescents, no such studies exist. Instead, breakfast composition studies are considered relative to the GI and/or GL (Table 5). Cooper and colleagues (2012) provided a low-GI muesli and a high-GI RTEC (and no breakfast) to adolescents in a school environment ( $n=41$ , 12-14 years) (Cooper et al., 2012).

Breakfasts were matched for available CHO (75g), and approximately matched for protein, fat and energy. Overall GR IAUC (2 hours) was significantly lower after the low-GI breakfast compared to the high-GI breakfast, which is consistent with findings in adult populations (Wolever & Bolognesi, 1996). In contrast to the current study, Cooper and colleagues (2012) reported no effect of either

breakfast on IR IAUC. Blood measures were collected at fewer time points (30, 60, 90 and 120 minutes) than the current study which may have influenced the mean and variation of their AUC values (Brouns et al., 2005). The authors attributed the finding to the inclusion of milk which is reported to have an insulintropic effect (Liljeberg Elmstahl & Bjorck, 2001). In the current study milk was present in the CB and the FB in similar amounts and yet there was a significantly lower IR to the FB. This suggests that the beneficial effects of the selected functional ingredients on IR were greater than the insulintropic effects of milk, although it is not possible to determine whether this was attributed to the overall fibre or polyphenol content, individual functional food ingredients, or a combination of factors.

Findings from other adolescent studies are more varied. Low-GI breakfasts produce a lower GR, potentially due to increases in fibre content (1.11.6.1); however, not all studies report the fibre content of breakfasts. Furthermore, when breakfasts are matched only on volume (Smith & Foster, 2008) or energy (Micha et al., 2011) it is difficult to determine where effects are coming from. Only one study in young adolescents ( $n=39$ , 10-12 years) reported the fibre content of energy-matched breakfasts varying in GL (Brindal et al., 2012), which conversely, was highest in the high-GL, high-GI breakfast. The consumption of the high GI/GL breakfast produced a higher GR compared to the medium and low GI/GL breakfasts, suggesting that the additional fibre was not effective in lowering the GR. This potentially supports that the inclusion of functional food ingredients in the FB were driving the beneficial effects on peak GR, compared to the CB, from which they were absent.

### **7.5.2 Cognitive performance, satiety and mood**

The secondary outcome of the current study was to investigate the effect of breakfast condition on cognitive performance, mood and satiety, and identify potential associations between cognitive

performance and blood measures. There were no significant effects of breakfast condition on map task performance, delayed recall performance, mood or satiety. Furthermore, there were no significant associations between cognitive performance and GR or IR.

To date, there are no studies reporting the effects of functional food ingredients, individually or in combination, on cognitive performance in adolescents. In a previous study in adults (chapter 6.4.1), there were no significant effects of a FB on cognitive performance compared to a CB. In children, two studies considered the effects of blueberries on cognition (Whyte et al., 2015; Whyte & Williams, 2015). Blueberries are a rich source of polyphenols and there are a number of animal studies promoting benefits to cognitive function, particularly spatial memory, although human interventions supporting this are limited (chapter 1.11.4). The consumption of a blueberry drink containing 143mg anthocyanin had no effect on spatial memory, but significantly improved delayed recall scores compared to a matched CHO control ( $n=14$ , 8-10 years) (Whyte & Williams, 2015). In the second study, children ( $n=21$ , 7-10 years) significantly improved performance on recall and delayed word recognition in a dose-responsive manner following blueberry consumption (Whyte et al., 2015). The total polyphenol contents were not reported, but based on the anthocyanin content this was lower than what was delivered in the FB, suggesting that in the current study external factors such as the sensitivity of the cognitive tests selected, may have contributed to the lack of effect. Developmental pilot work informed the timings and content of the map tasks for use in the current study; however, one participant achieved top marks thus, as with the adult study (chapter 6), a ceiling effect was evident. This represents a particular issue when working with young, healthy adults (Uttl, 2005) and adolescents (Cromer et al., 1990).

As with GR and IR, previous adolescent studies have considered the composition of breakfast and its effects on cognitive performance relative to its GI and/or GL (Table 5). In the aforementioned study by Cooper and colleagues (2012), a low-GI breakfast improved aspects of working memory,

executive function and attention, particularly on more demanding tasks and at the end of testing (120 minutes), compared to a high-GI breakfast (Cooper et al., 2012). Improvements in cognition were concurrent with a significantly lower GR compared to the high-GI breakfast at 30 and 120 minutes, supporting suggestions that postprandial GR may mediate effects on cognitive performance (Adolphus et al., 2016; Edefonti et al., 2014; Philippou & Constantinou, 2014); however, the analysis of individual time points may have increased the risk of type 1 errors. Conversely, Brindal and colleagues (2012) saw no effect of breakfast (low-versus high-GI) on cognitive function despite significant differences between continuously measured GR (Brindal et al., 2012). This further highlights the necessity of selecting appropriate cognitive tests, which may be more important for identifying effects on cognitive performance than effects from blood glucose control. Future studies in adolescents should consider using a secondary task to increase cognitive load (Smith & Foster, 2008), or introduce different levels which allow for the complexity of the tasks to be accounted for (Cooper et al., 2012).

In the current study there was a trend for performance on the map task and the delayed recall task to decrease over time, which was not observed in adults (both  $p > 0.05$ ) (chapter 6). This may have reflected a lack of motivation or boredom with completing multiple measures. This was consistent with changes in blood glucose which, as expected, also decreased over the testing period, potentially inducing fatigue. Other adolescent studies comparing breakfast composition report mixed findings. Some improvements in verbal memory were observed after consumption of low-GI, high-GI breakfasts (Micha et al., 2011); however, it was unclear whether it was the accuracy or the response time that had improved. Smith and Foster (2008) saw no effect of breakfast GI on cognitive function, although measures were taken for only 100 minutes (Smith & Foster, 2008). Mahoney and colleagues (2005) reported improvements in performance on the digit span backwards test in girls only, following consumption of the low-GI oat breakfast compared to the high-GI

RTEC (Mahoney et al., 2005); however, although breakfasts were reported as isocaloric, Hoyland and colleagues (2009) highlighted that this was wrongly calculated in the paper (Hoyland et al., 2009). Furthermore, breakfasts differed in macronutrient content. Comparing measures between the FB and CB over a three hour period was a strength of this study, although anthocyanin metabolites have been seen up to six hours after acute blueberry anthocyanin intervention (Rodriguez-Mateos et al., 2013); therefore, future studies comparing blueberries may wish to consider longer testing periods.

### **7.5.3 Mood & satiety**

The current study found no significant differences in adolescents' self-reported energy, tiredness or calmness ratings between the CB and the FB. There were increases in reported energy levels over the testing period, and participants became less tired and less calm regardless of which breakfast was consumed.

The effect of food on mood is delicate and can be easily altered depending on the psychological state of the respondent as well as the environmental surroundings (Hammersley et al., 2007). The novelty of taking part in this research study and being amongst peers and researchers may have contributed to increasing, rather than decreasing, levels of arousal. Furthermore, testing began earlier than the usual school day, disrupting some participants' sleeping patterns which may have affected their mood (and cognitive performance) (Lo, Ong, Leong, Gooley, & Chee, 2015); although, this was consistent across the testing period.

In contrast to the current study, where tension levels increased over the testing period following consumption of the FB compared to the CB, Cooper and colleagues (2011) reported a decrease in tension levels over the testing period (Cooper et al., 2011), despite decreasing blood glucose levels, which can promote feelings of tension (Benton, 2002). Increased tension levels and a trend for

increased desire to eat in the FB condition may have reflected the palatability of the breakfasts, particularly the FB. In adults the FB scored highly in terms of pleasantness of taste and appearance, but ratings were much lower for adolescents, highlighting the challenges of developing healthy, pleasant-tasting products for adolescents. It may benefit future studies to consider providing alternative food options to cater for the wide range of adolescent tastes.

Improvements in mood are associated with breakfast consumption (versus omission) in adolescents (Cooper et al., 2011; Defeyter & Russo, 2013), however, the potential effects of breakfast composition on mood are equivocal. No effect was reported when comparisons were made between: high vs. low energy breakfasts (Cromer et al., 1990), habitual vs. high energy breakfasts (Michaud et al., 1991), or high- vs. low-GI breakfasts (Mahoney et al., 2005; Smith & Foster, 2008). In contrast when both the GI and the GL of breakfast were considered there were measurable effects on mood following the consumption of a low-GI, high-GL breakfast compared to high-GI alternatives (Micha et al., 2011). In support of this there is some evidence to suggest that a refined breakfast increases reported laziness in children and adolescents (9-13 years) when compared to a breakfast based on whole-foods (Pereira et al., 2011).

Reported satiety levels did not differ between breakfasts despite the FB containing higher amounts of fibre than the CB, which was consistent with findings in adults (chapter 6). So far, there is little support in the adolescent literature for an effect of breakfast composition on satiety. There were no effects when comparing a high-GI to a low-GI breakfast (Mahoney et al., 2005; Smith & Foster, 2008), or between breakfasts with a high, medium or low GL, despite breakfasts containing varying amounts of protein (Brindal et al., 2012).

#### **7.5.4 Additional limitations**

The current study was adequately powered to observe an effect on GR and IR, but for cognitive measures power was based on a previous study observing a significant effect between the no breakfast and breakfast group (Mahoney et al., 2005). In future studies it would be prudent to base power calculations on studies where effects of breakfast composition are reported (where available).

Although the recruitment target was met, a larger number of participants than anticipated did not finish the breakfast at both time points. This potentially weakened the findings by reducing the overall power of the study and creating a small variation between the volumes of breakfast consumed. As with the chapter 6 study, the map task was used; however, as previously discussed (chapter 6.5.4) the limitations in the use of this task may have contributed to the null effect.

## **7.6 Conclusion**

These findings show that the inclusion of multiple functional food ingredients as part of breakfast can significantly improve GR and IR in adolescents, extending previous findings in adults.

The CB was based on ingredients that the public are encouraged to reduce in their diet, but which are present in popular RTEC consumed by adolescents (chapter 5). Incorporating functional foods into every meal and sustaining this over a long period could be unrealistic for adolescents, particularly where a general decline in healthy eating behaviours is observed (chapter 1.4). Findings from the current study are encouraging as they suggest that the inclusion of functional food ingredients into just one meal (breakfast) can promote enhanced gluco-regulation. Interventions promoting the regular consumption of breakfast should consider this as a preventative strategy for maintaining adequate gluco-regulation in adolescence, thus increasing the potential for optimal metabolic health in adulthood.

Selecting a cognitive test sensitive enough to detect differences between the compositions of breakfasts is a challenging aspect of this research. Cognitive domains influenced by individual

ingredients might be differentially affected when these ingredients are provided in combination. Future studies may show different effects using alternative cognitive tests; although it cannot be discounted that breakfast composition is unrelated to cognition.



## Chapter 8: General discussion

### 8.1 Summary of thesis findings

The primary aim of this thesis was to develop a breakfast based on multiple functional food ingredients (FB) (chapter 5) and measure the effects on GR and IR in adolescents in a school environment, when compared to a CB (chapter 7). As preparation for going into the school, the study was performed in adults in a laboratory environment (chapter 6) and included a third breakfast condition (RTEC), which was informed by findings from the adolescent questionnaire (chapter 4). Results from the adult study (chapter 6), and the time constraints identified from the preliminary studies with schools (chapter 4) resulted in the removal of the third breakfast condition (RTEC). A secondary outcome for both breakfast studies was to compare the effects of the FB on map task performance, delayed word recall, mood and satiety, measured using the same tasks and questionnaires in adults and adolescents.

The key findings of this thesis are:

1. Consumption of the FB resulted in a lower peak IR and IR AUC in adults, compared to a CB and RTEC (chapter 6).
2. In adolescents, peak glucose and peak insulin were lower, and IR AUC at T60 and T120, following consumption of the FB compared to the CB (chapter 7).
3. There were no differences between performance on cognitive tests, mood or satiety in adults or adolescents, regardless of which breakfast was consumed ( $p > .05$ ) (chapter 6 & 7).

Two preliminary studies contributed to the main hypothesis. The adolescent questionnaire informed the choice of the RTEC (chapter 4) and preliminary findings from the validation study of indirect calorimeters informed the removal of the DIT measure (chapter 3). The key findings of the studies in chapter 3 and chapter 4 relative to their sub-hypotheses are:

1. The ECAL was acceptable for use in a school environment and measures were collected successfully, but prediction equations significantly underestimated RMR compared to RMR measured on the ECAL (chapter 3).
2. The validation of the ECAL and the GEM was acceptable for repeatable measures, but were not accurate alternatives to the DT; however, the accuracy of the DT was questionable (chapter 3).
3. Adolescents' breakfast behaviours and attitudes towards breakfast consumption, are different between boys and girls (chapter 4)

### **8.1.1 Gluco-regulation**

There is considerable experimental evidence supporting the regular consumption of breakfast and benefits to metabolic parameters in adults (chapter 1.6.1.1). A series of studies by Macdonald and colleagues (2004, 2005a, 2005b), and more recently by Betts and colleagues (2014), support regular breakfast consumption (compared to breakfast skipping) and improved GR and IR to subsequent meals (Betts et al., 2014; Farshchi et al., 2004; Farshchi et al., 2005a, 2005b). It is well documented that consuming low-GI (Messier, 2004) or high-fibre foods (Tosh, 2013) produces a sustained GR and IR (chapter 1.6.2). Low-GI, high-fibre foods are also associated with increased satiety and prolonged satiation (Slavin & Green, 2007), and lower risk factors for CVD (Slavin, 2008), in adults and in young people (chapter 1.5.2.1), thus providing support for current recommendations promoting a low-GI breakfast to young people (Rampersaud, 2009; Szajewska & Ruszczynski, 2010).

The current study found that including functional food ingredients at breakfast significantly improved gluco-regulation in healthy adults and adolescents, compared to a CB (and to a RTEC in adults only). Maintaining normal, or even partially improved post-prandial glucose metabolism

promotes insulin sensitivity which could have long-term benefits to pancreatic  $\beta$ -cell function (Kallio et al., 2008). Furthermore, glycaemic control plays a crucial role in the prevention of T2DM (Philippou & Constantinou, 2014) which this has important implications in young people who are showing increasing rates of T2DM in the UK (Haines, Wan, Lynn, Barrett, & Shield, 2007).

Tovar and colleagues (2012, 2014, 2015) reported in a series of studies that the inclusion of multiple functional foods in the diet over a period of weeks was beneficial to metabolic markers of health (including insulin regulation) in healthy (but overweight or obese) adults (Tovar et al., 2015; Tovar et al., 2014; Tovar et al., 2012) (chapter 1.10.2). The current studies add to the literature by reporting the acute benefits to gluco-regulation that can be obtained from the inclusion of functional food ingredients at breakfast, in healthy (normal-weight) adults and adolescents.

The FB included blueberries, oats, cinnamon and baobab, selected for their positive effects on gluco-regulation in adults, which is generally attributed to their high polyphenol and/or fibre content (chapter 1.11). Törrönen and colleagues (2010, 2012a, 2012b, 2013) have shown in a series of studies that the addition of berries to CHO-based foods significantly lowered the GR and IR (Törrönen et al., 2012b; Törrönen et al., 2013; Törrönen et al., 2012a; Törrönen et al., 2010). This was attributed to the anthocyanins, which have been shown *in-vitro* to inhibit pancreatic  $\alpha$ -amylase, and intestinal  $\alpha$ -glucosidase (Johnson et al., 2011), and interact directly with glucose transporters (Alzaid et al., 2013) (chapter 1.11.1.1). Anthocyanins may also exhibit insulin-like actions, inducing secretion of insulin from pancreatic cells (McDougall & Stewart, 2005), although this is yet to be shown *in-vivo*. Two studies by Coe and colleagues (2013, 2015) saw GR- or IR-lowering effects of baobab when added to bread in varying doses (Coe & Ryan, 2015; Coe et al., 2013), attributed to the high-polyphenol or the high-fibre content of baobab. The provision of oats as a rich source of soluble fibre is well documented (Wood, 1990), and the  $\beta$ -glucan content is associated with significant reductions in GR and IR (Tosh, 2013). Oats also provide a rich source of

polyphenols (Ryan et al., 2011) which may contribute directly to a reduction in starch digestibility (Thondre et al., 2011). Research on cinnamon suggests that it may stimulate insulin activity (Anderson, 2008) as well as providing a rich source of polyphenols (Ho et al., 2013). A systematic review by Coe and colleagues (2016) identified overwhelming support for the beneficial effects of the addition of individual functional food ingredients which are rich in polyphenols, flavonoids, fibre and antioxidants, to CHO foods (Coe & Ryan, 2016). The studies in the current thesis support and further these findings, showing that the combination of selected functional food ingredients into a breakfast, provided in amounts that would be feasible to consume at breakfast, maintains positive effects on GR and IR. The CB and the RTEC were devoid of the functional food ingredients therefore, benefits may be attributed to their inclusion; however, the inclusion of multiple ingredients meant it was not possible to specify which ingredients or components.

### **8.1.2 Cognitive performance, mood and satiety**

A secondary outcome of the breakfast studies was to determine the effect of the FB on cognitive performance, mood and satiety; however, no significant differences were reported.

There is overall support in the literature that breakfast consumption can enhance cognitive performance in young people, particularly memory, when compared to no breakfast (Adolphus et al., 2016) (chapter 1.8.1.1). The evidence is less supportive of the effects of breakfast composition (chapter 1.8.2.1) potentially due to limited studies, or the lack of studies in naturalistic settings, but also relative to the difficulties facing researchers when trying to control for confounding factors.

There is a tendency for a variety of cognitive tests to be used, measuring a range of cognitive domains, making comparisons across studies difficult. Furthermore, studies do not match breakfast conditions (chapter 1.9.2.1), or withhold details of the breakfast (chapter 1.8.2.2), limiting the potential to make inferences.

Much of the adolescent research relates to the effects of low-GI and high-GI breakfasts due to established links between the GI of a food and its effects on blood glucose (Atkinson et al., 2008), the fuel source for the brain (Gold, 1995). Adolescent studies generally support a role for maintaining a lower GR and benefits to cognitive performance (chapter 1.9.2.1), but there are no definitive conclusions on the optimal breakfast, potentially due to the challenges in maintaining consistency across the whole meal content. Studies from the current thesis (chapter 6 and 7) were the first to consider the effect of a FB on map task and delayed recall performance, mood and satiety, in adults and adolescents. Extending work from Tovar and colleagues, Nilsson and colleagues (2013) reported a positive effect of a functional food based diet on cognitive function in adults, attributed to the high polyphenol content of blueberries, the omega-3 fatty acid content of fish and low-GI foods (Nilsson et al., 2013). The ingredients selected in the current thesis for the FB also included blueberries, although in smaller amounts (30g) than what other studies (chapter 6.5.2, 7.5.2) have provided (range 74.5g to 200g), perhaps contributing to the null effect on cognitive performance. There are a number of human studies associating improvements in cognitive performance with the consumption of blueberries, attributed to the ability of anthocyanins to cross the BBB and localise in areas of learning, which is well-documented in animal studies (Andres-Lacueva et al., 2005; Papandreou et al., 2009) (chapter 1.11.1.2). However, the support from human intervention studies is limited by methodological inadequacies and small samples of mostly at-risk individuals (Krikorian et al., 2010a; Krikorian et al., 2010b). Research on berry polyphenols and their effects on cognitive performance continues, more recently in healthy young adults (Dodd, 2012; Watson et al., 2015), and children (Whyte et al., 2015; Whyte & Williams, 2015), where improvements have been reported in memory performance, suggesting their potential even in individuals with no known risk of cognitive decline.

The cognitive tests in the current studies were carefully selected based on the cognitive domains most likely to be influenced by breakfast interventions (chapter 1.7.2), and which may be potentially enhanced using the selected functional ingredients (chapter 1.11); however, the breakfast and cognition literature so far only supports effects of breakfast versus no breakfast, or provides ingredients in much larger quantities than were used in the current study. Systematic reviews of the breakfast and cognition literature (Adolphus et al., 2016; Hoyland et al., 2009) also struggle to conclude which cognitive domain is the most sensitive which presents difficulties when selecting a cognitive test (chapter 1.8.1.1). This is a challenge for future research in this area.

## **8.2 Implications of the findings**

The findings from these studies have important implications that can help contribute to further research. Firstly, the beneficial effect of a FB on GR and IR in adults and adolescents was observed (chapter 6 and 7); therefore, their consumption could be promoted as part of a healthy breakfast. This could be through targeted interventions, as a preventative strategy to promote long-term metabolic health, based on in-depth understanding of the factors underpinning the consumption of breakfast, although more research specific to the composition of breakfast is necessary

Previous studies have shown that implementing interventions in adolescents in a rigorous manner is challenging (Shemilt et al., 2004) and there are a number of confounding factors (SES, ethnicity, nutritional status, age, gender) that studies frequently fail to consider (chapter 1.4.2). Furthermore, breakfast interventions in young people do not always increase breakfast consumption (chapter 4.2) and these challenges may also present in promotions considering the composition of breakfast.

However, the promotion of a breakfast that elicits beneficial effects on gluco-regulation may indirectly lead to improvements relative to overall nutritional status and potential increases in PA levels, which is pertinent to adolescents (chapter 1.5.3). Therefore, it is imperative that research in this area continues to improve the development and delivery of studies.

The amounts of functional food ingredients used in the FB represent feasible quantities that could be promoted to individuals as part of a healthy breakfast. Benefits from functional foods may come via direct actions relative to their high polyphenol, or fibre content (chapter 1.10.1.2), but may also come from the reduction of other non-healthy food items that the functional foods are replacing (Chiva-Blanch & Visioli, 2012). Furthermore, including functional foods could enhance breakfast quality in adolescents by adding to its soluble fibre content, thus contributing to improved glycaemic control and increasing low fibre intakes in adolescents (Beck et al., 2009a). Consuming food items that have potential to benefit cognitive function during adolescence (chapter 1.11) could enhance cognitive performance at a time of increased academic strain and cognitive development and there is emerging evidence in children and young adults from Whyte, Williams and colleagues to support this (chapter 6.5.2, 7.5.2); however, more research focused on adolescents is required.

Other important practical implications of the findings from this thesis relate to the use of novel methods to measure RMR (chapter 3). Collecting measures in adolescents is challenging; therefore, research using novel approaches that can enable researchers to do this efficiently and accurately is of great importance. Identifying the use of objective measures in a school environment can aid understanding of the inter-individual metabolic differences during adolescence, which is a critical period of growth (Epstein, 1986), and contribute to the measurement of the metabolic effects of food. Emerging research on breakfast habits and the effects on metabolic adaptations suggest that regularly skipping breakfast can be detrimental to gluco-regulation (chapter 1.6.1.2). However, the short- and long-term effects of breakfast habits and breakfast composition on DIT are still to be determined.

### **8.3 General strengths and limitations**

The current thesis reports the successful design and implementation of a school-based study which reported the beneficial effects of a FB on GR and IR compared to a CB. The development of the FB

included extensive laboratory analysis measuring the polyphenol content and *in-vitro* digestion abilities of the FB compared to the CB (chapter 5), which was a strength of the study. Studies are limited where comparisons are made in the absence of *in-vitro* analysis, as polyphenols are sensitive and can vary widely (chapter 1.10.1.2), although variations exist even within the same brand of polyphenol-rich sources and this remains a challenging aspect for future research in this area. In the current thesis the *in-vitro* analysis showed that the FB released sugar at a slower rate in the later stages of digestion and was higher in antioxidants and polyphenols, fibre and total sugars, suggesting that *in-vivo* there would be a lower GR and subsequently a lower IR. However, there was no effect on GR IAUC highlighting the difficulties translating *in-vitro* findings to *in-vivo*. The laboratory analysis in the current study did not measure the anthocyanin content of the breakfasts; however, future studies may consider this to help make comparisons with the existing literature. The breakfast studies presented in this thesis (chapter 6 and 7) collected blood measures at specified time points and if an effect on cognition, mood or satiety had been observed this may have given further insight into potential mechanisms, although detailed analysis of the mechanisms by which functional foods reduce GR and IR is not provided.

The inclusion of multiple functional food ingredients into the breakfast represented how ingredients are usually consumed (i.e. not in isolation); however, all breakfasts were a source of polyphenols and although breakfasts were closely matched for energy, available CHO, fat and protein, differences between the fibre content and total sugars meant it was unclear if it was the polyphenols, the fibre or indeed other components that were responsible for the reduction in GR and IR. Furthermore, using a range of ingredients provided a wide range of polyphenol classes and structures and the most abundant sources are not necessarily the most bioactive. Much of the research on gluco-regulation and cognition is centred on polyphenols and it cannot be discounted that there may be other influences that are not part of the research literature as yet.



The exclusion criteria were set in the adult and adolescent studies to exclude individuals who may be showing signs of IGT based on fasting glucose values between 6.1 - 6.9 mmol/L using WHO definitions (WHO, 1999). It is well-documented in reviews that impaired glucose regulation is associated with impaired cognition (Messier, 2004) and this is more prevalent in older adults (Lamport, Lawton, Mansfield, & Dye, 2009). The current study did not measure IGT, nor did it categorise participants based on GR and glucose control, as none of the baseline measures were outside recommendations; however, future studies may want to consider this. Furthermore, the habitual breakfast habits of individuals can affect GR to subsequent meals and performance on cognitive tests (Dye & Blundell, 2002) and mood (Lloyd et al., 1996); however, this was only measured in adolescents (not adults).

The selection of cognitive tests in the current thesis was limited by its reliance on the breakfast-no-breakfast literature. Future studies comparing breakfast composition may benefit from selecting a cognitive domain sensitive to the ingredients included, although this is challenging when there are a range of ingredients, and identifying a specific cognitive domain relative to breakfast composition represents a general limitation of research in this area (Adolphus et al., 2016). The map task was originally selected as a measure of spatial memory, based on its reporting in the literature (chapter 6.2.2); however, the test measures across domains and therefore the null effect was perhaps unsurprising. Furthermore, despite extensive pilot work the map task suffered from ceiling effects in both studies and its use in future studies in young populations should be considered.

## **8.4 Recommendations for future research**

This PhD highlights the importance of studying food as it would be consumed; however, research on individual functional food ingredients and their effects on gluco-regulation and cognition should continue, so as to contribute to the understanding of mechanisms which are mostly limited to animal and *in-vitro* models. To inform public health recommendations, further research considering the

benefits of consuming functional foods as part of a healthy breakfast, and the effects on gluco-regulation, cognitive performance, mood and satiety should be considered. It may be challenging for people to understand how to incorporate low-GI foods into the diet and although the same challenges remain for functional foods, the promotion of plant-based foods could be an easier message to promote to young people, thus forming the basis of future interventions aimed at promoting a healthy breakfast in young people.

Although there are a number of studies reporting on breakfast consumption, there is a lack of studies specific to adolescents and this balance should be addressed. Future studies should strive to be robust and harmonious in their reporting of age groups, breakfast types, definitions of breakfast and cognitive domains. Although research is emerging, more research to guide the selection of cognitive tests relative to meal composition is warranted, particularly around adolescents and children who are frequently targeted in cognitive studies due to potential impacts on academic performance and behaviour.

The null effect of the FB on cognitive test results, mood and satiety, suggest potential for future investigations using different tests and cognitive domains within adults and adolescents to develop a better understanding of the association between functional food intake and cognitive performance.

## **8.5 Overall conclusion**

This PhD adds relevant knowledge to the field of adolescent health research and contributes to the understanding of functional food ingredients and how they impact gluco-regulation, cognitive performance, mood and satiety. The results from this thesis provide support for the promotion of functional food ingredients as part of breakfast as a way to improve gluco-regulation in adults and adolescents. These findings will help to inform future research studies and support the development of cognitive tests that accurately measure the cognitive domain targeted.

## References

- Abraham, C., Sheeran, P., Norman, P., Conner, M., Vries, N. d., & Otten, W. (1999). When good intentions are not enough: Modeling postdecisional cognitive correlates of condom use. *Journal of Applied Social Psychology, 29*(12), 2591-2612. doi:10.1111/j.1559-1816.1999.tb00127.x
- Acheson, K. J. (2014). Indirect calorimetry: a case for improved standard operating procedures. *European Journal of Clinical Nutrition, 68*(1), 1. doi:10.1038/ejcn.2013.211
- Adolphus, K., Lawton, C. L., Champ, C. L., & Dye, L. (2016). The effects of breakfast and breakfast composition on cognition in children and adolescents: A systematic review. *Advances in Nutrition, 7*(3), 590S-612S. doi:10.3945/an.115.010256
- Adolphus, K., Lawton, C. L., & Dye, L. (2013). The effects of breakfast on behavior and academic performance in children and adolescents. *Frontiers in Human Neuroscience, 7*, 425. doi:10.3389/fnhum.2013.00425
- Adolphus, K., Lawton, C. L., & Dye, L. (2015). The relationship between habitual breakfast consumption frequency and academic performance in british adolescents. *Frontiers in Public Health, 3*, 68. doi:10.3389/fpubh.2015.00068
- Adriaanse, M. A., Vinkers, C. D. W., De Ridder, D. T. D., Hox, J. J., & De Wit, J. B. F. (2011). Do implementation intentions help to eat a healthy diet? A systematic review and meta-analysis of the empirical evidence. *Appetite, 56*(1), 183-193. doi:https://doi.org/10.1016/j.appet.2010.10.012
- Ahadi, Z., Qorbani, M., Kelishadi, R., Ardalan, G., Motlagh, M. E., Asayesh, H., . . . Heshmat, R. (2015). Association between breakfast intake with anthropometric measurements, blood pressure and food consumption behaviors among Iranian children and adolescents: the CASPIAN-IV study. *Public Health, 129*(6), 740-747. doi:http://dx.doi.org/10.1016/j.puhe.2015.03.019
- Ajzen, I. (2005). *Attitudes, Personality and Behaviour* (Second ed.). Maidenhead: Open University Press.
- Ajzen, I. (2006). Constructing a theory of planned behavior questionnaire. Retrieved from July 2, 2015: <http://people.umass.edu/aizen/pdf/tpb.measurement.pdf>
- Ajzen, I. (2012). The theory of planned behaviour. In Paul A M Van Lange, Arie W Kruglanski, & E Tory Higgins (Eds.), *Handbook of Theories of Social Psychology* (Vol. One, pp. 438-459). London: SAGE.

- Alam, D. S., Hulshof, P. J., Roordink, D., Meltzer, M., Yunus, M., Salam, M. A., & van Raaij, J. M. (2005). Validity and reproducibility of resting metabolic rate measurements in rural Bangladeshi women: comparison of measurements obtained by Medgem and by Deltatrac device. *European Journal of Clinical Nutrition*, *59*(5), 651-657.  
doi:10.1038/sj.ejcn.1602122
- Alzaid, F., Cheung, H.-M., Preedy, V. R., & Sharp, P. A. (2013). Regulation of glucose transporter expression in human intestinal caco-2 cells following exposure to an anthocyanin-rich berry extract. *PLoS One*, *8*(11), e78932. doi:10.1371/journal.pone.0078932
- Anderson, R. A. (2008). Chromium and polyphenols from cinnamon improve insulin sensitivity. *The Proceedings of the Nutrition Society*, *67*(1), 48-53. doi:10.1017/S0029665108006010
- Andres-Lacueva, C., Shukitt-Hale, B., Galli, R. L., Jauregui, O., Lamuela-Raventos, R. M., & Joseph, J. A. (2005). Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutritional Neuroscience*, *8*(2), 111-120.  
doi:10.1080/10284150500078117
- Arai, S. (1996). Studies on functional foods in Japan-State of the art. *Bioscience, Biotechnology, and Biochemistry*, *60*(1), 9-15. doi:10.1271/bbb.60.9
- Aranceta, J., Serra-Majem, L., Ribas, L., & Perez-Rodrigo, C. (2001). Breakfast consumption in Spanish children and young people. *Public Health Nutrition*, *4*(6a), 1439-1444.
- Armitage, C. C., M. (2001). Efficacy of the theory of planned behaviour: A meta-analytic review. *British Journal of Social Psychology*(40), 471-499.
- Armstrong, N., & Welsman, J. R. (2006). The physical activity patterns of European youth with reference to methods of assessment. *Sports Medicine*, *36*(12), 1067-1086.
- Astbury, N. M., Taylor, M. A., & Macdonald, I. A. (2011). Breakfast consumption affects appetite, energy intake, and the metabolic and endocrine responses to foods consumed later in the day in male habitual breakfast eaters. *Journal of Nutrition*, *141*(7), 1381-1389.  
doi:10.3945/jn.110.128645
- Atkinson, F. S., Foster-Powell, K., & Brand-Miller, J. C. (2008). International tables of glycemic index and glycemic load values: 2008. *Diabetes Care*, *31*(12), 2281-2283.  
doi:10.2337/dc08-1239
- Balding, A., & Regis, D. (2015). *Young People into 2015*. Retrieved from Devon, UK:  
<http://sheu.org.uk/content/page/young-people-2015>

- Ball, S. D., Keller, K. R., Moyer-Mileur, L. J., Ding, Y. W., Donaldson, D., & Jackson, W. D. (2003). Prolongation of satiety after low versus moderately high glycemic index meals in obese adolescents. *Pediatrics*, *111*(3), 488-494.
- Banks, W. A., Owen, J. B., & Erickson, M. A. (2012). Insulin in the brain: there and back again. *Pharmacology & therapeutics*, *136*(1), 82-93. doi:10.1016/j.pharmthera.2012.07.006
- Basu, A., Du, M., Leyva, M. J., Sanchez, K., Betts, N. M., Wu, M., . . . Lyons, T. J. (2010). Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *Journal of Nutrition*, *140*(9), 1582-1587. doi:10.3945/jn.110.124701
- Beck, E. J., Tosh, S. M., Batterham, M. J., Tapsell, L. C., & Huang, X. F. (2009a). Oat beta-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Molecular Nutrition & Food Research*, *53*(10), 1343-1351. doi:10.1002/mnfr.200800343
- Bell, L., Lamport, D. J., Butler, L. T., & Williams, C. M. (2015). A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. *Nutrients*, *7*(12), 10290-10306. doi:10.3390/nu7125538
- Belloc, N. B., & Breslow, L. (1972). Relationship of physical health status and health practices. *Preventive Medicine*, *1*(3), 409-421. doi:http://dx.doi.org/10.1016/0091-7435(72)90014-X
- Benton, D. (2002). Carbohydrate ingestion, blood glucose and mood. *Neuroscience & Biobehavioral Reviews*, *26*(3), 293-308.
- Benton, D. (2010). The influence of dietary status on the cognitive performance of children. *Molecular Nutrition & Food Research*, *54*(4), 457-470. doi:10.1002/mnfr.200900158
- Benton, D., Kallus, K. W., & Schmitt, J. A. J. (2005). How should we measure nutrition-induced improvements in memory? *European Journal of Nutrition*, *44*(8), 485-498. doi:10.1007/s00394-005-0583-6
- Benton, D., & Owens, D. (1993). Is raised blood glucose associated with the relief of tension? *J Psychosom Res*, *37*(7), 723-735.
- Benton, D., & Parker, P. Y. (1998). Breakfast, blood glucose, and cognition. *The American Journal of Clinical Nutrition*, *67*(4), 772s-778s.
- Benton, D., Parker, P. Y., & Donohoe, R. T. (1996). The supply of glucose to the brain and cognitive functioning. *Journal of Biosocial Science*, *28*(Special Issue 04), 463-479. doi:doi:10.1017/S0021932000022537

- Benton, D., Ruffin, M.-P., Lassel, T., Nabb, S., Messaoudi, M., Vinoy, S., . . . Lang, V. (2003). The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans. *Psychopharmacology*, *166*(1), 86-90. doi:10.1007/s00213-002-1334-5
- Benton, D., & Sargent, J. (1992). Breakfast, blood glucose and memory. *Biological Psychology*, *33*(2-3), 207-210. doi:http://dx.doi.org/10.1016/0301-0511(92)90032-P
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. doi:10.1006/abio.1996.0292
- Berg, C., Jonsson, I., & Conner, M. (2000). Understanding choice of milk and bread for breakfast among swedish children aged 11-15 years: An application of the theory of planned behaviour. *Appetite*, *34*(1), 5-19. doi:http://dx.doi.org/10.1006/appe.1999.0269
- Berkey, C. S., Rockett, H. R., Gillman, M. W., Field, A. E., & Colditz, G. A. (2003). Longitudinal study of skipping breakfast and weight change in adolescents. *International Journal of Obesity and Related Metabolic Disorders*, *27*(10), 1258-1266. doi:10.1038/sj.ijo.0802402
- Betts, J. A., Richardson, J. D., Chowdhury, E. A., Holman, G. D., Tsintzas, K., & Thompson, D. (2014). The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults. *The American Journal of Clinical Nutrition*, *100*, 539-547. doi:10.3945/ajcn.114.083402
- Bitar, A., Fellmann, N., Vernet, J., Coudert, J., & Vermorel, M. (1999). Variations and determinants of energy expenditure as measured by whole-body indirect calorimetry during puberty and adolescence. *The American Journal of Clinical Nutrition*, *69*(6), 1209-1216.
- Blacker, B. C., Snyder, S. M., Eggett, D. L., & Parker, T. L. (2013). Consumption of blueberries with a high-carbohydrate, low-fat breakfast decreases postprandial serum markers of oxidation. *British Journal of Nutrition*, *109*(9), 1670-1677. doi:10.1017/s0007114512003650
- Blakemore, S.-J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human Brain Mapping*, *31*(6), 926-933. doi:10.1002/hbm.21052
- Bland, J., & Altman, D. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, *327* (8476), 307-310.
- Blond, E., Maitrepierre, C., Normand, S., Sothier, M., Roth, H., Goudable, J., & Laville, M. (2011). A new indirect calorimeter is accurate and reliable for measuring basal energy expenditure, thermic effect of food and substrate oxidation in obese and healthy subjects. *e-SPEN, the*

*European e-Journal of Clinical Nutrition and Metabolism*, 6(1), e7-e15.

doi:10.1016/j.eclnm.2010.12.001

Blundell, J., de Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Luch, A., . . . Westerterp, M. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews*, 11(3), 251-270. doi:10.1111/j.1467-789X.2010.00714.x

Borges, G., Degeneve, A., Mullen, W., & Crozier, A. (2010). Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *Journal of Agricultural and Food Chemistry*, 58(7), 3901-3909. doi:10.1021/jf902263n

Braaten, J. T., Wood, P. J., Scott, F. W., Wolynetz, M. S., Lowe, M. K., Bradley-White, P., & Collins, M. W. (1994). Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition*, 48(7), 465-474.

Brindal, E., Baird, D., Danthiir, V., Wilson, C., Bowen, J., Slater, A., & Noakes, M. (2012). Ingesting breakfast meals of different glycaemic load does not alter cognition and satiety in children. *European Journal of Clinical Nutrition*, 66(10), 1166-1171. doi:10.1038/ejcn.2012.99

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. (2005). Glycaemic index methodology. *Nutrition Research Reviews*, 18(1), 145-171. doi:10.1079/nrr2005100

Buchholz, A. C., Rafii, M., & Pencharz, P. B. (2001). Is resting metabolic rate different between men and women? *British Journal of Nutrition*, 86(06), 641-646. doi:doi:10.1079/BJN2001471

Budson, A. E., & Price, B. H. (2005). Memory dysfunction. *New England Journal of Medicine*, 352(7), 692-699. doi:10.1056/NEJMra041071

Burton-Freeman, B., Davis, P. A., & Schneeman, B. O. (2002). Plasma cholecystokinin is associated with subjective measures of satiety in women. *The American Journal of Clinical Nutrition*, 76(3), 659-667.

Busch, C. R., Taylor, H. A., Kanarek, R. B., & Holcomb, P. J. (2002). The effects of a confectionery snack on attention in young boys. *Physiology & Behavior*, 77(2-3), 333-340.

Caldwell, K., Charlton, K. E., Roodenrys, S., & Jenner, A. (2016). Anthocyanin-rich cherry juice does not improve acute cognitive performance on RAVLT. *Nutritional Neuroscience*, 19(9), 423-424. doi:10.1179/1476830515Y.0000000005

- Carskadon, M. A. (2011). Sleep in Adolescents: The Perfect Storm. *Pediatric clinics of North America*, 58(3), 637-647. doi:10.1016/j.pcl.2011.03.003
- Cavallero, A., Empilli, S., Brighenti, F., & Stanca, A. M. (2002). High (1→3,1→4)-β-glucan barley fractions in bread making and their effects on human glyceemic response. *Journal of Cereal Science*, 36(1), 59-66. doi:http://dx.doi.org/10.1006/jcrs.2002.0454
- Ceriello, A. (2005). Postprandial hyperglycemia and diabetes complications. Is it time to treat? *Diabetes*, 54(1), 1-7. doi:10.2337/diabetes.54.1.1
- Ceriello, A., Colagiuri, S., Gerich, J., & Tuomilehto, J. (2008). Guideline for management of postmeal glucose. *Nutrition, Metabolism, and Cardiovascular Diseases*, 18(4), S17-33. doi:10.1016/j.numecd.2008.01.012
- Chan, K., & Tsang, L. (2011). Promote healthy eating among adolescents: A Hong Kong study. *Journal of Consumer Marketing*, 28(5), 354-362. doi:10.1108/07363761111150008
- Change4Life. (2014). Get off to a great start with a healthy breakfast! Retrieved from 20 May, 2014: <http://www.nhs.uk/Change4Life/Pages/breakfast-for-life.aspx>
- Chapman, G. E., Melton, C. L., & Hammond, G. K. (1998). College and university students' breakfast consumption patterns: Behaviours, beliefs, motivations and personal and environmental influences. *Canadian Journal of Dietetic Practice and Research*, 59(4), 176-182.
- Chiva-Blanch, G., & Visioli, F. (2012). Polyphenols and health: Moving beyond antioxidants. *Journal of Berry Research*, 2, 63–71.
- Cho, S., Dietrich, M., Brown, C. J., Clark, C. A., & Block, G. (2003). The effect of breakfast type on total daily energy intake and body mass index: results from the Third National Health and Nutrition Examination Survey (NHANES III). *The Journal of the American College of Nutrition*, 22(4), 296-302.
- Choo, J. J. (2003). Green tea reduces body fat accretion caused by high-fat diet in rats through β-adrenoceptor activation of thermogenesis in brown adipose tissue. *The Journal of Nutritional Biochemistry*, 14(11), 671-676. doi:https://doi.org/10.1016/j.jnutbio.2003.08.005
- Chun, O. K., Chung, S.-J., Claycombe, K. J., & Song, W. O. (2008). Serum c-reactive protein concentrations are inversely associated with dietary flavonoid intake in u.S. Adults. *The Journal of Nutrition*, 138(4), 753-760.



- Clegg, M. E., Golsorkhi, M., & Henry, C. J. (2013). Combined medium-chain triglyceride and chilli feeding increases diet-induced thermogenesis in normal-weight humans. *European Journal of Nutrition*, *52*(6), 1579-1585. doi:10.1007/s00394-012-0463-9
- Clegg, M. E., Pratt, M., Meade, C. M., & Henry, C. J. K. (2011). The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. *British Journal of Nutrition*, *106*(03), 335-338. doi:doi:10.1017/S0007114511001450
- Coe, S., & Ryan, L. (2015). White bread enriched with polyphenol extracts shows no effect on glycemic response or satiety, yet may increase postprandial insulin economy in healthy participants. *Nutrition Research*, *36*((2)), 193-200. doi:10.1016/j.nutres.2015.10.007
- Coe, S., & Ryan, L. (2016). The impact of polyphenol rich sources on acute postprandial glycaemia: a systematic review. *Journal of Nutritional Science*, *5*(e24).
- Coe, S. A., Clegg, M., Armengol, M., & Ryan, L. (2013). The polyphenol-rich baobab fruit (*Adansonia digitata* L.) reduces starch digestion and glycemic response in humans. *Nutrition Research*, *33*(11), 888-896. doi:10.1016/j.nutres.2013.08.002
- Cole, T. J., Freeman, J. V., & Preece, M. A. (1995). Body mass index reference curves for the UK, 1990. *Archives of Disease in Childhood*, *73*(1), 25-29.
- Compher, C., Frankenfield, D., Keim, N., & Roth-Yousey, L. (2006). Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *Journal of the American Dietetic Association*, *106*(6), 881-903. doi:10.1016/j.jada.2006.02.009
- Compher, C., Hise, M., Sternberg, A., & Kinosian, B. P. (2005). Comparison between Medgem and Deltatrac resting metabolic rate measurements. *European Journal of Clinical Nutrition*, *59*(10), 1136-1141. doi:10.1038/sj.ejcn.1602223
- Conner, M., Hugh-Jones, S., & Berg, C. (2011). Using the two-factor theory of planned behaviour to predict adolescent breakfast choices. *Educational and Child Psychology*, *28*(4), 37-50.
- Conner, M., Norman, P., & Bell, R. (2002). The theory of planned behavior and healthy eating. *Health Psychology*, *21*(2), 194-201.
- Cooper, J. A., Watras, A. C., O'Brien, M. J., Luke, A., Dobratz, J. R., Earthman, C. P., & Schoeller, D. A. (2009). Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *Journal of the American Dietetic Association*, *109*(1), 128-132. doi:10.1016/j.jada.2008.10.004

- Cooper, S. B., Bandelow, S., & Nevill, M. E. (2011). Breakfast consumption and cognitive function in adolescent schoolchildren. *Physiology & Behavior*, *103*(5), 431-439. doi:10.1016/j.physbeh.2011.03.018
- Cooper, S. B., Bandelow, S., Nute, M. L., Morris, J. G., & Nevill, M. E. (2012). Breakfast glycaemic index and cognitive function in adolescent school children. *The British Journal of Nutrition*, *107*(12), 1823-1832. doi:10.1017/S0007114511005022
- Cooper, S. B., Bandelow, S., Nute, M. L., Morris, J. G., & Nevill, M. E. (2015). Breakfast glycaemic index and exercise: Combined effects on adolescents' cognition. *Physiology & Behavior*, *139*, 104-111. doi:http://dx.doi.org/10.1016/j.physbeh.2014.11.024
- Corder, K., van Sluijs, E. M., Ridgway, C. L., Steele, R. M., Prynne, C. J., Stephen, A. M., . . . Ekelund, U. (2014). Breakfast consumption and physical activity in adolescents: daily associations and hourly patterns. *The American Journal of Clinical Nutrition*, *99*(2), 361-368. doi:10.3945/ajcn.111.027607
- Corder, K., van Sluijs, E. M., Steele, R. M., Stephen, A. M., Dunn, V., Bamber, D., . . . Ekelund, U. (2011). Breakfast consumption and physical activity in British adolescents. *The British Journal of Nutrition*, *105*(2), 316-321. doi:10.1017/S0007114510003272
- Crichton, G. E., Bryan, J., & Murphy, K. J. (2013). Dietary antioxidants, cognitive function and dementia--a systematic review. *Plant Foods for Human Nutrition*, *68*(3), 279-292. doi:10.1007/s11130-013-0370-0
- Cromer, B. A., Tarnowski, K. J., Stein, A. M., Harton, P., & Thornton, D. J. (1990). The school breakfast program and cognition in adolescents. *Journal of Developmental & Behavioral Pediatrics*, *11*(6), 295-300.
- Cueto, S., Jacoby, E., & Pollitt, E. (1998). Breakfast prevents delays of attention and memory functions among nutritionally at-risk boys. *Journal of Applied Developmental Psychology*, *19*(2), 219-233. doi:http://dx.doi.org/10.1016/S0193-3973(99)80037-9
- Davis, P. A., & Yokoyama, W. (2011). Cinnamon intake lowers fasting blood glucose: Meta-analysis. *Journal of Medicinal Food*, *14*(9), 884-889. doi:10.1089/jmf.2010.0180
- De Caluwé, E., Halamová, K., & Van Damme, P. (2010). *Adansonia digitata* L. – A review of traditional uses, phytochemistry and pharmacology. *Afrika Focus*, *Volume 23*(Nr. 1), pp. 11-51.

- de Jager, C. A., Dye, L., de Bruin, E. A., Butler, L., Fletcher, J., Lamport, D. J., . . . Wesnes, K. (2014). Criteria for validation and selection of cognitive tests for investigating the effects of foods and nutrients. *Nutrition Reviews*, *72*(3), 162-179. doi:10.1111/nure.12094
- de Jonee, L., & Bray, G. A. (1997). The thermic effect of food and obesity: A critical review. *Obesity Research*, *5*(6), 622-631. doi:10.1002/j.1550-8528.1997.tb00584.x
- de la Hunty, A., Gibson, S., & Ashwell, M. (2013). Does regular breakfast cereal consumption help children and adolescents stay slimmer? A systematic review and meta-analysis. *Obesity Facts*, *6*(1), 70-85. doi:10.1159/000348878
- Defeyter, M. A., & Russo, R. (2013). The effect of breakfast cereal consumption on adolescents' cognitive performance and mood. *Frontiers in Human Neuroscience*, *7*, 789. doi:10.3389/fnhum.2013.00789
- DeJong, C. S., van Lenthe, F. J., van der Horst, K., & Oenema, A. (2009). Environmental and cognitive correlates of adolescent breakfast consumption. *Preventive Medicine*, *48*(4), 372-377. doi:http://dx.doi.org/10.1016/j.ypmed.2009.02.009
- Delva, J., Johnston, L. D., & O'Malley, P. M. (2007). The epidemiology of overweight and related lifestyle behaviors: racial/ethnic and socioeconomic status differences among American youth. *American Journal of Preventive Medicine*, *33*(4 Suppl), S178-186. doi:10.1016/j.amepre.2007.07.008
- Department of Health (DOH). (2011). *Healthy lives, Healthy people: A call to action on obesity in England*. Retrieved from <https://www.gov.uk/government/publications/healthy-lives-healthy-people-a-call-to-action-on-obesity-in-england>
- Deshmukh-Taskar, P., Nicklas, T., O'Neil, C., Keast, D., Radcliffe, J., & Cho, S. (2010). The relationship of breakfast skipping and type of breakfast consumption with nutrient intake and weight status in children and adolescents: the National Health and Nutrition Examination Survey 1999-2006. *Journal of the American Dietetic Association*, *110*, 869 - 878.
- Devore, E., Jae Hee, K., Breteler, M. M. B., & Grodstein, F. (2012). Eating more berries may reduce cognitive decline in the elderly: flavonoid-rich blueberries and strawberries offer most benefit. *American journal of Alzheimer's disease and other dementias*, *27*(5), 358-358.
- Devore, E. E., Kang, J. H., Breteler, M. M. B., & Grodstein, F. (2012). Dietary intakes of berries and flavonoids in relation to cognitive decline. *Annals of Neurology*, *72*(1), 135-143. doi:10.1002/ana.23594

- Dhurandhar, N. V., Schoeller, D., Brown, A. W., Heymsfield, S. B., Thomas, D., Sorensen, T. I. A., . . . Allison, D. B. (2014). Energy balance measurement: when something is not better than nothing. *Int J Obes*, *39*(7), 1109-1113. doi:10.1038/ijo.2014.199
- Dickie, N. H., & Bender, A. E. (1982a). Breakfast and performance. *Human Nutrition. Applied Nutrition*, *36*(1), 46-56.
- Dikeman, C. L., & Fahey, G. C. (2006). Viscosity as related to dietary fiber: a review. *Critical Reviews in Food Science and Nutrition*, *46*(8), 649-663. doi:10.1080/10408390500511862
- Dodd, G. F. (2012). *The Acute Effects of Flavonoid-Rich Blueberries on Cognitive Function in Healthy Younger and Older Adults*. (Doctoral dissertation Doctoral dissertation), University of Reading, Reading, UK, Retrieved from <http://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.607157> (uk.bl.ethos.607157)
- Donin, A. S., Nightingale, C. M., Owen, C. G., Rudnicka, A. R., Perkin, M. R., Jebb, S. A., . . . Whincup, P. H. (2014). Regular breakfast consumption and type 2 diabetes risk markers in 9- to 10-year-old children in the child heart and health study in England (CHASE): A cross-sectional analysis. *PLOS medicine*, *11*(9). doi:10.1371/journal.pmed
- Duncan, M. J., Ravis, A., & Jordan, C. (2012). Brief report: Understanding intention to be physically active and physical activity behaviour in adolescents from a low socio-economic status background: An application of the theory of planned behaviour. *Journal of Adolescence*, *35*(3), 761-764. doi:http://dx.doi.org/10.1016/j.adolescence.2011.07.017
- Dye, L., & Blundell, J. (2002). Functional foods: psychological and behavioural functions. *The British Journal of Nutrition*, *88 Suppl 2*, S187-211. doi:10.1079/BJN2002684
- Dye, L., Lluch, A., & Blundell, J. E. (2000). Macronutrients and mental performance. *Nutrition*, *16*(10), 1021-1034.
- Ebbeling, C. B., Leidig, M. M., Sinclair, K. B., Hangen, J. P., & Ludwig, D. S. (2003). A reduced-glycemic load diet in the treatment of adolescent obesity. *Archives of Pediatrics & Adolescent Medicine*, *157*(8), 773-779. doi:10.1001/archpedi.157.8.773
- ECAL. (2013). ECAL User Manual. In. Singapore: Lionstar Technologies Ltd.
- Edefonti, V., Rosato, V., Parpinel, M., Nebbia, G., Fiorica, L., Fossali, E., . . . Agostoni, C. (2014). The effect of breakfast composition and energy contribution on cognitive and academic performance: a systematic review. *The American Journal of Clinical Nutrition*, *100*(2), 626-656. doi:10.3945/ajcn.114.083683

- Ells, L. J., Hillier, F. C., Shucksmith, J., Crawley, H., Harbige, L., Shield, J., . . . Summerbell, C. D. (2008). A systematic review of the effect of dietary exposure that could be achieved through normal dietary intake on learning and performance of school-aged children of relevance to UK schools. *The British Journal of Nutrition*, *100*(5), 927-936.  
doi:10.1017/S0007114508957998
- Englyst, H. N., & Hudson, G. J. (1987). Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. *Food Chemistry*, *24*(1), 63-76. doi:http://dx.doi.org/10.1016/0308-8146(87)90084-7
- Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1999). Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. *The American Journal of Clinical Nutrition*, *69*(3), 448-454.
- Epstein, H. T. (1986). Stages in human brain development. *Developmental Brain Research*, *30*(1), 114-119. doi:http://dx.doi.org/10.1016/0165-3806(86)90139-2
- Erlund, I., Koli, R., Alfthan, G., Marniemi, J., Puukka, P., Mustonen, P., . . . Jula, A. (2008). Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *The American Journal of Clinical Nutrition*, *87*(2), 323-331.
- European Food Safety Authority (EFSA). (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley. *EFSA Journal*, *9*(6), 2207.
- European Food Safety Authority (EFSA). (2013). Scientific opinion on dietary reference values for energy. *EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)*(11), 112.
- Farshchi, H. R., Taylor, M. A., & Macdonald, I. A. (2004). Regular meal frequency creates more appropriate insulin sensitivity and lipid profiles compared with irregular meal frequency in healthy lean women. *European Journal of Clinical Nutrition*, *58*(7), 1071-1077.
- Farshchi, H. R., Taylor, M. A., & Macdonald, I. A. (2005a). Beneficial metabolic effects of regular meal frequency on dietary thermogenesis, insulin sensitivity, and fasting lipid profiles in healthy obese women. *The American Journal of Clinical Nutrition*, *81*(1), 16-24.
- Farshchi, H. R., Taylor, M. A., & Macdonald, I. A. (2005b). Deleterious effects of omitting breakfast on insulin sensitivity and fasting lipid profiles in healthy lean women. *The American Journal of Clinical Nutrition*, *81*(2), 388-396.
- Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175-191. doi:10.3758/bf03193146

- Fernandez Morales, I., Aguilar Vilas, M. V., Mateos Vega, C. J., & Martinez Para, M. C. (2008). Relation between the breakfast quality and the academic performance in adolescents of Guadalajara (Castilla-La Mancha). *Nutricion Hospitalaria*, 23(4), 383-387.
- Fernstrom, J. D., & Wurtman, R. J. (1971). Brain serotonin content: Physiological dependence on plasma tryptophan levels. *Science*, 173(3992), 149-152. doi:10.1126/science.173.3992.149
- Ferrannini, E. (1988). The Theoretical Bases of Indirect Calorimetry: A Review. *Metabolism*, 37(No 3 (March)), pp 287-301.
- Field, A. E., Haines, J., Rosner, B., & Willett, W. C. (2010). Weight-control behaviors and subsequent weight change among adolescents and young adult females. *The American Journal of Clinical Nutrition*, 91(1), 147-153. doi:10.3945/ajcn.2009.28321
- Fields, D. A., Kearney, J. T., & Copeland, K. C. (2006). MedGem hand-held indirect calorimeter is valid for resting energy expenditure measurement in healthy children. *Obesity*, 14(10), 1755-1761. doi:10.1038/oby.2006.202
- Fila, S., & Smith, C. (2006). Applying the theory of planned behavior to healthy eating behaviors in urban Native American youth. *International Journal of Behavioral Nutrition and Physical Activity*, 3(1), 11.
- File, S. E., Jarrett, N., Fluck, E., Duffy, R., Casey, K., & Wiseman, H. (2001). Eating soya improves human memory. *Psychopharmacology (Berl)*, 157(4), 430-436. doi:10.1007/s002130100845
- Fischer, K., Colombani, P. C., Langhans, W., & Wenk, C. (2002). Carbohydrate to protein ratio in food and cognitive performance in the morning. *Physiology & Behavior*, 75(3), 411-423. doi:http://dx.doi.org/10.1016/S0031-9384(01)00676-X
- Fletcher, P. C., & Henson, R. N. (2001). Frontal lobes and human memory: insights from functional neuroimaging. *Brain*, 124(Pt 5), 849-881.
- Francis, S. T., Head, K., Morris, P. G., & Macdonald, I. A. (2006). The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *Journal of Cardiovascular Pharmacology*, 47 Suppl 2, S215-220.
- Frankenfield, D., Roth-Yousey, L., & Compher, C. (2005). Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: A systematic review. *Journal of the American Dietetic Association*, 105(5), 775-789. doi:http://dx.doi.org/10.1016/j.jada.2005.02.005

- Frankenfield, D. C. (2013). Bias and accuracy of resting metabolic rate equations in non-obese and obese adults. *Clinical Nutrition*, 32(6), 976-982. doi:10.1016/j.clnu.2013.03.022
- Frankenfield, D. C., & Coleman, A. (2013). An evaluation of a handheld indirect calorimeter against a standard calorimeter in obese and nonobese adults. *Journal of Parenteral and Enteral Nutrition*. doi:10.1177/0148607112473340
- Frydman-Marom, A., Levin, A., Farfara, D., Benromano, T., Scherzer-Attali, R., Peled, S., . . . Ovadia, M. (2011). Orally administrated cinnamon extract reduces  $\beta$ -amyloid oligomerization and corrects cognitive impairment in Alzheimer's disease animal models. *PLoS One*, 6(1), e16564. doi:10.1371/journal.pone.0016564
- Fukushima, Y., Ohie, T., Yonekawa, Y., Yonemoto, K., Aizawa, H., Mori, Y., . . . Kondo, K. (2009). Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. *Journal of Agricultural and Food Chemistry*, 57(4), 1253-1259. doi:10.1021/jf802418j
- Gharlipour, Z., Ghaffari, M., Hoseini, Z., Heidarabadi, A. B., Tavassoli, E., Hozuri, M., . . . Sahraiyani, M. (2015). Investigation of educational intervention based on theory of planned behavior on breakfast consumption among middle school students of Qom City in 2012. *Journal of Education and Health Promotion*, 4, 39. doi:10.4103/2277-9531.157224
- Giacalone, M., Di Sacco, F., Traupe, I., Topini, R., Forfori, F., & Giunta, F. (2011). Antioxidant and neuroprotective properties of blueberry polyphenols: a critical review. *Nutritional Neuroscience*, 14(3), 119-125. doi:10.1179/1476830511y.0000000007
- Giavarina, D. (2015). Understanding bland altman analysis. *Biochemia Medica*, 25(2), 141-151. doi:10.11613/BM.2015.015
- Gibbons, M. R. D., Henry, C. J. K., Ulijaszek, S. J., & Lightowler, H. J. (2004). Intra-individual variation in RMR in older people. *British Journal of Nutrition*, 91(3), 485-489. doi:10.1079/bjn20031071
- Gibson, S. (2003). Micronutrient intakes, micronutrient status and lipid profiles among young people consuming different amounts of breakfast cereals: further analysis of data from the National Diet and Nutrition Survey of Young People aged 4 to 18 years. *Public Health Nutrition*, 6(8), 815-820.
- Gold, P. E. (1995). Role of glucose in regulating the brain and cognition. *The American Journal of Clinical Nutrition*, 61(4), 987S-995S.

- Goran, M. I., & Gower, B. A. (2001). Longitudinal study on pubertal insulin resistance. *Diabetes*, 50(11), 2444-2450.
- Graf, S., Karsegard, V. L., Viatte, V., Maisonneuve, N., Pichard, C., & Genton, L. (2013). Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study. *Clinical Nutrition*, 32(6), 1067-1072. doi:http://dx.doi.org/10.1016/j.clnu.2013.08.012
- Grønhoj, A., Bech-Larsen, T., Chan, K., & Tsang, L. (2013). Using theory of planned behavior to predict healthy eating among Danish adolescents. *Health Education*, 113(1), 4-17. doi:10.1108/09654281311293600
- Gummesson, L., Jonsson, I., & Conner, M. (1997). Predicting intentions and behaviour of Swedish 10 to 16 year olds at breakfast. *Food Quality and Preference*, 8(4), 297-306.
- Haddock, G., & Maio, G. R. (2004). *Contemporary perspectives on the psychology of attitudes*. East Sussex, New York: Psychology Press.
- Haines, L., Wan, K. C., Lynn, R., Barrett, T. G., & Shield, J. P. (2007). Rising incidence of type 2 diabetes in children in the U.K. *Diabetes Care*, 30(5), 1097-1101. doi:10.2337/dc06-1813
- Hallstrom, L., Vereecken, C. A., Labayen, I., Ruiz, J. R., Le Donne, C., Garcia, M. C., . . . Sjoström, M. (2012). Breakfast habits among European adolescents and their association with sociodemographic factors: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. *Public Health Nutrition*, 15(10), 1879-1889. doi:10.1017/S1368980012000341
- Hammersley, R., Reid, M., & Atkin, S. L. (2014). How to measure mood in nutrition research. *Nutrition Research Reviews*, 27(02), 284-294. doi:doi:10.1017/S0954422414000201
- Hammersley, R., Reid, M., & Duffy, M. (2007). How may refined carbohydrates affect satiety and mood? *Nutrition Bulletin*, 32, 61-70. doi:10.1111/j.1467-3010.2007.00605.x
- Hanhineva, K., Torronen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkanen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*, 11(4), 1365-1402. doi:10.3390/ijms11041365
- Harada, U., Chikama, A., Saito, S., Takase, H., Nagao, T., Hase, T., & Tokimitsu, I. (2005). Effects of the long-term ingestion of tea catechins on energy expenditure and dietary fat oxidation in healthy subjects. *Journal of Health Science*, 51(2), 248-252. doi:10.1248/jhs.51.248



- Harris, J. A., & Benedict, F. G. (1918). A biometric study of human basal metabolism. *Proceedings of the National Academy of Sciences of the United States of America National Academy of Sciences (US); National Research Council (US)*, 4(12), 370-373.
- Hassenstab, J. J., Sweat, V., Bruehl, H., & Convit, A. (2010). Metabolic syndrome is associated with learning and recall impairment in middle age. *Dementia and Geriatric Cognitive Disorders*, 29(4), 356-362. doi:10.1159/000296071
- Hasson, R. E., Howe, C. A., Jones, B. L., & Freedson, P. S. (2011). Accuracy of four resting metabolic rate prediction equations: effects of sex, body mass index, age, and race/ethnicity. *Journal of Science and Medicine in Sport*, 14(4), 344-351. doi:10.1016/j.jsams.2011.02.010
- Haug, E., Rasmussen, M., Samdal, O., Iannotti, R., Kelly, C., Borraccino, A., . . . Ahluwalia, N. (2009). Overweight in school-aged children and its relationship with demographic and lifestyle factors: results from the WHO-Collaborative Health Behaviour in School-aged Children (HBSC) study. *International Journal of Public Health*, 54 Suppl 2, 167-179. doi:10.1007/s00038-009-5408-6
- Hendrickson, S. J., & Mattes, R. D. (2008). No acute effects of grape juice on appetite, implicit memory and mood. *Food & Nutrition Research*, 52, 10.3402/fnr.v3452i3400.1891. doi:10.3402/fnr.v52i0.1891
- Henes, S. T., Cummings, D. M., Hickner, R. C., Houmard, J. A., Kolasa, K. M., Lazorick, S., & Collier, D. N. (2013). Comparison of predictive equations and measured resting energy expenditure among obese youth attending a pediatric healthy weight clinic: One size does not fit all. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*, 28(5), 10.1177/0884533613497237. doi:10.1177/0884533613497237
- Henes, S. T., Johnson, A., Toner, M., Mamaril, K., Kelkar, M., Xiao, Y., & Warren, G. L. (2015). Assessing resting metabolic rate in overweight and obese adolescents with a portable indirect calorimeter: A pilot study for validation and reliability. *Nutrition in Clinical Practice*. doi:10.1177/0884533615603966
- Henry, C. J. (2005). Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutrition*, 8(7a), 1133-1152.
- Herrero Lozano, R., & Fillat Ballesteros, J. C. (2006). A study on breakfast and school performance in a group of adolescents. *Nutricion Hospitalaria*, 21(3), 346-352.

- Hewitt, A. M., & Stephens, C. (2007). Healthy eating among 10 - 13-year-old New Zealand children: understanding choice using the theory of planned behaviour and the role of parental influence. *Psychology, Health & Medicine, 12*(5), 526-535.  
doi:10.1080/13548500601164396
- Ho, S. C., Chang, K. S., & Chang, P. W. (2013). Inhibition of neuroinflammation by cinnamon and its main components. *Food Chemistry, 138*(4), 2275-2282.  
doi:10.1016/j.foodchem.2012.12.020
- Höglund, D., Samuelson, G., & Mark, A. (1998). Food habits in Swedish adolescents in relation to socioeconomic conditions. *European Journal of Clinical Nutrition, 52*(11), 784-789.
- Holliday, M. A. (1971). Metabolic rate and organ size during growth from infancy to maturity and during late gestation and early infancy. *Pediatrics, 47*(1), Suppl 2:169+.
- Holt, S. H. A., Delargy, H. J., Lawton, C. L., & Blundell, J. E. (1999). The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. *International Journal of Food Sciences and Nutrition, 50*, 13-28.
- Hooper, L., Kroon, P. A., Rimm, E. B., Cohn, J. S., Harvey, I., Le Cornu, K. A., . . . Cassidy, A. (2008). Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition, 88*(1), 38-50.
- Hosseini, Z., Aghamolaei, T., Gharlipour Gharghani, Z., & Ghanbarnejad, A. (2015). Effect of educational interventions based on theory of planned behavior to promote breakfast consumption behavior in students. *Hormozgan Medical Journal, 19*(1).
- Hoyland, A., Dye, L., & Lawton, C. L. (2009). A systematic review of the effect of breakfast on the cognitive performance of children and adolescents. *Nutrition Research Reviews, 22*(02), 220-243. doi:doi:10.1017/S0954422409990175
- Hoyland, A., McWilliams, K. A., Duff, R. J., & Walton, J. L. (2012). Breakfast consumption in UK schoolchildren and provision of school breakfast clubs. *Nutrition Bulletin, 37*(3), 232-240.  
doi:10.1111/j.1467-3010.2012.01973.x
- Hursel, R., Viechtbauer, W., Dulloo, A. G., Tremblay, A., Tappy, L., Rumpler, W., & Westerterp-Plantenga, M. S. (2011). The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obesity reviews, 12*(7), e573-e581. doi:10.1111/j.1467-789X.2011.00862.x

- Inchley, J., Currie, D., Young, T., Samdal, O., Torsheim, T., Augustson, L., . . . Barnekow, V. (2016). *Growing up unequal: gender and socioeconomic differences in young people's health and well-being*: World Health Organisation.
- Ingoldsby, M. E. (1951). Be smart: Eat a good breakfast. *The American Journal of Nursing*, *51*(9), 559-560. doi:10.2307/3468423
- Ingwersen, J., Defeyter, M. A., Kennedy, D. O., Wesnes, K. A., & Scholey, A. B. (2007). A low glycaemic index breakfast cereal preferentially prevents children's cognitive performance from declining throughout the morning. *Appetite*, *49*(1), 240-244. doi:10.1016/j.appet.2006.06.009
- International Organization for Standardization (ISO). (2010). *Food products - Determination of the glycaemic index (GI) and recommendation for food classification*. Retrieved from [http://www.iso.org/iso/catalogue\\_detail.htm?csnumber=43633](http://www.iso.org/iso/catalogue_detail.htm?csnumber=43633)
- Jenkins, D. J., Kendall, C. W., Faulkner, D. A., Nguyen, T., Kemp, T., Marchie, A., . . . Singer, W. (2006). Assessment of the longer-term effects of a dietary portfolio of cholesterol-lowering foods in hypercholesterolemia. *The American Journal of Clinical Nutrition*, *83*(3), 582-591.
- Jenkins, D. J., Kendall, C. W., Marchie, A., Faulkner, D. A., Wong, J. M., de Souza, R., . . . Connelly, P. W. (2005). Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. *The American Journal of Clinical Nutrition*, *81*(2), 380-387.
- Jenkins, D. J. A., Ghafari, H., Wolever, T. M. S., Taylor, R. H., Jenkins, A. L., Barker, H. M., . . . Bowling, A. C. (1982). Relationship between rate of digestion of foods and post-prandial glycaemia. *Diabetologia*, *22*(6), 450-455. doi:10.1007/bf00282589
- Johnson, M. H., Lucius, A., Meyer, T., & de Mejia, E. G. (2011). Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of alpha-amylase and alpha-glucosidase by highbush blueberry (*Vaccinium corombosum*). *Journal of Agricultural and Food Chemistry*, *59*(16), 8923-8930. doi:10.1021/jf201720z
- Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Bielinski, D., Martin, A., McEwen, J. J., & Bickford, P. C. (1999). Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *Journal of Neuroscience*, *19*(18), 8114-8121.
- Kafatos, A., Linardakis, M., Bertsiyas, G., Mammias, I., Fletcher, R., & Bervanaki, F. (2005). Consumption of ready-to-eat cereals in relation to health and diet indicators among school

adolescents in Crete, Greece. *Annals of Nutrition and Metabolism*, 49(3), 165-172.  
doi:10.1159/000086880

- Kallio, P., Kolehmainen, M., Laaksonen, D. E., Pulkkinen, L., Atalay, M., Mykkänen, H., . . . Niskanen, L. (2008). Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. *The American Journal of Clinical Nutrition*, 87(5), 1497-1503.
- Kennedy, C., Hajek, A., Morris, D., Linnell, S., & Gines, J. (2005). Strategies to increase breakfast consumption in a residential university population: an environmental intervention. *American Journal of Health Promotion*, 19(No. 6.), pp. 457-458
- Kennedy, D. O. (2014). Polyphenols and the human brain: Plant “secondary metabolite” ecologic roles and endogenous signaling functions drive benefits. *Advances in Nutrition: An International Review Journal*, 5(5), 515-533. doi:10.3945/an.114.006320
- Keski-Rahkonen, A., Kaprio, J., Rissanen, A., Virkkunen, M., & Rose, R. J. (2003). Breakfast skipping and health-compromising behaviors in adolescents and adults. *European Journal of Clinical Nutrition*, 57(7), 842-853.
- Kessels, R. P. C., Nys, G. M. S., Brands, A. M. A., van den Berg, E., & Van Zandvoort, M. J. E. (2006). The modified Location Learning Test: Norms for the assessment of spatial memory function in neuropsychological patients. *Archives of Clinical Neuropsychology*, 21(8), 841-846. doi:http://dx.doi.org/10.1016/j.acn.2006.06.015
- Khan, A., Bryden, N. A., Polansky, M. M., & Anderson, R. A. (1990). Insulin potentiating factor and chromium content of selected foods and spices. *Biological Trace Element Research*, 24(2), 183-188. doi:10.1007/bf02917206
- Kim, J. H., & So, W. Y. (2012). Association between frequency of breakfast eating and obesity in korean adolescents. *Iranian Journal of Public Health*, 41(6), 50-57.
- Kopelman, M. D. (1986). The cholinergic neurotransmitter system in human memory and dementia: A review. *The Quarterly Journal of Experimental Psychology Section A*, 38(4), 535-573. doi:10.1080/14640748608401614
- Kothe, E. J., Mullan, B. A., & Amaratunga, R. (2011). Randomised controlled trial of a brief theory-based intervention promoting breakfast consumption. *Appetite*, 56(1), 148-155. doi:10.1016/j.appet.2010.12.002
- Kowalski, K., Crocker, P., & Kowalski, N. (1997). Convergent validity of the physical activity questionnaire for adolescents. *Pediatr Exerc Sci*(9), 342-352.

- Kral, T. V., Whiteford, L. M., Heo, M., & Faith, M. S. (2011). Effects of eating breakfast compared with skipping breakfast on ratings of appetite and intake at subsequent meals in 8- to 10-year-old children. *The American Journal of Clinical Nutrition*, *93*(2), 284-291.  
doi:10.3945/ajcn.110.000505
- Krikorian, R., Nash, T. A., Shidler, M. D., Shukitt-Hale, B., & Joseph, J. A. (2010a). Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *The British Journal of Nutrition*, *103*(5), 730-734.  
doi:10.1017/S0007114509992364
- Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., & Joseph, J. A. (2010b). Blueberry supplementation improves memory in older adults. *Journal of Agricultural and Food Chemistry*, *58*(7), 3996-4000. doi:10.1021/jf9029332
- Lamport, D. J., Dye, L., Wightman, J. D., & Lawton, C. L. (2012). The effects of flavonoid and other polyphenol consumption on cognitive performance: A systematic research review of human experimental and epidemiological studies. *Nutrition and Aging*, *1*(1), 5-25.  
doi:10.3233/NUA-2012-0002
- Lamport, D. J., Lawton, C. L., Mansfield, M. W., & Dye, L. (2009). Impairments in glucose tolerance can have a negative impact on cognitive function: a systematic research review. *Neuroscience & Biobehavioral Reviews*, *33*(3), 394-413.  
doi:10.1016/j.neubiorev.2008.10.008
- Lan-Pidhainy, X., Brummer, Y., Tosh, S. M., Wolever, T. M., & Wood, P. J. (2007). Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. *Cereal Chemistry Journal*, *84*(5), 512-517. doi:10.1094/CCHEM-84-5-0512
- Lawrence, F. (2008). *Eat Your Heart Out: Why the Food Business Is Bad for the Planet and Your Health*. (Vol. Vol.1.). London: Penguin Books.
- Lawrence, J. C., Lee, H.-M., Kim, J.-H., & Kim, E.-K. (2009). Variability in results from predicted resting energy needs as compared to measured resting energy expenditure in Korean children. *Nutrition Research*, *29*(11), 777-783.  
doi:http://dx.doi.org/10.1016/j.nutres.2009.10.017
- Lazzeri, G., Ahluwalia, N., Niclasen, B., Pammolli, A., Vereecken, C., Rasmussen, M., . . . Kelly, C. (2016). Trends from 2002 to 2010 in daily breakfast consumption and its socio-

- demographic correlates in adolescents across 31 countries participating in the HBSC study. *PLoS One*, *11*(3), e0151052. doi:10.1371/journal.pone.0151052
- Lazzeri, G., Giacchi, M. V., Spinelli, A., Pammolli, A., Dalmasso, P., Nardone, P., . . . Cavallo, F. (2014). Overweight among students aged 11-15 years and its relationship with breakfast, area of residence and parents' education: results from the Italian HBSC 2010 cross-sectional study. *Nutrition Journal*, *13*, 69. doi:10.1186/1475-2891-13-69
- Leach, M. J., & Kumar, S. (2012). Cinnamon for diabetes mellitus. *Cochrane Database of Systematic Reviews*(9). doi:10.1002/14651858.CD007170.pub2
- Leech, R., McNaughton, S., & Timperio, A. (2014). The clustering of diet, physical activity and sedentary behavior in children and adolescents: a review. *International Journal of Behavioral Nutrition and Physical Activity*, *11*(1), 4.
- Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry*, *125*(2), 288-306. doi:http://dx.doi.org/10.1016/j.foodchem.2010.08.012
- Letenneur, L., Proust-Lima, C., Le Gouge, A., Dartigues, J. F., & Barberger-Gateau, P. (2007). Flavonoid intake and cognitive decline over a 10-year period. *American Journal of Epidemiology*, *165*(12), 1364-1371. doi:10.1093/aje/kwm036
- Lieberman, H. R., Caballero, B., & Finer, N. (1986). The composition of lunch determines afternoon plasma tryptophan ratios in humans. *Journal of Neural Transmission*, *65*(3), 211-217. doi:10.1007/bf01249083
- Liljeberg Elmstahl, H., & Björck, I. (2001). Milk as a supplement to mixed meals may elevate postprandial insulinaemia. *European Journal of Clinical Nutrition*, *55*(11), 994-999. doi:10.1038/sj.ejcn.1601259
- Liljeberg, H. G., Åkerberg, A. K., & Björck, I. M. (1999). Effect of the glycemic index and content of indigestible carbohydrates of cereal-based breakfast meals on glucose tolerance at lunch in healthy subjects. *The American Journal of Clinical Nutrition*, *69*(4), 647-655.
- Littlewood, R. (2002). Comparison of the Cosmed K4 b2 and the Deltatrac II metabolic cart in measuring resting energy expenditure in adults. *Clinical Nutrition*, *21*(6), 491-497. doi:10.1054/clnu.2002.0580
- Lloyd, H. M., Rogers, P. J., Hedderley, D. I., & Walker, A. F. (1996). Acute effects on mood and cognitive performance of breakfasts differing in fat and carbohydrate content. *Appetite*, *27*(2), 151-164. doi:10.1006/appe.1996.0042

- Lo, J. C., Ong, J. L., Leong, R. L., Gooley, J. J., & Chee, M. W. (2015). Cognitive performance, sleepiness, and mood in partially sleep deprived adolescents: The need for sleep study. *Sleep, 39*((3)), 687–698.
- Lopez-Sobaler, A. M., Ortega, R. M., Quintas, M. E., Navia, B., & Requejo, A. M. (2003). Relationship between habitual breakfast and intellectual performance (logical reasoning) in well-nourished schoolchildren of Madrid (Spain). *European Journal of Clinical Nutrition, 57*(S1), S49-S53.
- Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics, 103*(3), e26-e26. doi:10.1542/peds.103.3.e26
- Lusk, G. (1928). The elements of the science of nutrition. In *The Elements of the Science of Nutrition*. London: WB Saunders Co.
- Macready, A. L., Kennedy, O. B., Ellis, J. A., Williams, C. M., Spencer, J. P. E., & Butler, L. T. (2009). Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. *Genes & Nutrition, 4*(4), 227-242. doi:10.1007/s12263-009-0135-4
- Mahoney, C. R., Taylor, H. A., & Kanarek, R. B. (2007). Effect of an afternoon confectionery snack on cognitive processes critical to learning. *Physiology & Behavior, 90*(2-3), 344-352. doi:10.1016/j.physbeh.2006.09.033
- Mahoney, C. R., Taylor, H. A., Kanarek, R. B., & Samuel, P. (2005). Effect of breakfast composition on cognitive processes in elementary school children. *Physiology & Behavior, 85*(5), 635-645. doi:10.1016/j.physbeh.2005.06.023
- Martens, M. K., van Assema, P., & Brug, J. (2005). Why do adolescents eat what they eat? Personal and social environmental predictors of fruit, snack and breakfast consumption among 12-14-year-old Dutch students. *Public Health Nutrition, 8*(8), 1258-1265.
- Mazza, G., Kay, C. D., Cottrell, T., & Holub, B. J. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agricultural and Food Chemistry, 50*(26), 7731-7737.
- McClave, S. A., & Snider, H. L. (1992). Invited review: Use of indirect calorimetry in clinical nutrition. *Nutrition in Clinical Practice, 7*(5), 207-221. doi:10.1177/0115426592007005207

- McCrary, M. A., Howarth, N. C., Roberts, S. B., & Huang, T. T.-K. (2011). Eating frequency and energy regulation in free-living adults consuming self-selected diets. *The Journal of Nutrition*, *141*(1), 148-153. doi:10.3945/jn.109.114991
- McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *Journal of Agricultural and Food Chemistry*, *53*(7), 2760-2766. doi:10.1021/jf0489926
- McDougall, G. J., & Stewart, D. (2005). The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors*, *23*(4), 189-195.
- McEachan, R. R. C., Conner, M., Taylor, N. J., & Lawton, R. J. (2011). Prospective prediction of health-related behaviours with the theory of planned behaviour: A meta-analysis. *Health Psychology Review*, *5*(2), 97-144. doi:10.1080/17437199.2010.521684
- McLean, J. A., & Tobin, G. (1987). *Animal and human calorimetry*. Cambridge: Cambridge University Press.
- McNulty, H., Eaton-Evans, J., Cran, G., Woulahan, G., Boreham, C., Savage, J. M., . . . Strain, J. J. (1996). Nutrient intakes and impact of fortified breakfast cereals in schoolchildren. *Archives of Disease in Childhood*, *75*(6), 474-481. doi:10.1136/adc.75.6.474
- Medagama, A. B. (2015). The glycaemic outcomes of Cinnamon, a review of the experimental evidence and clinical trials. *Nutrition Journal*, *14*(1), 1-12. doi:10.1186/s12937-015-0098-9
- Messier, C. (2004). Glucose improvement of memory: a review. *European Journal of Pharmacology*, *490*(1-3), 33-57. doi:http://dx.doi.org/10.1016/j.ejphar.2004.02.043
- Micha, R., Rogers, P. J., & Nelson, M. (2010). The glycaemic potency of breakfast and cognitive function in school children. *European Journal of Clinical Nutrition*, *64*(9), 948-957. doi:10.1038/ejcn.2010.96
- Micha, R., Rogers, P. J., & Nelson, M. (2011). Glycaemic index and glycaemic load of breakfast predict cognitive function and mood in school children: a randomised controlled trial. *British Journal of Nutrition*, *106*(10), 1552-1561. doi:doi:10.1017/S0007114511002303
- Michaud, C., Musse, N., Nicolas, J. P., & Mejean, L. (1991). Effects of breakfast-size on short-term memory, concentration, mood and blood glucose. *The Journal of Adolescent Health*, *12*(1), 53-57.
- Michels, N., De Henauw, S., Breidenassel, C., Censi, L., Cuenca-García, M., Cuenca-Garcí, M., . . . Claessens, M. (2015). European adolescent ready-to-eat-cereal (RTEC) consumers have a



- healthier dietary intake and body composition compared with non-RTEC consumers. *European Journal of Nutrition*, 54(4), 653-664. doi:10.1007/s00394-014-0805-x
- Michie, S., Abraham, C., Whittington, C., McAteer, J., & Gupta, S. (2009). Effective techniques in healthy eating and physical activity interventions: a meta-regression. *Health Psychology*, 28(6), 690-701. doi:10.1037/a0016136
- Miller, D. P., Waldfogel, J., & Han, W.-J. (2012). Family meals and child academic and behavioral outcomes. *Child Development*, 83(6), 2104-2120. doi:10.1111/j.1467-8624.2012.01825.x
- Miller, M. G., & Shukitt-Hale, B. (2012). Berry fruit enhances beneficial signaling in the brain. *Journal of Agricultural and Food Chemistry*, 60(23), 5709-5715. doi:10.1021/jf2036033
- Mishra, S., Monro, J., & Hedderley, D. (2008). Effect of processing on slowly digestible starch and resistant starch in potato. *Starch - Stärke*, 60(9), 500-507. doi:10.1002/star.200800209
- Mishra, S., & Monro, J. A. (2009). Digestibility of starch fractions in wholegrain rolled oats. *Journal of Cereal Science*, 50(1), 61-66. doi:http://dx.doi.org/10.1016/j.jcs.2009.03.002
- Molnár, D., & Schutz, Y. (1997). The effect of obesity, age, puberty and gender on resting metabolic rate in children and adolescents. *European Journal of Pediatrics*, 156(5), 376-381. doi:10.1007/s004310050618
- Moore, G. F., Moore, L., & Murphy, S. (2009). Normative and cognitive correlates of breakfast skipping in 9–11-year-old schoolchildren in Wales. *Appetite*, 53(3), 332-337. doi:http://dx.doi.org/10.1016/j.appet.2009.07.012
- Moore, G. F., Tapper, K., Murphy, S., Lynch, R., Raisanen, L., Pimm, C., & Moore, L. (2007). Associations between deprivation, attitudes towards eating breakfast and breakfast eating behaviours in 9–11-year-olds. *Public Health Nutrition*, 10(06), 582-589. doi:doi:10.1017/S1368980007699558
- Morgan, K. J., Zabik, M. E., & Stampley, G. L. (1986). The role of breakfast in diet adequacy of the U.S. adult population. *The Journal of the American College of Nutrition*, 5(6), 551-563.
- Most, J., Goossens, G. H., Jocken, J. W., & Blaak, E. E. (2014). Short-term supplementation with a specific combination of dietary polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects. *Int J Obes*, 38(5), 698-706. doi:10.1038/ijo.2013.231
- Mullan, B., Wong, C., & Kothe, E. (2013). Predicting adolescent breakfast consumption in the UK and Australia using an extended theory of planned behaviour. *Appetite*, 62(0), 127-132. doi:http://dx.doi.org/10.1016/j.appet.2012.11.021

- Mullan, B. A., & Singh, M. (2010). A systematic review of the quality, content, and context of breakfast consumption. *Nutrition & Food Science*, 40(1), 81-114.  
doi:10.1108/00346651011015953
- Mullan, B. A., Wong, C. L., Kothe, E. J., & MacCann, C. (2013). Predicting breakfast consumption: A comparison of the theory of planned behaviour and health action process approach. *British Food Journal*, Vol. 115(11), 1638-1657.
- Müller, M. J., & Bosy-Westphal, A. (2003). Assessment of energy expenditure in children and adolescents. *Current Opinion in Clinical Nutrition & Metabolic Care*, 6(5), 519-530.
- Murata, M. (2000). Secular trends in growth and changes in eating patterns of Japanese children. *The American Journal of Clinical Nutrition*, 72(5 Suppl), 1379S-1383S.
- Murphy, S., Moore, G., Tapper, K., Lynch, R., Raisanen, L. and Clark, R. . (2007). An evaluation of the Welsh assembly government's primary school free breakfast initiative. *Cardiff Institute for Society, Health and Ethics, Cardiff*.
- Mursu, J., Virtanen, J. K., Tuomainen, T.-P., Nurmi, T., & Voutilainen, S. (2014). Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: The Kuopio ischaemic heart disease risk factor study. *The American Journal of Clinical Nutrition*, 99(2), 328-333.  
doi:10.3945/ajcn.113.069641
- Nabb, S., & Benton, D. (2006). The influence on cognition of the interaction between the macro-nutrient content of breakfast and glucose tolerance. *Physiology & Behavior*, 87(1), 16-23.  
doi:http://dx.doi.org/10.1016/j.physbeh.2005.08.034
- National Health and Medical Research Council. (2013). *Australian Dietary Guidelines*. Retrieved from <https://www.nhmrc.gov.au/guidelines-publications/n55>
- Nestler, J. E., Barlascini, C. O., Clore, J. N., & Blackard, W. G. (1988). Absorption characteristic of breakfast determines insulin sensitivity and carbohydrate tolerance for lunch. *Diabetes Care*, 11(10), 755-760.
- Neumark-Sztainer, D., Story, M., Hannan, P. J., Perry, C. L., & Irving, L. M. (2002). Weight-related concerns and behaviors among overweight and nonoverweight adolescents: implications for preventing weight-related disorders. *Archives of Pediatrics & Adolescent Medicine*, 156(2), 171-178.
- Nicklas, T. A., O'Neil, C. E., & Berenson, G. S. (1998). Nutrient contribution of breakfast, secular trends, and the role of ready-to-eat cereals: a review of data from the Bogalusa Heart Study. *The American Journal of Clinical Nutrition*, 67(4), 757S-763S.

- Niemeier, H. M., Raynor, H. A., Lloyd-Richardson, E. E., Rogers, M. L., & Wing, R. R. (2006). Fast food consumption and breakfast skipping: predictors of weight gain from adolescence to adulthood in a nationally representative sample. *The Journal of Adolescent Health, 39*(6), 842-849. doi:10.1016/j.jadohealth.2006.07.001
- Nilsson, A., Radeborg, K., & Bjorck, I. (2012). Effects on cognitive performance of modulating the postprandial blood glucose profile at breakfast. *European Journal of Clinical Nutrition, 66*(9), 1039-1043. doi:http://www.nature.com/ejcn/journal/v66/n9/supinfo/ejcn201280s1.html
- Nilsson, A., Tovar, J., Johansson, M., Radeborg, K., & Bjorck, I. (2013). A diet based on multiple functional concepts improves cognitive performance in healthy subjects. *Nutrition & Metabolism, 10*(49).
- Nilsson, A. C., Östman, E. M., Granfeldt, Y., & Björck, I. M. (2008). Effect of cereal test breakfasts differing in glycemic index and content of indigestible carbohydrates on daylong glucose tolerance in healthy subjects. *The American Journal of Clinical Nutrition, 87*(3), 645-654.
- Nooyens, A. C. J., Milder, I. E. J., van Gelder, B. M., Bueno-de-Mesquita, H. B., van Boxtel, M. P. J., & Verschuren, W. M. M. (2015). Diet and cognitive decline at middle age: the role of antioxidants. *British Journal of Nutrition, 113*(09), 1410-1417. doi:doi:10.1017/S0007114515000720
- Nour, A. A., Magboul, B. I., & Kheiri, N. H. (1980). Chemical Composition of Baobab Fruit. *Tropical Science, 22*(4), 383-388.
- Nurul-Fadhilah, A., Teo, P. S., Huybrechts, I., & Foo, L. H. (2013). Infrequent breakfast consumption is associated with higher body adiposity and abdominal obesity in Malaysian school-aged adolescents. *PLoS One, 8*(3), e59297. doi:10.1371/journal.pone.0059297
- Odegaard, A. O., Jacobs, D. R., Steffen, L. M., Van Horn, L., Ludwig, D. S., & Pereira, M. A. (2013). Breakfast frequency and development of metabolic risk. *Diabetes Care, 36*(10), 3100-3106. doi:10.2337/dc13-0316
- Ogden, J. (2008). *Essential Readings In Health Psychology*. Maidenhead, England: Open University Press.
- Othman, R. A., Moghadasian, M. H., & Jones, P. J. (2011). Cholesterol-lowering effects of oat beta-glucan. *Nutrition Reviews, 69*(6), 299-309. doi:10.1111/j.1753-4887.2011.00401.x
- Otten, J. J., Hellwig, J. P., & Meyers, L. D. (2006). *Dietary reference intakes: the essential guide to nutrient requirements*. Washington, DC: National Academies Press.

- Oxford Brookes University. (2011). Random Order Generator. Retrieved from <http://psych.brookes.ac.uk/research/order.asp>
- Pan, H., & Cole, T. J. (2012). LMSgrowth, a Microsoft Excel add-in to access growth references based on the LMS method. Version 2.77. <http://www.healthforallchildren.co.uk/>; (Version 2.77).
- Papandreou, M. A., Dimakopoulou, A., Linardaki, Z. I., Cordopatis, P., Klimis-Zacas, D., Margarity, M., & Lamari, F. N. (2009). Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behavioural Brain Research, 198*(2), 352-358. doi:10.1016/j.bbr.2008.11.013
- Pearson, N., Biddle, S. J. H., & Gorely, T. (2009). Family correlates of breakfast consumption among children and adolescents. A systematic review. *Appetite, 52*(1), 1-7. doi:<http://dx.doi.org/10.1016/j.appet.2008.08.006>
- Pereira, M. A., Erickson, E., McKee, P., Schrankler, K., Raatz, S. K., Lytle, L. A., & Pellegrini, A. D. (2011). Breakfast frequency and quality may affect glycemia and appetite in adults and children. *The Journal of Nutrition, 141*(1), 163-168. doi:10.3945/jn.109.114405
- Pfeifer, J. H., & Blakemore, S.-J. (2012). Adolescent social cognitive and affective neuroscience: past, present, and future. *Social Cognitive and Affective Neuroscience, 7*(1), 1-10. doi:10.1093/scan/nsr099
- Philippou, E., & Constantinou, M. (2014). The influence of glycemic index on cognitive functioning: A systematic review of the evidence. *Advances in Nutrition, 5*(2), 119-130. doi:10.3945/an.113.004960
- Pollitt, E., Cueto, S., & Jacoby, E. R. (1998). Fasting and cognition in well- and undernourished schoolchildren: a review of three experimental studies. *The American Journal of Clinical Nutrition, 67*(4), 779S-784S.
- Pollitt, E., & Mathews, R. (1998). Breakfast and cognition: an integrative summary. *The American Journal of Clinical Nutrition, 67*(4), 804S-813S.
- Powers, H. J., Stephens, M., Russell, J., & Hill, M. H. (2016). Fortified breakfast cereal consumed daily for 12 wk leads to a significant improvement in micronutrient intake and micronutrient status in adolescent girls: a randomised controlled trial. *Nutrition Journal, 15*(1), 69. doi:10.1186/s12937-016-0185-6

- Pribis, P., & Shukitt-Hale, B. (2014). Cognition: the new frontier for nuts and berries. *The American Journal of Clinical Nutrition*, 100(Supplement 1), 347S-352S.  
doi:10.3945/ajcn.113.071506
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290-4302. doi:10.1021/jf0502698
- Public Health England (PHE). (2013, August 2013). How healthy behaviour supports children's wellbeing. Retrieved from  
[https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/232978/Smart\\_Restart\\_280813\\_web.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/232978/Smart_Restart_280813_web.pdf)
- Public Health England (PHE). (2016). *The Eatwell Guide: Helping you eat a healthy, balanced diet*. Retrieved from <https://www.gov.uk/government/publications/the-eatwell-guide>.
- Rampersaud, G. C. (2009). Benefits of breakfast for children and adolescents: Update and recommendations for practitioners. *American Journal of Lifestyle Medicine*, 3(2), 86-103.  
doi:10.1177/1559827608327219
- Rampersaud, G. C., Pereira, M. A., Girard, B. L., Adams, J., & Metz, J. D. (2005). Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *Journal of the American Dietetic Association*, 105(5), 743-760; quiz 761-742.  
doi:10.1016/j.jada.2005.02.007
- Rebello, C. J., Johnson, W. D., Martin, C. K., Xie, W., O'Shea, M., Kurilich, A., . . . Greenway, F. L. (2013). Acute effect of oatmeal on subjective measures of appetite and satiety compared to a ready-to-eat breakfast cereal: A randomized crossover trial. *Journal of the American College of Nutrition*, 32(4), 272-279. doi:10.1080/07315724.2013.816614
- Regand, A., Chowdhury, Z., Tosh, S. M., Wolever, T. M. S., & Wood, P. (2011). The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chemistry*, 129(2), 297-304.  
doi:10.1016/j.foodchem.2011.04.053
- Regand, A., Tosh, S. M., Wolever, T. M., & Wood, P. J. (2009). Physicochemical properties of beta-glucan in differently processed oat foods influence glycemic response. *Journal of Agricultural and Food Chemistry*, 57(19), 8831-8838. doi:10.1021/jf901271v

- Rendeiro, C., Rhodes, J. S., & Spencer, J. P. E. (2015). The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. *Neurochemistry International*, 89, 126-139. doi:http://dx.doi.org/10.1016/j.neuint.2015.08.002
- Rendeiro, C., Vauzour, D., Rattray, M., Waffo-Téguo, P., Mérillon, J. M., Butler, L. T., . . . Spencer, J. P. E. (2013). Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. *PLoS One*, 8(5), e63535. doi:10.1371/journal.pone.0063535
- Riby, L. M. (2004). The impact of age and task domain on cognitive performance: A meta-analytic review of the glucose facilitation effect. *Brain Impairment*, 5(02), 145-165. doi:doi:10.1375/brim.5.2.145.58253
- Riddoch, C. J., Mattocks, C., Deere, K., Saunders, J., Kirkby, J., Tilling, K., . . . Ness, A. R. (2007). Objective measurement of levels and patterns of physical activity. *Archives of Disease in Childhood*, 92(11), 963-969. doi:10.1136/adc.2006.112136
- Riebl, S. K., Estabrooks, P. A., Dunsmore, J. C., Savla, J., Frisard, M. I., Dietrich, A. M., . . . Davy, B. M. (2015). A systematic literature review and meta-analysis: The theory of planned behavior's application to understand and predict nutrition-related behaviors in youth. *Eating Behaviors*, 18, 160-178. doi:10.1016/j.eatbeh.2015.05.016
- Rios, L. Y., Bennett, R. N., Lazarus, S. A., Rémésy, C., Scalbert, A., & Williamson, G. (2002). Cocoa procyanidins are stable during gastric transit in humans. *The American Journal of Clinical Nutrition*, 76(5), 1106-1110.
- Riso, P., Klimis-Zacas, D., Del Bo, C., Martini, D., Campolo, J., Vendrame, S., . . . Porrini, M. (2013). Effect of a wild blueberry (*Vaccinium angustifolium*) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. *European Journal of Nutrition*, 52(3), 949-961. doi:10.1007/s00394-012-0402-9
- Rivis, A., Sheeran, P., & Armitage, C. J. (2006). Augmenting the theory of planned behaviour with the prototype/willingness model: Predictive validity of actor versus abstainer prototypes for adolescents' health-protective and health-risk intentions. *British Journal of Health Psychology*, 11(3), 483-500. doi:10.1348/135910705X70327
- Rodrigues, A. S., Pérez-Gregorio, M. R., García-Falcón, M. S., & Simal-Gándara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs.

*Food Research International*, 42(9), 1331-1336.

doi:<http://dx.doi.org/10.1016/j.foodres.2009.04.005>

- Rodriguez-Mateos, A., Rendeiro, C., Bergillos-Meca, T., Tabatabaee, S., George, T. W., Heiss, C., & Spencer, J. P. (2013). Intake and time dependence of blueberry flavonoid-induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. *The American Journal of Clinical Nutrition*, 98, 1179–1191. doi:10.3945/ajcn.113.066639
- Rodriguez, G., Moreno, L. A., Sarría, A., Fleta, J., & Bueno, M. (2002). Resting energy expenditure in children and adolescents: agreement between calorimetry and prediction equations. *Clinical Nutrition*, 21(3), 255-260. doi:<http://dx.doi.org/10.1054/clnu.2001.0531>
- Roffey, D. M., Byrne, N. M., & Hills, A. P. (2006). Day-to-day variance in measurement of resting metabolic rate using ventilated-hood and mouthpiece & nose-clip indirect calorimetry systems. *Journal of Parenteral and Enteral Nutrition*, 30(5), 426-432. doi:10.1177/0148607106030005426
- Rogers, P. J., & Lloyd, H. M. (1994). Nutrition and mental performance. *Proceedings of the Nutrition Society*, 53(02), 443-456. doi:10.1079/PNS19940049
- Rothwell, N. J., & Stock, M. J. (1983). Diet-Induced Thermogenesis. In Lucien Girardier & Michael J. Stock (Eds.), *Mammalian Thermogenesis* (pp. 208-233). Dordrecht: Springer Netherlands.
- Ryan, L., & Carolan, S. (2011). Determination of the total antioxidant capacity and total polyphenol content of commercially available green tea. *Proceedings of the Nutrition Society*, 70(OCE4). doi:10.1017/S002966511100190X
- Ryan, L., Thondre, P. S., & Henry, C. J. K. (2011). Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential. *Journal of Food Composition and Analysis*, 24(7), 929-934. doi:<http://dx.doi.org/10.1016/j.jfca.2011.02.002>
- Samuelson, G. (2000). Dietary habits and nutritional status in adolescents over Europe. An overview of current studies in the Nordic countries. *European Journal of Clinical Nutrition*, 54 Suppl 1, S21-28.
- Sandercock, G. R., Voss, C., & Dye, L. (2010). Associations between habitual school-day breakfast consumption, body mass index, physical activity and cardiorespiratory fitness in English schoolchildren. *European Journal of Clinical Nutrition*, 64(10), 1086-1092. doi:10.1038/ejcn.2010.145

- Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition*, 81(1), 215S-217S.
- Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45(4), 287-306. doi:10.1080/1040869059096
- Scalbert, A., & Williamson, G. (2000). Dietary Intake and Bioavailability of Polyphenols. *The Journal of Nutrition, Supplement*(0022-3166/00), 2073-2085S.
- Schmitt, J. A. J., Benton, D., & Kallus, K. W. (2005). General methodological considerations for the assessment of nutritional influences on human cognitive functions. *European Journal of Nutrition*, 44(8), 459-464. doi:10.1007/s00394-005-0585-4
- Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition Clinical Nutrition*, 39 Suppl 1, 5-41.
- Sharma, P., & Gujral, H. S. (2010). Antioxidant and polyphenol oxidase activity of germinated barley and its milling fractions. *Food Chemistry*, 120(3), 673-678. doi:http://dx.doi.org/10.1016/j.foodchem.2009.10.059
- Shemilt, I., Harvey, I., Shephstone, L., Swift, L., Reading, R., Mugford, M., . . . Robinson, J. (2004). A national evaluation of school breakfast clubs: evidence from a cluster randomized controlled trial and an observational analysis. *Child: Care, Health and Development*, 30(5), 413-427. doi:10.1111/j.1365-2214.2004.00453.x
- Shetty, P. S., Henry, C. J., Black, A. E., & Prentice, A. M. (1996). Energy requirements of adults: an update on basal metabolic rates (BMRs) and physical activity levels (PALs). *European Journal of Clinical Nutrition*, 50 Suppl 1, S11-23.
- Shukitt-Hale, B., Bielinski, D. F., Lau, F. C., Willis, L. M., Carey, A. N., & Joseph, J. A. (2015). The beneficial effects of berries on cognition, motor behaviour and neuronal function in ageing. *The British Journal of Nutrition*, 114(10), 1542-1549. doi:10.1017/s0007114515003451
- Siahanidou, T., Margeli, A., Kappis, A., Papassotiriou, I., & Mandyla, H. (2011). Circulating visfatin levels in healthy preterm infants are independently associated with high-density lipoprotein cholesterol levels and dietary long-chain polyunsaturated fatty acids. *Metabolism*, 60(3), 389-393. doi:10.1016/j.metabol.2010.03.002



- Siega-Riz, A. M., Popkin, B. M., & Carson, T. (1998). Trends in breakfast consumption for children in the United States from 1965-1991. *The American Journal of Clinical Nutrition*, 67(4), 748s-756s.
- Sinha, R., Fisch, G., Teague, B., Tamborlane, W. V., Banyas, B., Allen, K., . . . Caprio, S. (2002). Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *New England Journal of Medicine*, 346(11), 802-810. doi:10.1056/NEJMoa012578
- Slavin, J., & Green, H. (2007). Dietary fibre and satiety. *Nutrition Bulletin*, 32, 32-42. doi:10.1111/j.1467-3010.2007.00603.x
- Slavin, J. L. (2008). Position of the American Dietetic Association: health implications of dietary fiber. *Journal of the American Dietetic Association*, 108(10), 1716-1731.
- Smith, A., & Kendrick, A. (1992). Meals and performance. In *Handbook of Human Performance* (1st ed., Vol. 2). London: Academic Press.
- Smith, K. J., Gall, S. L., McNaughton, S. A., Blizzard, L., Dwyer, T., & Venn, A. J. (2010). Skipping breakfast: Longitudinal associations with cardiometabolic risk factors in the childhood determinants of adult health study. *The American Journal of Clinical Nutrition*, 92(6), 1316-1325. doi:10.3945/ajcn.2010.30101
- Smith, M. A., & Foster, J. K. (2008). The impact of a high versus a low glycaemic index breakfast cereal meal on verbal episodic memory in healthy adolescents. *Nutritional Neuroscience*, 11(5), 219-227. doi:10.1179/147683008x344110
- Sniehotta, F. F., Pesseau, J., & Araújo-Soares, V. (2013). Time to retire the theory of planned behaviour. *Health Psychology Review*, 8(1), 1-7. doi:10.1080/17437199.2013.869710
- Sniehotta, F. F., Scholz, U., & Schwarzer, R. (2005). Bridging the intention-behaviour gap: Planning, self-efficacy, and action control in the adoption and maintenance of physical exercise. *Psychology and Health*, 20(2), 143-160.
- Spears, K. E., Kim, H., Behall, K. M., & Conway, J. M. (2009). Hand-held indirect calorimeter offers advantages compared with prediction equations, in a group of overweight women, to determine resting energy expenditures and estimated total energy expenditures during research screening. *Journal of the American Dietetic Association*, 109(5), 836-845. doi:http://dx.doi.org/10.1016/j.jada.2009.02.016
- Spencer, J. P. (2010a). Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. *The Proceedings of the Nutrition Society*, 69(2), 244-260. doi:10.1017/s0029665110000054

- Spencer, J. P. (2010b). The impact of fruit flavonoids on memory and cognition. *The British Journal of Nutrition*, *104 Suppl 3*, S40-47. doi:10.1017/s0007114510003934
- Spencer, J. P., Abd El Mohsen, M. M., Minihane, A. M., & Mathers, J. C. (2008). Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *The British Journal of Nutrition*, *99*(1), 12-22. doi:10.1017/s0007114507798938
- Spencer, J. P. E. (2008). Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society*, *67*(02), 238-252. doi:doi:10.1017/S0029665108007088
- Spieth, L. E., Harnish, J. D., Lenders, C. M., Raezer, L. B., Pereira, M. A., Hangen, S. J., & Ludwig, D. S. (2000). A low-glycemic index diet in the treatment of pediatric obesity. *Archives of Pediatrics & Adolescent Medicine*, *154*(9), 947-951.
- St-Onge, M. P., Rubiano, F., Jones, A., & Heymsfield, S. B. (2004). A new hand-held indirect calorimeter to measure postprandial energy expenditure. *Obesity Research*, *12*(4), 704-709. doi:10.1038/oby.2004.82
- Stewart, C. L., Goody, C. M., & Branson, R. (2005). Comparison of two systems of measuring energy expenditure. *Journal of Parenteral and Enteral Nutrition*, *29*(3), 212-217. doi:10.1177/0148607105029003212
- Stewart, L. K., Soileau, J. L., Ribnicky, D., Wang, Z. Q., Raskin, I., Poulev, A., . . . Gettys, T. W. (2008). Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. *Metabolism: clinical and experimental*, *57*(7 Suppl 1), S39-S46. doi:10.1016/j.metabol.2008.03.003
- Sun, Q., Wedick, N. M., Tworoger, S. S., Pan, A., Townsend, M. K., Cassidy, A., . . . van Dam, R. M. (2015). Urinary excretion of select dietary polyphenol metabolites is associated with a lower risk of type 2 diabetes in proximate but not remote follow-up in a prospective investigation in 2 cohorts of US women. *The Journal of Nutrition*, *145*(6), 1280-1288. doi:10.3945/jn.114.208736
- Sundstrom, M., Tjader, I., Rooyackers, O., & Wernerman, J. (2013). Indirect calorimetry in mechanically ventilated patients. A systematic comparison of three instruments. *Clinical Nutrition*, *32*(1), 118-121. doi:10.1016/j.clnu.2012.06.004
- Szajewska, H., & Ruszczyński, M. (2010). Systematic review demonstrating that breakfast consumption influences body weight outcomes in children and adolescents in Europe.

*Critical Reviews in Food Science and Nutrition*, 50(2), 113-119.

doi:10.1080/10408390903467514

- Tagliamonte, A., DeMontis, M. G., Olianias, M., Onali, P. L., & Gessa, G. L. (1975). Possible role of insulin in the transport of tyrosine and tryptophan from blood to brain. *Pharmacological Research Communications*, 7(6), 493-499. doi:http://dx.doi.org/10.1016/0031-6989(75)90010-7
- Tanko, Y., Yerima, M., Mahdi, M. A., Yaro, A. H., Musa, K. Y., & Mohammed, A. (2008). Hypoglycemic activity of methanolic stem bark of *adansonia digitata* extract on blood glucose levels of streptozocin-induced diabetic wistar rats. *International Journal of Applied Research in Natural Products*, 1(2), 5.
- Tapper, K., Murphy, S., Lynch, R., Clark, R., Moore, G. F., & Moore, L. (2008). Development of a scale to measure 9-11-year-olds' attitudes towards breakfast. *European Journal of Clinical Nutrition*, 62(4), 511-518. doi:10.1038/sj.ejcn.1602735
- Tappy, L., Gugolz, E., & Wursch, P. (1996). Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. *Diabetes Care*, 19(8), 831-834. doi:10.2337/diacare.19.8.831
- Teff, K. L., Young, S. N., & Blundell, J. E. (1989). The effect of protein or carbohydrate breakfasts on subsequent plasma amino acid levels, satiety and nutrient selection in normal males. *Pharmacology Biochemistry and Behavior*, 34(4), 829-837. doi:http://dx.doi.org/10.1016/0091-3057(89)90282-7
- Thayer, R. E. (1989). *The biopsychology of mood and arousal*. New York Oxford: Oxford University Press.
- The Food and Agriculture Organization (FAO). (1998). *Carbohydrates in human nutrition, a Joint FAO/WHO Report*. Retrieved from Rome: <http://www.fao.org/docrep/w8079E/w8079e00.htm>
- The Food and Agriculture Organization (FAO). (2001). *Human energy requirements*. Retrieved from Rome: <http://www.fao.org/docrep/007/y5686e/y5686e00.htm>
- Thondre, P. S., & Henry, C. J. K. (2009). High-molecular-weight barley  $\beta$ -glucan in chapatis (unleavened Indian flatbread) lowers glycemic index. *Nutrition Research*, 29(7), 480-486. doi:http://dx.doi.org/10.1016/j.nutres.2009.07.003

- Thondre, P. S., Ryan, L., & Henry, C. J. K. (2011). Influence of polyphenols on in vitro starch digestibility of oat porridges. *Proceedings of the Nutrition Society*, 70(OCE4). doi:doi:10.1017/S0029665111001893
- Thondre, P. S., Shafat, A., & Clegg, M. E. (2013). Molecular weight of barley beta-glucan influences energy expenditure, gastric emptying and glycaemic response in human subjects. *The British Journal of Nutrition*, 110(12), 2173-2179. doi:10.1017/s0007114513001682
- Timlin, M. T., & Pereira, M. A. (2007). Breakfast frequency and quality in the etiology of adult obesity and chronic diseases. *Nutrition Reviews*, 65(6 Pt 1), 268-281.
- Timlin, M. T., Pereira, M. A., Story, M., & Neumark-Sztainer, D. (2008). Breakfast eating and weight change in a 5-year prospective analysis of adolescents: Project EAT(eating among teens). *Pediatrics*, 121(3), e638-e645. doi:10.1542/peds.2007-1035
- Tolfrey, K., & Zakrzewski, J. K. (2012). Breakfast, glycaemic index and health in young people. *Journal of Sport and Health Science*, 1(3), 149-159. doi:http://dx.doi.org/10.1016/j.jshs.2012.09.001
- Törrönen, R., Kolehmainen, M., Sarkkinen, E., Mykkänen, H., & Niskanen, L. (2012b). Postprandial glucose, insulin, and free fatty acid responses to sucrose consumed with blackcurrants and lingonberries in healthy women. *The American Journal of Clinical Nutrition*, 96(3), 527-533. doi:10.3945/ajcn.112.042184
- Törrönen, R., Kolehmainen, M., Sarkkinen, E., Poutanen, K., Mykkänen, H., & Niskanen, L. (2013). Berries reduce postprandial insulin responses to wheat and rye breads in healthy women. *The Journal of Nutrition*, 143(4), 430-436. doi:10.3945/jn.112.169771
- Törrönen, R., Sarkkinen, E., Niskanen, T., Tapola, N., Kilpi, K., & Niskanen, L. (2012a). Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects. *British Journal of Nutrition*, 107(10), 1445-1451. doi:10.1017/s0007114511004557
- Törrönen, R., Sarkkinen, E., Tapola, N., Hautaniemi, E., Kilpi, K., & Niskanen, L. (2010). Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. *The British Journal of Nutrition*, 103(8), 1094-1097. doi:10.1017/s0007114509992868
- Tosh, S. M. (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition*, 67(4), 310-317. doi:http://www.nature.com/ejcn/journal/v67/n4/supinfo/ejcn201325s1.html

- Tovar, J., Johansson, M., & Björck, I. (2015). A multifunctional diet improves cardiometabolic-related biomarkers independently of weight changes: an 8-week randomized controlled intervention in healthy overweight and obese subjects. *European Journal of Nutrition*, 1-12. doi:10.1007/s00394-015-1039-2
- Tovar, J., Nilsson, A., Johansson, M., & Björck, I. (2014). Combining functional features of whole-grain barley and legumes for dietary reduction of cardiometabolic risk: a randomised cross-over intervention in mature women. *British Journal of Nutrition*, 111(04), 706-714. doi:doi:10.1017/S000711451300305X
- Tovar, J., Nilsson, A., Johansson, M., Ekesbo, R., Aberg, A. M., Johansson, U., & Bjorck, I. (2012). A diet based on multiple functional concepts improves cardiometabolic risk parameters in healthy subjects. *Nutr Metab (Lond)*, 9, 29. doi:10.1186/1743-7075-9-29
- Tuttle, W. W., Wilson, M., & Daum, K. (1949). Effect of Altered Breakfast Habits on Physiologic Response. *Journal of Applied Physiology*, 1(8), 545-559.
- United States Department of Agriculture (USDA). (2010). *Dietary Guidelines for Americans*. Retrieved from <http://health.gov/dietaryguidelines/2015/guidelines/search/?query=breakfast>.
- Unusan, N., Sanlier, N., & Danisik, H. (2006). Comparison of attitudes towards breakfast by Turkish fourth graders living in Turkey and Germany. *Appetite*, 46(3), 248-253. doi:http://dx.doi.org/10.1016/j.appet.2006.01.009
- Utter, J., Scragg, R., Mhurchu, C. N., & Schaaf, D. (2007). At-home breakfast consumption among New Zealand children: associations with body mass index and related nutrition behaviors. *Journal of the American Dietetic Association*, 107(4), 570-576. doi:10.1016/j.jada.2007.01.010
- Uttl, B. (2005). Measurement of individual differences: Lessons from memory assessment in research and clinical practice. *Psychological Science*, 16(6), 460-467. doi:10.1111/j.0956-7976.2005.01557.x
- Vaisman, N., Voet, H., Akivis, A., & Vakil, E. (1996). Effect of breakfast timing on the cognitive functions of elementary school students. *Archives of Pediatrics & Adolescent Medicine*, 150(10), 1089-1092.
- van der Horst, K., Oenema, A., Ferreira, I., Wendel-Vos, W., Giskes, K., van Lenthe, F., & Brug, J. (2007). A systematic review of environmental correlates of obesity-related dietary behaviors in youth. *Health Education Research*, 22(2), 203-226. doi:10.1093/her/cyl069

- van Mil, E. G., Westerterp, K. R., Kester, A. D., & Saris, W. H. (2001). Energy metabolism in relation to body composition and gender in adolescents. *Archives of Disease in Childhood*, *85*, 73–78.
- Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M. J., & Spencer, J. P. E. (2010). Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients*, *2*(11), 1106-1131.
- Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., & Spencer, J. P. E. (2008). The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes & Nutrition*, *3*(3-4), 115-126. doi:10.1007/s12263-008-0091-4
- Ventham, J. C., & Reilly, J. J. (1999). Reproducibility of resting metabolic rate measurement in children. *The British Journal of Nutrition*, *81*(6), 435-437.
- Vereecken, C., Dupuy, M., Rasmussen, M., Kelly, C., Nansel, T. R., Al Sabbah, H., . . . Ahluwalia, N. (2009). Breakfast consumption and its socio-demographic and lifestyle correlates in schoolchildren in 41 countries participating in the HBSC study. *International Journal of Public Health*, *54 Suppl 2*, 180-190. doi:10.1007/s00038-009-5409-5
- Viner, R. M., & Macfarlane, A. (2005). Health Promotion. In Russell M. Viner (Ed.), *ABC of Adolescence* (pp. 19-21). Oxford, UK: Blackwell Publishing Ltd.
- Viner, R. M., Ozer, E. M., Denny, S., Marmot, M., Resnick, M., Fatusi, A., & Currie, C. (2012). Adolescence and the social determinants of health. *The Lancet*, *379*(9826), 1641-1652.
- Wahlqvist, M. L., Lee, M. S., Lee, J. T., Hsu, C. C., Chou, Y. C., Fang, W. H., . . . Andrews, Z. B. (2016). Cinnamon users with prediabetes have a better fasting working memory: a cross-sectional function study. *Nutrition Research*, *36*(4), 305-310. doi:10.1016/j.nutres.2015.12.005
- Wahrlich, V., Anjos, L. A., Going, S. B., & Lohman, T. G. (2006). Validation of the VO2000 calorimeter for measuring resting metabolic rate. *Clinical Nutrition*, *25*(4), 687-692. doi:10.1016/j.clnu.2006.01.002
- Warren, J. M., Henry, C. J., & Simonite, V. (2003). Low glycemic index breakfasts and reduced food intake in preadolescent children. *Pediatrics*, *112*(5), e414.
- Watson, A. W., Haskell-Ramsay, C. F., Kennedy, D. O., Cooney, J. M., Trower, T., & Scheepens, A. (2015). Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults. *Journal of Functional Foods*, *17*, 524-539. doi:http://dx.doi.org/10.1016/j.jff.2015.06.005

- Weickert, M. O., Möhlig, M., Schöfl, C., Arafat, A. M., Otto, B., Viehoff, H., . . . Pfeiffer, A. F. H. (2006). Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. *Diabetes Care*, *29*(4), 775-780. doi:10.2337/diacare.29.04.06.dc05-2374
- Weinsier, R. L., Schutz, Y., & Bracco, D. (1992). Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *The American Journal of Clinical Nutrition*, *55*(4), 790-794.
- Weissman, C., Sardar, A., & Kemper, M. (1990). In vitro evaluation of a compact metabolic measurement instrument. *Journal of Parenteral and Enteral Nutrition*, *14*(2), 216-221.
- Wells, J., & Fuller, N. (1998). Precision and accuracy in a metabolic monitor for indirect calorimetry. *European Journal of Clinical Nutrition*, *52*, 536-540.
- Wennberg, M., Gustafsson, P. E., Wennberg, P., & Hammarstrom, A. (2014). Poor breakfast habits in adolescence predict the metabolic syndrome in adulthood. *Public Health Nutrition*, *18*(1), 122-129. doi:10.1017/S1368980013003509
- Wesnes, K. A. (2010). Evaluation of techniques to identify beneficial effects of nutrition and natural products on cognitive function. *Nutrition Reviews*, *68 Suppl 1*, S22-28. doi:10.1111/j.1753-4887.2010.00328.x
- Wesnes, K. A., Pincock, C., Richardson, D., Helm, G., & Hails, S. (2003). Breakfast reduces declines in attention and memory over the morning in schoolchildren. *Appetite*, *41*(3), 329-331.
- Wesnes, K. A., Pincock, C., & Scholey, A. (2012). Breakfast is associated with enhanced cognitive function in schoolchildren. An internet based study. *Appetite*, *59*(3), 646-649. doi:10.1016/j.appet.2012.08.008
- Westerterp, K. (2004). Diet induced thermogenesis. *Nutrition & Metabolism*, *1*(1), 5.
- Whitton, C., Nicholson, S. K., Roberts, C., Prynne, C. J., Pot, G., Olson, A., . . . Stephen, A. M. (2011). National Diet and Nutrition Survey: UK food consumption and nutrient intakes from the first year of the rolling programme and comparisons with previous surveys. *The British Journal of Nutrition*, *106*(12), 1899-1914. doi:10.1017/S0007114511002340
- Whyte, A. R., Schafer, G., & Williams, C. M. (2015). Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. *European Journal of Nutrition*, *55*(6), 2151-2162. doi:10.1007/s00394-015-1029-4

- Whyte, A. R., & Williams, C. M. (2015). Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10y old children. *Nutrition, 31*(3), 531-534.  
doi:10.1016/j.nut.2014.09.013
- Widenhorn-Müller, K., Hille, K., Klenk, J., & Weiland, U. (2008). Influence of having breakfast on cognitive performance and mood in 13- to 20-year-old high school students: Results of a crossover trial. *Pediatrics, 122*(2), 279-284. doi:10.1542/peds.2007-0944
- Williams, A. F. (2013). Paediatric Nutrition. In Marinos Elia, Olle Ljungqvist, Rebecca Stratton, Susan A. Lanham-New, & Julie Dowsett (Eds.), *Clinical Nutrition* (2 ed., pp. 430-434). Somerset: Wiley. Retrieved from <https://ebookcentral-proquest-com.oxfordbrookes.idm.oclc.org/lib/brookes/reader.action?docID=1152684#>.
- Williams, C. M., El Mohsen, M. A., Vauzour, D., Rendeiro, C., Butler, L. T., Ellis, J. A., . . . Spencer, J. P. (2008). Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radical Biology & Medicine, 45*(3), 295-305.  
doi:10.1016/j.freeradbiomed.2008.04.008
- Williamson, G. (2013). Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular Nutrition & Food Research, 57*(1), 48-57. doi:10.1002/mnfr.201200511
- Wilmore, J. H., Costill, D. L., & Kennedy, L. W. (2007). *Physiology of Sport and Exercise* (4th ed.).
- Wolever, T. M. (2003). Carbohydrate and the regulation of blood glucose and metabolism. *Nutrition Reviews, 61*(5 Pt 2), S40-48.
- Wolever, T. M. S., & Bolognesi, C. (1996). Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *The Journal of Nutrition, 126*(11), 2798-2806.
- Wong, C. L., & Mullan, B. A. (2009). Predicting breakfast consumption: an application of the theory of planned behaviour and the investigation of past behaviour and executive function. *British Journal of Health Psychology, 14*(Pt 3), 489-504. doi:10.1348/135910708x360719
- Wood, P. J. (1986). *Oat  $\beta$ -Glucan: Structure, location and properties*. St Paul, MN: AACC International.
- Wood, P. J. (1990). Physicochemical properties and physiological effects of the (1----3)(1----4)-beta-D-glucan from oats. *Advances in Experimental Medicine and Biology, 270*, 119-127.



- World Health Organisation (WHO). (1999). *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications*. Retrieved from [http://www.staff.ncl.ac.uk/philip.home/who\\_dmg.pdf](http://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf)
- World Health Organisation (WHO). (2011). Adolescent development. Retrieved from [http://www.who.int/maternal\\_child\\_adolescent/topics/adolescence/dev/en/](http://www.who.int/maternal_child_adolescent/topics/adolescence/dev/en/)
- World Health Organisation (WHO). (2016). Obesity and overweight: Fact sheet 311. Fact sheet N°311. Retrieved from <http://www.who.int/mediacentre/factsheets/fs311/en/>
- Wu, Y., Chen, Z., Li, X., & Li, M. (2009). Effect of tea polyphenols on the retrogradation of rice starch. *Food Research International*, 42(2), 221-225. doi:<http://dx.doi.org/10.1016/j.foodres.2008.11.001>
- Xu, R. (2012). Oat fibre: overview on their main biological properties. *European Food Research & Technology*, 234(4), 563-569. doi:10.1007/s00217-012-1666-2
- Yau, P. L., Javier, D. C., Ryan, C. M., Tsui, W. H., Ardekani, B. A., Ten, S., & Convit, A. (2010). Preliminary evidence for brain complications in obese adolescents with type 2 diabetes mellitus. *Diabetologia*, 53(11), 2298-2306. doi:10.1007/s00125-010-1857-y
- Zakrzewski, J. K., Gillison, F. B., Cumming, S., Church, T. S., Katzmarzyk, P. T., Broyles, S. T., . . . Group, I. R. (2015). Associations between breakfast frequency and adiposity indicators in children from 12 countries. *International Journal of Obesity Supplements*, 5(Suppl 2), S80-88. doi:10.1038/ijosup.2015.24
- Zakrzewski, J. K., Stevenson, E. J., & Tolfrey, K. (2012). Effect of breakfast glycemic index on metabolic responses during rest and exercise in overweight and non-overweight adolescent girls. *European Journal of Clinical Nutrition*, 66(4), 436-442. doi:10.1038/ejcn.2011.175
- Zamora-Ros, R., Knaze, V., Lujan-Barroso, L., Romieu, I., Scalbert, A., Slimani, N., . . . Gonzalez, C. A. (2013). Differences in dietary intakes, food sources and determinants of total flavonoids between Mediterranean and non-Mediterranean countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The British Journal of Nutrition*, 109(8), 1498-1507. doi:10.1017/s0007114512003273
- Zhao, W. Q., & Alkon, D. L. (2001). Role of insulin and insulin receptor in learning and memory. *Molecular and Cellular Endocrinology*, 177(1-2), 125-134.

## Appendix 1: Publication arising from chapter two

S. Kennedy, L. Ryan, A. Fraser and M. E. Clegg, 'Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis', *Journal of Nutritional Science* (2014), vol. 3, e52.

<https://doi.org/10.1017/jns.2014.58>

Accessible via the following links:

<https://radar.brookes.ac.uk/radar/items/b0e03ab8-f04e-44fe-a2fa-946f08311f94/1/>

<https://www.cambridge.org/core/journals/journal-of-nutritional-science/article/comparison-of-the-gem-and-the-ecal-indirect-calorimeters-against-the-deltatrac-for-measures-of-rmr-and-diet-induced-thermogenesis/E3B2181332CE913008B01E8F51BCDEE0>

## **Participant information sheet**

### **Project title: Lifestyle habits of adolescents**

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take your time to read the following information carefully and discuss any concerns with your parent(s) or guardian(s).

#### **The purpose of the study**

There is a large amount of scientific research that looks at the associations between what we eat and physical activity and the risk of developing diseases such as heart disease, type 2 diabetes and certain types of cancer. Recommendations are in place by the Government around amounts of physical activity as well as dietary components to promote health.

This study aims to investigate the eating habits and physical activity of young people in Oxfordshire. To gather this information we would like you to complete a questionnaire which will ask some questions about you, your family, about the types of foods you eat, what you think about some of these foods, your physical activity patterns and how these factors affect your lifestyle.

#### **Why have I been invited to participate?**

All schools in the Oxfordshire area have been invited to take part to ensure a wide variety of participants. Everyone who takes part will be in year 9, 10 or 11 and attending a school in Oxfordshire.

#### **Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. Your decision on whether to take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies. All questionnaires will remain completely anonymous.

#### **What will happen to me if I take part?**

Once you have read this information sheet and had time to discuss it you can decide whether or not to take part. If you wish to take part you will be given the questionnaire to fill out. The questionnaire contains 70-75 questions which should take you around 20-30 minutes to complete. If you need extra time due to reading or writing difficulties this will be arranged with your teacher. Your school may be able to offer access to a computer for completion of an online version of the questionnaire.

#### **What are the possible benefits of taking part?**

By letting us use the information you provide in the questionnaire, we can gain essential insights into the lifestyle habits of young people and see what issues are important to you and how other young people like you can improve their health and wellbeing.

#### **What are the possible disadvantages of taking part?**

The questionnaire will ask some general questions about your eating habits and behaviours. If you have any concerns about any aspect of eating and would like to talk to someone the school counsellor can offer you help and advice on this.

#### **Will what I say in this study be kept confidential?**

Any information you provide will be anonymous and totally confidential (subject to legal limitations). Only the researcher working on this study will have access to the data. Computer files or paper questionnaires will be password protected or kept in locked cabinets. The data generated in the course of the research must be kept securely in paper or electronic form for a

period of ten years after the completion of a research project retained in accordance with the University's policy on Academic Integrity.

Due to participation taking place during school hours, it may be difficult to maintain anonymity of who is taking part but your responses will be returned anonymously to ensure confidentiality.

#### **What should I do if I want to take part?**

If you would like to take part in this research study your teacher will provide you with a questionnaire or access to an online version. Please complete this in full. Completion of the questionnaire will be taken as indicating your consent to participate.

#### **What will happen to the results of the research?**

The information you provide will be used to write a research paper on the lifestyle habits of young people. Your identity will not be recognisable from this. The results generated from the study will also be included in the write up of a PhD thesis and may form the basis of further research in the area.

A summary of the results of the study will be presented to your school.

#### **Who is organising and funding the research?**

This research is being conducted as part of a PhD research project and is funded by the Faculty of Health and Life Sciences at Oxford Brookes University

#### **Who has reviewed the study?**

This research has been approved by the University Research Ethics Committee, Oxford Brookes University

If you have any concerns about the way in which the study has been conducted, please contact the Chair of the University Research Ethics Committee on [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet

If you have any questions regarding this study, you can contact either:

Dr Miriam Clegg  
Functional Food Centre  
Oxford Brookes University  
Gipsy Lane Campus  
Oxford  
OX3 0BP  
Email: [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Sarah Kennedy  
Functional Food Centre  
Oxford Brookes University  
Gipsy Lane Campus  
Oxford  
OX3 0BP  
Email: [sarah.kennedy2013a@brookes.ac.uk](mailto:sarah.kennedy2013a@brookes.ac.uk)

## Appendix 2b Letter to the head teacher



### **Project title: Lifestyle habits of adolescents**

Dear Head teacher,

We are writing to ask if you would be interested in taking part in a new research project investigating dietary and physical activity habits of young adults in Oxfordshire schools. Below is an outline of the research project and what your involvement would be if you decide to take part.

#### **The Research**

As young people become independent adults they begin to develop lifestyle habits that determine their adult health status. Regular exercise and eating healthily are important to ensure that we enjoy long term health. However, young people tend not to eat the recommended portions of fruit and vegetables each day, nor consume regular meals, and physical activity levels drop (especially in girls) around this age.

We have developed a questionnaire that will allow us to gain some insight into the lifestyle habits surrounding meals and activity levels of young people in Oxfordshire. The questionnaire will ask about the types of foods they eat, what they think about some of these foods, their physical activity patterns and how these factors affect their lifestyle.

From each school we are looking to recruit from 30 to 100+ students from years 9, 10 and 11. The questionnaire will take around 20-30 minutes to complete on one day before the 31<sup>st</sup> January 2014. A link to the testing mode version of the questionnaire is provided at the bottom of this letter.

#### **The Process**

With your approval we will provide information sheets to your students and teachers explaining the research. Given the low risk nature of this study we propose that parents are informed of the research by issue of a fair processing notice along with a letter from us explaining the research and a copy of the student's information sheet. Participation by the student in the study is voluntary and the questionnaire will be completely anonymous with students having the option to 'opt-in' to the study through voluntary completion or submission of the questionnaire. We suggest that the questionnaire is completed during tutor time or assembly however, we are happy to discuss the best way to incorporate it into the school day with minimum disruption. If access allows online completion by the students is possible, or the questionnaire can be provided in a paper format.

Although we are not collecting data on disordered eating behaviours, as the questionnaire asks some general questions on eating habits and behaviours we recommend that the school counsellor is informed of the study. We have provided a letter which can be passed to them should they wish to contact us directly (see copy attached).

#### **The Benefits**

The questionnaire asks for the student's height and weight measurements if known. As some children may not know this information we can arrange for equipment to be provided for use by the students during the study (with or without the researchers present). We are happy to discuss this further if you perceive this to be a beneficial activity.

The results from this research will be used to inform the development of intervention models that correspond with wider government initiatives for health promotion in adolescents. We would be happy to discuss the results of the study with the students to help them understand the process of scientific research. Additionally we can provide a report for the school based on the results which could be used to form part of the school's self-evaluation report.

### **The Researchers**

This research is being conducted by the Faculty of Health and Life Sciences at Oxford Brookes University. Mrs Sarah Kennedy is a PhD research student, an associate nutritionist and has experience of working with young people. The project supervisors are Dr Miriam Clegg and Dr Helen Lightowler.

Mrs Sarah Kennedy  
PhD Researcher  
[sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)

Dr Miriam Clegg  
Senior Lecturer in Nutrition  
[mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Dr Helen Lightowler  
Senior Lecturer in Nutrition  
[hlightowler@brookes.ac.uk](mailto:hlightowler@brookes.ac.uk)

If you are interested in taking part in this research please respond to Mrs Sarah Kennedy using any of the contact details below. I will contact you by phone next week so I can answer any questions you might have, or please feel free contact me if you require further information.

Yours Sincerely,

Mrs Sarah Kennedy BSc, *ANutr*  
PhD Research Student  
Functional Food Centre,  
Faculty of Health & Life Sciences,  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP.  
Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)  
Tel: 01865 483283

Questionnaire link: <http://edu.surveymzmo.com/s3/1216484/testversiondec>

## Appendix 2c Letter to the parents



### **Project Title: Lifestyle habits of adolescents**

Dear Parent(s)/Guardian(s),

Your child's school is supporting a new research project investigating eating habits and physical activity of young people in Oxfordshire schools. Below is an outline of the research project and what your child's involvement would be if they decide to take part.

#### **What is the purpose of the study?**

There is a large amount of scientific research that looks at the associations between what we eat and physical activity and the risk of developing diseases such as heart disease, type 2 diabetes and certain types of cancer. Recommendations are in place by the Government around time spent doing physical activity as well as dietary components to promote health.

This study aims to investigate the eating habits and physical activity of young people in Oxfordshire. To gather this information we will be asking your child to complete a questionnaire which will ask them about the types of foods they eat, what they think about some of these foods and how these factors affect their lifestyle now. The questionnaire will also ask your child's ethnicity as well as parent's or guardian's level of education, marital status and employment status, as these factors have been shown to have an effect on dietary habits.

#### **Why has my child been invited to participate?**

All schools in the Oxfordshire area have been invited to take part. Everyone who takes part will be between in year 9, year 10 or year 11 and attending a school in Oxfordshire.

#### **Does my child have to take part?**

It is up to your child to decide whether or not to take part. They will be given an information sheet one week before the questionnaire is distributed to allow time for them to discuss with you if they wish to take part. If they decide to take part they are still free to withdraw at any time and without giving a reason. Their decision on whether to take part or not take part in the study or to withdraw at any time will have no impact on their marks, assessments or future studies.

#### **What will happen to my child if they take part?**

Your child will be asked to fill out a questionnaire. The questionnaire will be completed during class tutor time and contains 70-75 questions, which should take around 20-30 minutes to complete. If they need extra time due to reading or writing difficulties this will be arranged with their teacher. We can provide you with a copy of the questionnaire if required. As the questionnaire will be completed during class tutor time there will be no impact on teaching time as part of the normal school day.

#### **What are the possible benefits of taking part?**

By letting us use the information provided in the questionnaire, we can gain essential insights into the lifestyle habits of young people and see what issues are important to them and how other young people like them can improve their health and wellbeing.

#### **What are the possible disadvantages of taking part?**

The questionnaire will ask some general questions about your child's eating habits and behaviours. Although we are not collecting data on disordered eating behaviours we acknowledge that some children might be experiencing concerns around aspects of their eating. If required the school counsellor can offer further advice and support to families on these issues.

### **Will what my child says in this study be kept confidential?**

All information provided will be anonymous and totally confidential (subject to legal limitations). Only the researcher working on this study will have access to the data. Computer files or paper questionnaires will be password protected or kept in locked cabinets. The data generated in the course of the research must be kept securely in paper or electronic form for a period of ten years after the completion of a research project retained in accordance with the University's policy on Academic Integrity. Due to participation taking place during school hours, it may be difficult to maintain anonymity of who is taking part but your child's responses will be returned anonymously to ensure confidentiality.

### **What will happen to the results of the research study?**

The information provided will be used to write a research paper on the lifestyle habits of young people. Your child's identity will not be recognisable from this. The results generated from the study will also be included in the write up of a PhD thesis and may form the basis of further research in the area. A summary of the results of the study will be presented to the school.

### **Who is organising and funding the research?**

This research is being conducted as part of a PhD research project and is funded by the Faculty of Health and Life Sciences at Oxford Brookes University.

### **Who has reviewed the study?**

This research has been approved by the University Research Ethics Committee at Oxford Brookes University. If you have any concerns about the way in which the study has been conducted, you should contact the Chair of the University Research Ethics Committee on [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

If you require any further information about the study please contact one of the researchers:

Mrs Sarah Kennedy  
PhD Researcher  
[sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)

Dr Miriam Clegg  
Senior Lecturer in Nutrition  
[mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Dr Helen Lightowler  
Senior Lecturer in Nutrition  
[hlightowler@brookes.ac.uk](mailto:hlightowler@brookes.ac.uk)

Thank you for taking time to read this information sheet.

Yours Sincerely,

Mrs Sarah Kennedy BSc, *ANutr*  
PhD Research Student  
Functional Food Centre,  
Faculty of Health & Life Sciences,  
Oxford Brookes University,  
Gypsy Lane, Oxford, OX3 0BP.  
Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)  
Tel: 01865 483283



## **Teacher Information sheet**

### **Project title: Lifestyle habits of adolescents**

Your students are being invited to take part in a research study as supported by your head teacher. Below is an outline of the research project and what your involvement would be, if you decide to take part.

#### **The purpose of the study**

There is a large amount of scientific research that looks at the associations between diet and physical activity and the risk of developing diseases such as heart disease, type 2 diabetes and certain types of cancer. Recommendations are in place by the Government around amounts of physical activity as well as dietary components to promote health.

This study aims to investigate the dietary and physical activity habits of young people in Oxfordshire. To gather this information we would like your students to complete a questionnaire which will ask them about the types of foods they eat, what they think about some of these foods, their physical activity patterns and how these factors affect their lifestyle now. The questionnaire will also ask about ethnicity as well as parent's or guardian's level of education, marital status and employment status, as these factors have been shown to have an effect on dietary habits.

#### **Why have we been invited to participate?**

All schools in the Oxfordshire area have been invited to take part to ensure a wide variety of participants. Everyone who takes part will be in year 9, 10 or 11 and attending a school in Oxfordshire.

#### **Does every child have to take part?**

All students and their parents will be provided with information sheets (see attached) explaining the research study. Each student can decide whether or not to take part. If they decide to take part they are still free to withdraw at any time and without giving a reason. Their decision on whether or not to take part or to withdraw at any time should not impact on their marks, assessments or future studies.

#### **What will happen to the students who do take part?**

They will be asked to fill out a questionnaire (a paper or an online version is available). We recommend the questionnaire be completed during tutor time or assembly so that a wide variety of responses can be collected. For students not taking part we suggest they are provided with alternative reading material.

The questionnaire contains 70-75 questions and takes around 20-30 minutes to complete. We ask that you arrange extra time for students who have reading or writing difficulties. We will provide you with copies or access to the questionnaire in advance as well as secure questionnaire collection boxes (if paper versions are being used). We will also provide the opportunity to ask any questions about the study. A link to the testing mode of the questionnaire is provided here: <http://edu.surveygizmo.com/s3/1216484/testversiondec>

#### **What are the possible benefits of taking part?**

By letting us use the information the students provide we can gain essential insights into the lifestyle habits of young people and see what issues are important to them and how other young people like them can improve their health and wellbeing.

#### **What are the possible disadvantages of taking part?**

If the questionnaire is completed during class time we expect this to cause minimal disruption to their normal school day.

The questionnaire asks some general questions about the student's eating habits and behaviours. Although we are not collecting data on disordered eating behaviours we acknowledge that some children might be experiencing concerns around aspects of their eating.

For help or advice on eating we suggest on their information sheet that they talk to the school counsellor. A separate information sheet will be sent to the school counsellor informing them of the project.

### **Will what the students say in this study be kept confidential?**

Due to participation taking place during school hours, it may be difficult to maintain anonymity of who is taking part but the questionnaire is to be returned anonymously to ensure participant confidentiality.

All the information provided will be anonymous and confidential (subject to legal limitations). Only the researchers working on this study will have access to the data. Computer files or paper questionnaires will be password protected or kept in locked cabinets. Data generated by the study must be retained in accordance with the University's policy on Academic Integrity. The data generated in the course of the research must be kept securely in paper or electronic form for a period of ten years after the completion of a research project.

### **What will happen to the results of the research?**

The information provided will be used to write a research paper on the lifestyle habits of young people. The student's identity will not be recognisable from this. The information will also be included in the write up of a PhD thesis and may form the basis of further research in the area. A summary of the results of the project will be provided to the school. Any requests for copies of the published research should be made to the contact details provided below.

### **Who is organising and funding the research?**

This research is being conducted as part of a PhD research project and is funded by the Faculty of Health and Life Sciences at Oxford Brookes University

### **Who has reviewed the study?**

This research has been approved by the University Research Ethics Committee, Oxford Brookes University

If you have any concerns about the way in which the study has been conducted, please contact the Chair of the University Research Ethics Committee on [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet

If you have any questions regarding this study, you can contact either:

Dr Miriam Clegg  
Functional Food Centre  
Oxford Brookes University  
Gypsy Lane Campus  
Oxford  
OX3 0BP  
Email: [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Sarah Kennedy  
Functional Food Centre  
Oxford Brookes University  
Gypsy Lane Campus  
Oxford  
OX3 0BP  
Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)

## **School counsellor information sheet**

### **Project title: Lifestyle habits of adolescents**

Your students are being invited to take part in a research study as supported by your head teacher. Below is an outline of the research project and what your involvement would be, if you decide to take part.

#### **The purpose of the study**

There is a large amount of scientific research that looks at the associations between diet and physical activity and the risk of developing diseases such as heart disease, type 2 diabetes and certain types of cancer. Recommendations are in place by the Government around amounts of physical activity as well as dietary components to promote health.

This study aims to investigate the dietary and physical activity habits of young people in Oxfordshire. To gather this information we would like your students to complete a questionnaire which will ask them about the types of foods they eat, what they think about some of these foods, their physical activity patterns and how these factors affect their lifestyle now. The questionnaire will also ask about ethnicity as well as parent's or guardian's level of education, marital status and employment status, as these factors have been shown to have an effect on dietary habits.

#### **Why have we been invited to participate?**

All schools in the Oxfordshire area have been invited to take part to ensure a wide variety of participants. Everyone who takes part will be in year 9, 10 or 11 and attending a school in Oxfordshire. All questionnaires will remain completely anonymous.

#### **Does every child have to take part?**

All students and their parents will be provided with information sheets (see attached) explaining the research study. Each student can decide whether or not to take part. If they decide to take part they are still free to withdraw at any time and without giving a reason. Their decision on whether or not to take part or to withdraw at any time should not impact on their marks, assessments or future studies.

#### **What will happen to the students who do take part?**

They will be asked to fill out a questionnaire (a paper or an online version is available). We recommend the questionnaire be completed during tutor time or assembly so that a wide variety of responses can be collected. For students not taking part we suggest they are provided with alternative reading material.

The questionnaire contains 70-75 questions and takes around 20-30 minutes to complete. We ask that you arrange extra time for students who have reading or writing difficulties. We will provide you with copies or access to the questionnaire in advance as well as secure questionnaire collection boxes (if paper versions are being used). We will also provide the opportunity to ask any questions about the study. A link to the testing mode of the questionnaire is provided here: <http://edu.surveymzmo.com/s3/1216484/testversiondec>

#### **What are the possible benefits of taking part?**

By letting us use the information the students provide we can gain essential insights into the lifestyle habits of young people and see what issues are important to them and how other young people like them can improve their health and wellbeing.

### **What are the possible disadvantages of taking part?**

If the questionnaire is completed during class time we expect this to cause minimal disruption to their normal school day.

The questionnaire asks some general questions about the student's eating habits and behaviours. Although we are not collecting data on disordered eating behaviours we acknowledge that some children might be experiencing concerns around aspects of their eating. For help or advice on eating we suggest on their information sheet that they talk to the school counsellor. A separate information sheet will be sent to the school counsellor informing them of the project.

### **Will what the students say in this study be kept confidential?**

Due to participation taking place during school hours, it may be difficult to maintain anonymity of who is taking part but the questionnaire is to be returned anonymously to ensure participant confidentiality.

All the information provided will be anonymous and confidential (subject to legal limitations). Only the researchers working on this study will have access to the data. Computer files or paper questionnaires will be password protected or kept in locked cabinets. Data generated by the study must be retained in accordance with the University's policy on Academic Integrity. The data generated in the course of the research must be kept securely in paper or electronic form for a period of ten years after the completion of a research project.

### **What will happen to the results of the research?**

The information provided will be used to write a research paper on the lifestyle habits of young people. The student's identity will not be recognisable from this. The information will also be included in the write up of a PhD thesis and may form the basis of further research in the area.

A summary of the results of the project will be provided to the school. Any requests for copies of the published research should be made to the contact details provided below.

### **Who is organising and funding the research?**

This research is being conducted as part of a PhD research project and is funded by the Faculty of Health and Life Sciences at Oxford Brookes University

### **Who has reviewed the study?**

This research has been approved by the University Research Ethics Committee, Oxford Brookes University

If you have any concerns about the way in which the study has been conducted, please contact the Chair of the University Research Ethics Committee on [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet

If you have any questions regarding this study, you can contact either:

Dr Miriam Clegg  
Functional Food Centre  
Oxford Brookes University  
Gypsy Lane Campus  
Oxford  
OX3 0BP  
Email: [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Sarah Kennedy  
Functional Food Centre  
Oxford Brookes University  
Gypsy Lane Campus  
Oxford  
OX3 0BP  
Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)


## Lifestyle Habits of Adolescents

---

### Thanks for agreeing to fill out this questionnaire!

The information you share with us will be used to gain a better understanding of the current lifestyle habits and attitudes of adolescents. The data we collect will go on to be used as part of a PhD research project to help develop a health intervention for your age group.

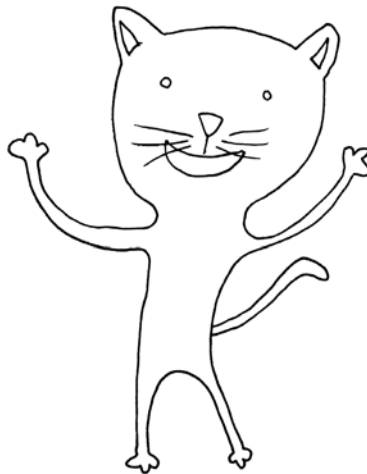
Please take the time to read and answer the questions as honestly and accurately as you can, this is very important for our research.

The questionnaire should take you about 20-30 minutes to complete. Please answer every question. Instructions are given throughout on how to complete the questionnaire, look out for the pencil icon  If you are not clear about something then please ask.


Remember it is NOT a test, there are no right or wrong answers. The questionnaire is anonymous. Your name is not required so no one can identify who you are.

By completing this questionnaire you are indicating your consent to participate in this research

Many thanks! Your help with this project is really appreciated.



## Your general EATING HABITS...when and what?

 Please select your answer by ticking the box and select one answer only.

*Breakfast = food item/smoothie consumed before the first lesson of the school day, or at the weekend before 11am.*

1. During the past 7 days, on how many days did you eat *breakfast*?

- never
  - 1-2 days
  - 3-4 days
  - 5-6 days
  - everyday
- 

2. Did you eat breakfast Saturday morning?

- yes
  - no (go to Question 5)
- 

3. What did you have for breakfast Saturday morning? (please give as much detail as possible including brand names, for example 'Kellogg's Cornflakes')

I ate

I drank

---

4. Where did you have breakfast Saturday morning?

- at home
  - other: please give details
- 

5. What was the reason (or reasons) for you not eating breakfast Saturday morning? (skip this question if you DID eat breakfast)

1

2

3

---

6. Did you eat breakfast this morning?

- yes
  - no (go to question 9)
-

7. What did you have for breakfast this morning? (please give as much detail as possible including brand names, for example 'Kellogg's Cornflakes')

I ate

I drank

---

8. Where did you have breakfast this morning?

at home

on the way to school

school breakfast club

other: please give details

---

9. What was the reason (or reasons) for you not eating breakfast this morning? (skip this question if you DID eat breakfast)

1

2

3

---

*Lunch = the meal eaten in the middle of the day*

10. During the past 7 days, on how many days did you eat *lunch*?

never

1-2 days

3-4 days

5-6 days

everyday

---

*Dinner = the meal eaten in the evening*

11. During the past 7 days, on how many days did you eat *dinner*?

never

1-2 days

3-4 days

5-6 days

everyday

---

12. During the past 7 days, how many times did you eat from a fast food outlet (like McDonald's, Subway, KFC, etc.)?

- never
  - 1-2 times
  - 3-4 times
  - 5-6 times
  - everyday
  - twice on everyday
  - more than twice on everyday
- 

13. During the past 7 days, how many times did you eat high sugar/fat snacks (like crisps, cakes, biscuits etc.)?

- never
  - 1-2 times
  - 3-4 times
  - 5-6 times
  - everyday
  - twice on everyday
  - three times on everyday
  - more than three times on everyday
- 

14. During the past 7 days, how many times did you drink fizzy drinks? (excluding fizzy water).

- never
  - 1-2 times
  - 3-4 times
  - 5-6 times
  - everyday
  - twice on everyday
  - three times on everyday
  - more than three times on everyday
- 

*1 portion e.g. = 1 medium banana, 7 strawberries, 1 heaped tablespoon raisins, 150ml fruit juice, 150ml smoothie*

15. How many portions of fruit did you eat yesterday?

- none
  - 1 portion
  - 2-3 portions
  - 4-5 portions
  - more than 5 portions
-



**1 portion e.g.= 3 heaped tablespoons of peas/baked beans/lentils, 1 tomato, 2 pieces of broccoli, 150ml vegetable juice**

**16. How many portions of vegetables did you eat yesterday?**

- none
  - 1 portion
  - 2-3 portions
  - 4-5 portions
  - more than 5 portions
- 


**17. During the past 7 days, how many times did all, or most of your family living in your house eat a meal together? (breakfast, lunch or dinner)**

- never
  - 1-2 times
  - 3-4 times
  - 5-6 times
  - 7 times
  - more than 7 times
- 

**18. Which meal is eaten together most frequently by all, or most of your family living in your house?**

- breakfast
  - lunch
  - dinner
  - I never eat a meal with any of my family
-

## We would like to know more about your BREAKFAST habits...

 How much do you agree or disagree with the following statements? Please read the answer options carefully. Please select your answer by ticking the box and select one answer only.

**19. Most of my family members eat breakfast regularly (i.e. on 5 or more days of the week)**

- disagree a lot       disagree a bit       don't agree or disagree       agree a bit       agree a lot
- 

**20. Most people my age eat breakfast regularly (i.e. on 5 or more days of the week)**

- disagree a lot       disagree a bit       don't agree or disagree       agree a bit       agree a lot
- 

**21. In general, how *similar* are you to the type of person your age who regularly eats breakfast?**

- not at all similar       not that similar       neither similar or  
unsimilar       a bit similar       very similar
- 

**22. People who are important to me think I should eat breakfast regularly (i.e. on 5 or more days of the week)**

- definitely do not       do not a bit       neither do or do not       do a bit       definitely do
- 

**23. For me, eating breakfast regularly (i.e. on 5 or more days of the week) would be...**

- very difficult       a bit difficult       neither easy or difficult       a bit easy       very easy
- 

**24. I am confident I can eat breakfast regularly (i.e. on 5 or more days of the week) if I want to**

- disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot
- 

**25. Over the next week, I intend to eat breakfast on the following days...(tick all that apply)**

- Monday  
 Tuesday  
 Wednesday  
 Thursday  
 Friday  
 Saturday  
 Sunday  
 All of the above

26. To what extent would you be motivated to eat breakfast regularly over the next 7 days?

- not at all motivated       not that motivated       neither motivated or unmotivated       a bit motivated       very motivated
- 


27. Do you think its important to eat breakfast regularly?

- yes  
 no
- 

28. Please give a reason (or reasons) for your answer to Question 27

- 1
- 2
- 3
-

## We would like to know more about your ATTITUDES towards breakfast...

 How much do you agree or disagree with the following statements? Please read the answer options carefully. Please select your answer by ticking the box and select one answer only.

**29. I often miss breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**30. Its OK to miss breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**31. I hardly eat anything for breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**32. I hate eating breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**33. I can concentrate in class even when I have missed breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**34. I usually have a snack at morning break instead of breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**35. I feel okay in the mornings even if I haven't eaten breakfast**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

**36. Eating breakfast is boring**

disagree a lot       disagree a bit       neither agree or disagree       agree a bit       agree a lot

---

37. I'd rather have a snack at morning break than eat breakfast

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

38. If I miss breakfast I feel more tired in the morning

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

*Healthy = cereals (without sugar or chocolate), toast, fruit, yogurt, cooked items which were not fried or deep fried*

39. I usually eat a *healthy* breakfast

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

40. Today I ate a *healthy* breakfast

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

41. I am too rushed in the morning to eat breakfast

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

42. I am too rushed in the mornings to eat a healthy breakfast

disagree a lot

disagree a bit

neither agree or disagree

agree a bit

agree a lot

---

43. Teenagers don't need to be concerned about their eating habits

disagree a lot

disagree a bit


neither agree or disagree

agree a bit


agree a lot

---

We would like to find out about your PHYSICAL ACTIVITY in the last 7 days

 For example, sports or dance that made you sweat, or your legs feel tired, or games that made you breathe hard, like tag, skipping, running or climbing.  
Please select your answer by ticking the box and select one answer only.

44. In the last 7 days, have you done any of the following activities? If yes, how many times?

 There are blank boxes at the end if your activity is not listed. Please select only one answer per activity.

	None	1-2 times	3-4 times	5-6 times	7 times or more
skipping					
rowing/canoeing					
roller-skating					
skateboarding					
tag					
walking for exercise					
bicycling					
jogging					
sprinting/athletics					
circuit training					
aerobics					
swimming					
dance					
football					
badminton or tennis					
hockey (field, street or ice)					
trampolining					
basketball					
ice-skating					
scooter					
<input type="text"/>					
<input type="text"/>					
<input type="text"/>					
<input type="text"/>					

45. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)?

- Hardly ever
  - Sometimes
  - Quite often
  - Always
  - I don't do PE
- 

46. In the last 7 days, what did you normally do at *morning break*?

- Sat down (talking, reading, doing schoolwork)
  - Stood around or walked around
  - Ran or played a little bit
  - Ran around and played quite a bit
  - Ran and played hard most of the time
- 

47. In the last 7 days, what did you normally do at *lunch*? (besides eating lunch)

- Sat down (talking, reading, doing schoolwork)
  - Stood around or walked around
  - Ran or played a little bit
  - Ran around and played quite a bit
  - Ran and played hard most of the time
- 

48. In the last 7 days, on how many days *right after school*, did you do sports, dance, or play games in which you were very active?

- None
  - 1 day last week
  - 2 or 3 days last week
  - 4 days last week
  - 5 days last week
- 

49. In the last 7 days, on how many *evenings* did you do sports, dance, or play games in which you were very active?

- None
  - 1 evening last week
  - 2 or 3 evenings last week
  - 4 or 5 evenings last week
  - 6 or 7 evenings last week
-

50. On the last weekend, how many times did you do sports, dance, or play games in which you were very active?

- None
  - 1 time
  - 2 - 3 times
  - 4 - 5 times
  - 6 or more times
- 

51. Which one of the following describes you best for the last 7 days? read all five statements before deciding on the one answer that describes you

- All or most of my free time was spent doing things that involve little physical effort
  - I sometimes (1-2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics)
  - I often (3-4 times last week) did physical things in my free time
  - I quite often (5-6 times last week) did physical things in my free time
  - I very often (7 or more times last week) did physical things in my free time
- 

52. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

	None	Little bit	Medium	Often	Very often
Monday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tuesday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wednesday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Thursday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Friday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Saturday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sunday	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

53. Last week, did anything prevent you from doing your normal physical activities?

- yes
  - no (go to Question 55)
- 

54. Please tell us what prevented you?

---

55. Do you have any mobility issues that restrict your physical activity?

- yes
  - no (go to Question 57)
-



56. Please can you tell us a bit more about this?

57. In your freetime on an average weekday (monday to friday), how many hours per day do you spend...

	0hr	30mins	1 hr	2 hrs	3 hrs	4 hrs	5+ hrs
watching TV & movies	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
reading & doing homework	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
using a computer (not for homework)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

58. On an average weekend day (Saturday or Sunday), how many hours per day do you spend...

	0hr	30mins	1 hr	2 hrs	3 hrs	4 hrs	5+ hrs
watching TV & movies	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
reading & doing homework	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
using a computer (not for homework)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

59. How did you travel to school this morning?

walk


cycle

bus

car

other

## FINALLY, let's finish with some GENERAL QUESTIONS about YOU and your FAMILY...

 Please select your answer by ticking the box and select one answer only.

### 60. What is the name of the school you attend?

- Bartholomew School
- Bicester Community College
- Cokethorpe School
- Fitzharry's School
- Gosford Hill School
- John Mason School
- King Alfred's
- Matthew Arnold School
- North Oxfordshire Academy
- Rye St Antony
- St Gregory The Great Catholic School
- The Cooper School
- The Oxford Academy
- Tudor Hall School
- Other: please give details

### 61. How many brothers and/or sisters do you have?

- none
- 1
- 2
- 3
- 4
- 5
- more than 5

### 62. What is the highest level of academic study that your mum/guardian completed?

- did not finish secondary school
- finished secondary school
- did some college or training after secondary school
- finished college
- went to university (degree level)
- went to university (master's or PhD level)
- I don't know

**63. Does your mum/guardian...**

- work full-time for pay
  - work part-time for pay
  - not work for pay
  - I don't know
- 

**64. What is the highest level of academic study that your dad/guardian completed?**

- did not finish secondary school
  - finished secondary school
  - did some college or training after secondary school
  - finished college
  - went to university (degree level)
  - went to university (master's or PhD level)
  - I don't know
- 

**65. Does your dad/guardian...**

- work full-time for pay
  - work part-time for pay
  - not work for pay
  - I don't know
- 

**66. Are your parents/guardians:**

- married
  - divorced
  - living together (not married)
  - separated
  - one or both of my parents/guardians has died
- 

**67. Do you, or someone in your household, own a car?**

- yes
  - no (go to Question 69)
-

**68. How many cars do your household own?**

- 1
  - 2
  - 3
  - 4
  - more than 4
- 

**69. How would you describe your level of fitness?**

- poor
  - fair
  - good
  - excellent
- 

**70. Approximately how tall are you? (use feet or metres)**

height

---

**71. Approximately how much do you weigh? (use kilograms or stone/pounds)**

weight

---

**72. What is your age?**

- 13 years
  - 14 years
  - 15 years
  - 16 years
  - 17 years
  - other:
- 

**73. Are you...?**

- male
  - female
-

**74. Would you describe yourself as....**

- Arab
  - Asian / Asian British
  - Black / Black African / Black British / Black Caribbean
  - Mixed / Multiple ethnic groups
  - White
  - Other ethnic group:
- 

Thank you for taking the time to complete this survey!



Your responses are very important to our research.

---

---

## **Information sheet**

### **Project title: The effect of a functional food based breakfast on blood glucose, insulin, satiety, mood and cognitive performance**

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

#### **The purpose of the study**

Functional foods demonstrate health benefits beyond their basic nutritional function and their role in protecting against several chronic diseases including the development of cancers, cardiovascular diseases (CVDs), diabetes and osteoporosis is well established. Popular functional foods include those rich in antioxidant polyphenols, low glycemic index (GI) foods and foods high in dietary fibre (for e.g. beta-glucan). These foods have been suggested to lower blood glucose and insulin response, enhance satiety and improve mood and cognitive performance.

This study will look at the effect of a breakfast muffin enriched with low GI ingredients and polyphenols when compared with a control muffin and a ready to eat breakfast cereal on measures of blood glucose and insulin response, satiety, mood and cognitive performance.

- The glycaemic response (GR) is the degree to which a food raises the blood glucose levels compared with a standard food
- The insulinaemic response (IR) is the degree to which a food raises the blood insulin level compared with a standard food
- Satiety is the feeling of fullness after the consumption of a meal
- Cognitive tests to measure memory

#### **Who is eligible to participate?**

- Aged 18-65 years
- Body mass index (BMI) between 18.5kg/m<sup>2</sup> and 30kg/m<sup>2</sup>
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- No use of steroids, protease inhibitors or antipsychotics
- Fasting blood glucose < 6.1 mmol/l

#### **Do I have to take part?**

- It is up to you to decide whether or not to take part
- If you do decide to take part you will be asked to sign a consent form
- If you are a student at Oxford Brookes, choosing to take part or not to take part will have no impact on your marks, assessments or future studies or relationships with any of the principal investigators of the study
- If you do decide to take part you are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied

#### **What will happen to me if I take part?**

- You will be invited to attend a screening session, in a fasted state, where we will collect your body composition measurements, your fasting blood glucose levels and ask you to complete:
  - A health questionnaire
  - A food frequency questionnaire
  - A consent form

We will also measure:

- Your height and weight (in order to estimate your body mass index (BMI) – a measure of weight status)
- Your blood pressure
- If you fulfil the eligibility criteria (stipulated above) during the screening you will be invited to stay and complete the first session, or, if you require more time to consider your participation in the study then you will be invited to return another day. Each session will last for approximately 3.5 hours. You can decide how many test sessions you do in a week – it could be 1, 2 or 3, but you need to leave at least 1 day between tests
- Each test session will involve the following:
  - Two fasting blood glucose and insulin measurements (at -5 minutes and 0 minutes) – this involves two small finger-pricks using disposable individual lancets
  - Consumption of the standard or test food
  - Further finger-prick blood samples at 15, 30, 45, 60, 90, 120, 150 and 180 minutes after the start of the meal
  - Each test session will require a **maximum** of 10 finger-pricks.
  - At the same time intervals, you will be asked how hungry you feel and what your mood is at that time
  - The researchers will also ask you to complete two cognitive tests so that they can compare performance on memory tasks during each test condition. The first test involves the completion of a map task which involves recalling the location of items on a map. A second task will ask you to recall a list of words. You will do these 3 times each during each testing session (9 times each in total). The total time spent completing cognitive tests will be ~36 minutes per test. This time is included in the total session timings (~3.5 hours).
- Testing will take place at the Functional Food Centre (FFC) lab. In-between measures you can sit in the FFC area and read, revise or watch movies on your laptop.
- Once you have finished a test you will be offered a snack

#### How to prepare for the study

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, although you are allowed to drink water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at around 8.30 pm the previous evening
- On your first session we will ask you to record what you had for dinner and to try and consume a meal as similar as possible the night before subsequent tests
- On the day before a test
  - limit your caffeine intake (maximum of 2-3 cups of coffee/tea)
  - limit your alcohol intake (maximum of 1 glass of wine/1 pint of beer)
  - restrict participating in intense physical activity (e.g. long periods at the gym, excessive swimming, running, aerobics)
- Do not smoke on the morning of the test

#### What are the possible disadvantages and risks of taking part?

- Each measurement requires only a few drops of blood, therefore the finger-prick will be small, with minimal discomfort. A light bruising may also occur in some people, but this should disappear itself within a couple of days and will not affect your ability to work.
- Trained personnel will take finger-prick blood samples using standard procedures and finger-pricks are made using disposable individual lancets to avoid the possibility of contamination
- If you have any concerns around aspects of your mood please refer to the following website which gives information on where to get professional advice  
<http://www.nhs.uk/Conditions/stress-anxiety-depression/Pages/low-mood-and-depression.aspx>

### **Benefits of the study**

- The study will provide valuable information on identifying the potential benefits between breakfast varying in nutritional composition and will inform the development of future breakfast based interventions
- At the time of collection we can provide you with your anthropometric and body composition measurements
- You will receive £25 of Amazon or book vouchers on completion of your 3 test sessions

### **Will what I say in this study be kept confidential?**

- All information collected will be kept strictly confidential (subject to legal limitations) and confidentiality, privacy and anonymity will be ensured in the collection, storage and publication of research material. Data generated by the study must be retained in accordance with the University's policy on Academic Integrity.
- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work.
- All data from the study will be owned by Oxford Brookes University and will be kept securely in paper or electronic form at the University for a minimum of 10 years.
- Data will be used in scientific publications and presentations at conferences. However, your name will never appear in any of these.

### **What should I do if I want to take part?**

- Please email Sarah Kennedy to arrange for your first screening visit on [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)

### **What will happen to the results of the research?**

- Data will be used to form part of a PhD thesis. It may also be presented in scientific publications and at conferences. However, your name will never appear in any of these.
- If you require a copy of any published research please let the research team know

### **Who is organising and funding the research?**

- This research is being conducted as part of a PhD research project
- Funding is provided by the Department of Sports and Health Science, Faculty of Health and Life Sciences at Oxford Brookes University

### **Who has reviewed the study?**

- This research has been approved by the University Research Ethics Committee, Oxford Brookes University
- If you have any concerns about the way in which the study has been conducted, please contact the Chair of the University Research Ethics Committee on [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet

If you have any questions regarding this study, you can contact either:

Dr Miriam Clegg  
Functional Food Centre  
Oxford Brookes University  
Gipsy Lane Campus  
Oxford  
OX3 0BP  
Email: [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Sarah Kennedy  
Functional Food Centre  
Oxford Brookes University  
Gipsy Lane Campus  
Oxford  
OX3 0BP  
Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)



## Appendix 3b Health questionnaire

Subject

### Health Questionnaire

(Please circle as appropriate)

- Are you allergic to any foods? Yes or No  
If yes which one(s)? \_\_\_\_\_
- Do you have a genetic or metabolic disease? Yes or No
- Are you taking any medication? Yes or No  
If yes, which one(s)? \_\_\_\_\_
- Have you undergone any major medical/ surgical event in the last 3 months? Yes or No
- Are you a smoker? Yes or No  
If yes, cigarettes/day: \_\_\_\_\_
- Are you following a special diet? Yes or No  
If yes, which one(s)? \_\_\_\_\_
- Do you exercise or participate in any sports? Yes or No  
How often a week? \_\_\_\_\_ Duration: \_\_\_\_\_ Intensity: \_\_\_\_\_
- Are there any foods you dislike? \_\_\_\_\_
- Do you usually eat breakfast? Yes or No  
How many days per week? \_\_\_\_\_

**Women only:** please answer the following questions:

- Do you use oral contraceptive pills? Yes or No
- If yes, which type of pill? \_\_\_\_\_
- What is the average length of your menstrual cycle? \_\_\_\_\_
- Number of days since the end of your last menstruation? \_\_\_\_\_

## Appendix 3c Food frequency questionnaire

### Food Frequency Questionnaire (FFQ)

PLEASE READ THE INSTRUCTION CAREFULLY BEFORE FILLING THE QUESTIONNAIRE.

- This questionnaire asks for some background information about your diet
- We would like you to answer each question as best as you can.
- If you are unsure about how to answer a question do the best you can and please do not leave a question blank.
- For each food item there is an amount shown, either an average portion size or usual household unit such as 1 slice of bread, 1 teaspoon of sugar. Please put a tick (✓) in the box to indicate how often, on average you have eaten the specified amount of each food over the last month.

#### Example

For white bread the average amount is one slice, so if you ate 5 slices in a week, you need to put a tick in the column headed 5 per week.

BREAD AND ROLLS	Once a week	2 per week	3 per week	4 per week	5 per week	6 per week	7 per week	Every 2 to 3 weeks	Rarely or never
White bread					✓				

This food frequency questionnaire was adapted from: Broadfield E, McKeever T, Fogarty A, Britton J. Measuring dietary fatty acid intake: validation of a food-frequency questionnaire against 7 d weighed records. *Br J Nutr.* 2003; 90(1):215-20.

## Appendix 3c Food frequency questionnaire

Food and amount	Average consumption over last month								
	rarely/never	every 2-3 wk	1/wk	2/wk	3/wk	4/wk	5/wk	6/wk	7+/wk
<b>Drink (200 mL)</b>									
Black tea									
Green tea									
Oolong/other tea									
Coffee									
Red wine									
Beer (regular)									
Beer (dark)									
Cider									
Hot chocolate									
Fruit juice (not pure)									
Pure orange juice									
Pure apple juice									
Pure pomegranate juice									
Pure cranberry juice									
Pure blueberry juice									
Pure mango juice									
Pure tomato juice									
Pure beetroot juice									
Pure prune juice									
Pure carrot juice									
Other vegetable juice									
<b>Fruits</b>									
Dark plums (2 units)									
Apple (1 whole)									
Cherries (14 units)									
Peach (1 unit)									
Pear (1 unit)									
Melon (1 slice)									
Kiwi (2 whole)									
Banana (1)									
Pineapple (1 slice)									
Avocado (1/2 whole)									
Grapefruit (1/2)									
Orange/Lemon (1 whole)									
Blackberry (10 units)									
Pomegranate (1/2)									
Cranberries (1/2 handful)									
Blueberry (20 units)									
Raspberry (20)									

	rarely/never	every 2-3wk	1/wk	2/wk	3/wk	4/wk	5/wk	6/wk	7+/wk
Redcurrants (20)									
Blackcurrants (20)									
White grapes (10)									
Red grapes (10)									
Strawberries (6)									
Apricot (3)									
Dried fruit (small handful)									
<b>Vegetables</b>									
White onion (1 whole)									
Garlic (3 cloves)									
Red onion (1 whole)									
Leek (1)									
Kale (1 handful)									
Pepper (1/2)									
Tomatoes (2 whole)									
Broccoli (4 stems)									
Red cabbage (1 handful)									
Salad leaves (1/4 bag)									
Rhubarb (1 stick)									
Spinach (1 handful)									
Cabbage/sprouts (1 handful)									
Parsnip (1)									
Carrots (1 handful)									
Red beetroot (1)									
Asparagus (5 spears)									
Celery (3 sticks)									
Artichokes (1 whole)									
Aubergines (1 whole)									
Cauliflower (4 stems)									
Cucumber (1/4)									
Sweetcorn (1 handful)									
Green bean (1 handful)									
Courgette (1/2)									
Peas (1 handful)									
Mushrooms (4 whole)									
Chillies (1 whole)									
Potatoes (5 medium)									

	rarely/never	every 2-3wk	1/wk	2/wk	3/wk	4/wk	5/wk	6/wk	7+/wk
<b>Chips (10 average)</b>									
<b>Frozen vegetables (1 medium handful)</b>									
<b>Herbs and spices</b>									
<b>Cinnamon (1/2 tsp)</b>									
<b>Turmeric (1/2 tsp)</b>									
<b>Cumin (1/2 tsp)</b>									
<b>Basil (1 tbsp)</b>									
<b>Parsley (1 tbsp)</b>									
<b>Nuts seeds &amp; pulses</b>									
<b>Chestnuts (10 kernels)</b>									
<b>Hazelnuts (6 whole)</b>									
<b>Walnuts (6 halves)</b>									
<b>Peanuts butter (1 tbsp)</b>									
<b>Brazil nuts (3 whole)</b>									
<b>Peanuts (10 whole)</b>									
<b>Almonds (6 whole)</b>									
<b>Macadamia (10)</b>									
<b>Cashew (17 whole)</b>									
<b>Lentils (boiled-1 tbsp)</b>									
<b>Chickpeas (1 tbsp-cooked)</b>									
<b>Red kidney beans (1 tbsp)</b>									
<b>Baked beans (2 tbsp)</b>									
<b>Bread</b>									
<b>Wholemeal (1 slice)</b>									
<b>Multigrain (1 slice)</b>									
<b>Rye (1 slice)</b>									
<b>White (1 slice)</b>									
<b>Brown (1 slice)</b>									
<b>Sorghum (1 slice)</b>									
<b>Cereals</b>									
<b>Weetabix (1 biscuit)</b>									
<b>Allbran (1 portion)</b>									
<b>Muesli (1 portion)</b>									
<b>Porridge (1 portion)</b>									
<b>Shredded wheat (1 biscuit)</b>									
<b>Blueberry wheat (1 portion)</b>									

Oils	Rarely/never	every 2-3wk	1/wk	2/wk	3/wk	4/wk	5/wk	6/wk	7+/wk
Olive oil (1 tbsp)									
<b>Soy products</b>									
Soy beans (1 handful)									
<b>Puddings &amp; sweets</b>									
Milk chocolate (4 units)									
Dark chocolate (4 units)									
Oat biscuits (2)									
Chocolate biscuit (2)									
Cereal bars (1)									
Jams/jellies (1 tbsp)									
Is there any other fruit, vegetable, cereal, bread or drink item (such as smoothies, home baking) or supplement (i.e. vitamin C tablet) that you ate more than once a week? If yes, please list them below									
<b>Other foods</b>									
<b>Supplements</b>									

*Additional questions:*

Do you eat mostly organic foods Yes or No

Do you peel the skin off of fruits and vegetables? Yes or No

Do you eat your vegetables mostly raw or cooked?

\_\_\_\_\_

Do you add milk to your tea and coffee? Yes or No

**Consent form**

Project title: The effect of a functional food based breakfast on blood glucose, insulin, satiety, mood and cognitive performance

**Contacts:**

Ms Sarah Kennedy, PhD Research Student  
Dr Miriam Clegg, Senior Lecturer

Functional Food Centre  
Department of Sport and Health Sciences  
Faculty of Health and Life Sciences  
Oxford Brookes University  
Gipsy Lane Campus  
Oxford OX3 0BP  
Tel: 01865 483818 / 01865 483988/ 01865 484365  
Email: [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk) / [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	<input type="checkbox"/>	<input type="checkbox"/>
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	<input type="checkbox"/>	<input type="checkbox"/>
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	<input type="checkbox"/>	<input type="checkbox"/>
4. I understand that confidentiality of information provided can only be protected within the limits of the law and due to the small sample size of this study there may be implications for maintaining full anonymity of my participation in the study.	<input type="checkbox"/>	<input type="checkbox"/>
5. I agree to take part in the above research.	<input type="checkbox"/>	<input type="checkbox"/>

Name of Participant ..... Date .....  
(block capitals)

Signature .....

Contact number: ..... email: .....

Name of Researcher ..... Date .....  
(block capitals)

Signature .....

## Appendix 3e Word recall task

Write down as many words as you can remember from the last map task

1)

2)

3)

4)

5)

6)

7)

8)

9)

10)

11)

12)

13)

14)

15)

16)

17)

18)

19)

20)

21)

22)

23)

24)

25)

26)

27)

28)

29)

30)



## Appendix 3f Mood questionnaire

Each of the words below describes feelings or mood. Please use the rating scale next to each word to describe your feelings at this moment. For e.g. If you definitely do not feel 'active' at the moment then you would tick the box in column 1 against the word 'active'. Work rapidly, but please mark all the words. Your first reaction is best. This should take only a minute or two.

<b>0:00 (baseline)</b>				
	1	2	3	4
	You <b>definitely</b> feel ' _ ' at the moment	You feel <b>slightly</b> ' _ ' at the moment	Word <b>does</b> <b>not apply</b> or <b>you cannot</b> <b>decide</b> if you feel ' ___ ' at the moment.	You <b>definitely</b> <b>do not</b> feel ' _ ' at the moment
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

0:15

	1 You <b>definitely</b> feel ' _ ' at the moment	2 You feel <b>slightly</b> ' _ ' at the moment	3 Word <b>does</b> <b>not apply</b> or <b>you cannot</b> <b>decide</b> if you feel '___' at the moment.	4 You <b>definitely</b> <b>do not</b> feel ' _ ' at the moment
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1:00

	1	2	3	4
	You <b>definitely</b> feel ' _ ' at the moment	You feel <b>slightly</b> ' _ ' at the moment	Word <b>does not apply</b> or <b>you cannot decide</b> if you feel ' ___ ' at the moment.	You <b>definitely do not</b> feel ' _ ' at the moment
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

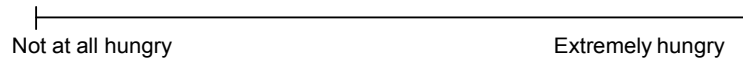
2:00

	1	2	3	4
	You <b>definitely</b> feel ' _ ' at the moment	You feel <b>slightly</b> ' _ ' at the moment	Word <b>does not apply</b> or <b>you cannot decide</b> if you feel ' ___ ' at the moment.	You <b>definitely do not</b> feel ' _ ' at the moment
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

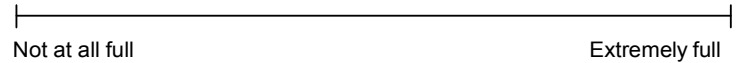
## Appendix 3g Appetite and palatability scale (VAS)

0:00

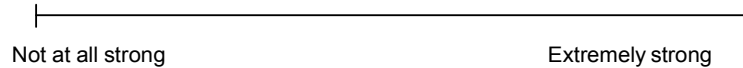
**How hungry do you feel?**



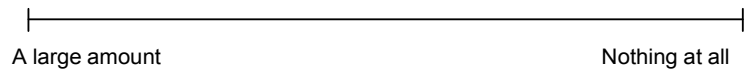
**How full do you feel?**



**How strong is your desire to eat?**

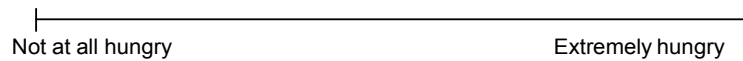


**How much food do you think you can eat?**

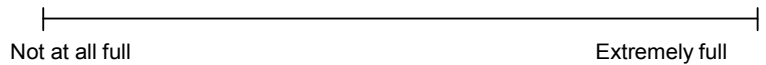


0:15

**How hungry do you feel?**



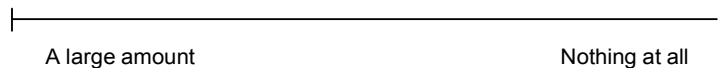
**How full do you feel?**



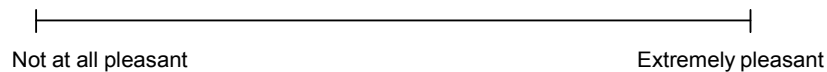
**How strong is your desire to eat?**



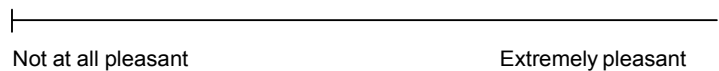
**How much food do you think you can eat?**



**How pleasant was the taste of the breakfast?**

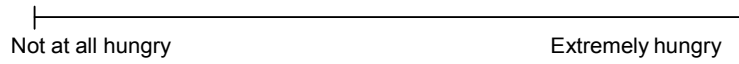


**How pleasant was the appearance of the breakfast?**

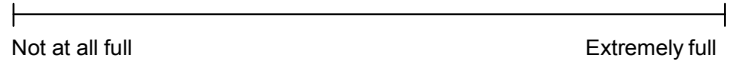


0:30

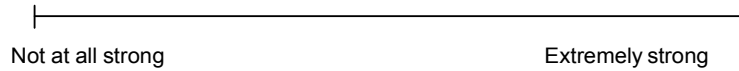
**How hungry do you feel?**



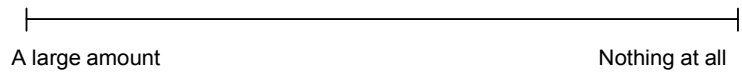
**How full do you feel?**



**How strong is your desire to eat?**

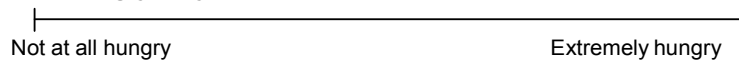


**How much food do you think you can eat?**

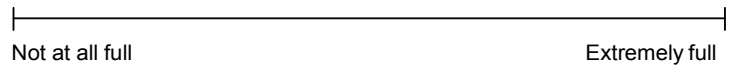


1:00

**How hungry do you feel?**



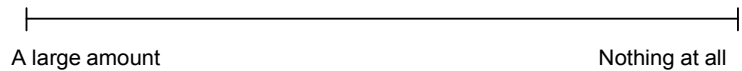
**How full do you feel?**



**How strong is your desire to eat?**

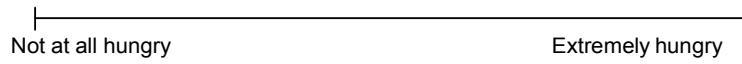


**How much food do you think you can eat?**

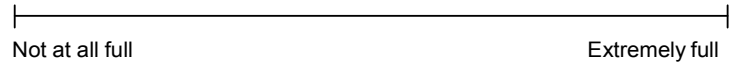


1:30

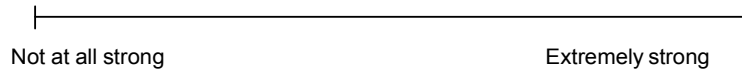
**How hungry do you feel?**



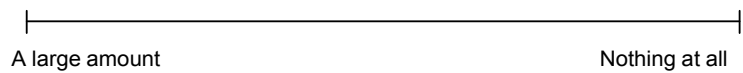
**How full do you feel?**



**How strong is your desire to eat?**

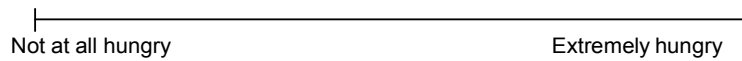


**How much food do you think you can eat?**

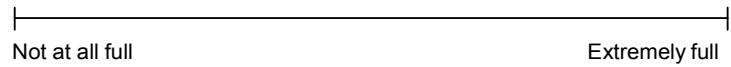


2:00

**How hungry do you feel?**



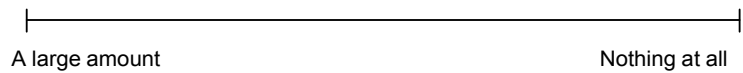
**How full do you feel?**



**How strong is your desire to eat?**



**How much food do you think you can eat?**

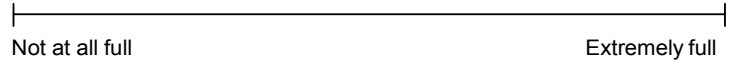


2:30

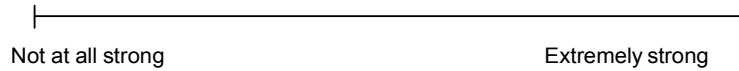
**How hungry do you feel?**



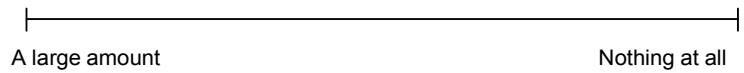
**How full do you feel?**



**How strong is your desire to eat?**

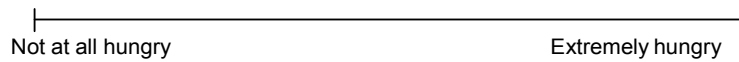


**How much food do you think you can eat?**

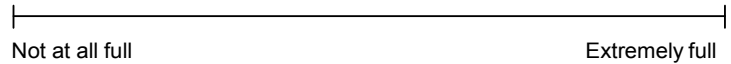


3:00

**How hungry do you feel?**



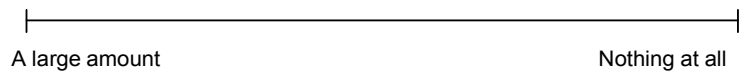
**How full do you feel?**



**How strong is your desire to eat?**



**How much food do you think you can eat?**





Project title: The effect of a functional food based breakfast on blood glucose, insulin, satiety, mood and cognitive performance in adolescents.

Dear Head teacher,

As part of our continued research into the benefits of breakfast in adolescents, Oxford Brookes University are running an exciting project investigating the effects of a breakfast enriched with functional food ingredients on health outcomes. Below is an outline of the research project and what your school's involvement would be if you decide to sign up.

### **Background**

There is a large amount of scientific research that looks at the associations between functional foods and their role in protecting against chronic diseases, including cancer, cardiovascular diseases and type 2 diabetes. A functional food gives health benefits in addition to the vitamins and minerals it contains. Popular functional foods include those rich in antioxidants (e.g. blueberries) and high in dietary fibre (e.g. oats). Research suggests that these foods help stabilise blood glucose levels and improve the efficiency of insulin to control glucose levels in the blood. They are also reported to increase feelings of fullness (satiety), improve mood and improve cognitive performance.

This research study aims to investigate the effect of a breakfast muffin enriched with functional food ingredients on the above factors when compared with a control breakfast muffin (without the functional ingredients).

### **The study protocol**

The study will start in the morning, before school, and will continue over three and a half hours on two separate days. During each session students would spend around one and a half hours having measures collected by the researchers (see participant information sheet - PIS). As this would interrupt lesson time we propose that the study runs parallel to the timetabled lessons in a separate 'study classroom'. This way, students who choose not to take part can go to their lesson as normal. Alternatively, the school may wish to incorporate the study as part of a timetabled lesson (for e.g. food technology) in which case students who have provided parental consent would attend the lesson and arrangements would need to be made for non-participants to complete set work in an alternative lesson.

In-between the measures we are collecting students would have around two hours per session to complete other work. This may be work provided by the teacher, alternatively (or additionally) the researchers would deliver themed workshops covering areas such as (i) careers (life at university/career profiling), (ii) mentoring (study skills in science, math, and nutrition) and (iii) health and nutrition (food groups, metabolism etc). We would work closely with teaching staff to ensure that workshops are tailored to complement the student's learning and career choice.

### **How would the school be involved?**

We would ask that the school supports the study in three ways:

- (i) The study has the sanction of the school as well permission of absence for students (where necessary) from timetabled lesson(s) until morning break
- (ii) Provide a space where researchers can screen students and run the study
- (iii) Recruitment of students to the study

### **Space**

We would require exclusive use of a space (i.e. a classroom) large enough to accommodate 30 people with desk space on two mornings. If screens were available we would use these to divide the space into three areas for each experimental group. We would also require three additional tables that we can use for

blood measures. A dedicated clean area for the collection of blood will be sectioned off by curtains brought by the researchers. In addition, we would require a room where we could carry out health screenings of potential participants prior to their recruitment to the study. This would take place in the morning before school and last around 20 minutes. The number of days this was needed would depend on the number of interested students.

### **Recruitment**

We would be hoping to recruit around 20 adolescents from the same year group (year 9, year 10 or year 11). We would ask the school to distribute the participant information sheets to students and to email parents a parent information sheet, child health questionnaire and consent form (see attached). Please note that the study would only run with a minimum of nine participants.

### **What is the process for students that want to take part?**

Participation is voluntary and it will be up to the children and parents to decide if they want to take part. Parents will need to provide written consent and return a child health questionnaire. If they choose to take part they are still free to withdraw at any time and without giving a reason. Their decision on whether to take part, or not take part or to withdraw at any time will have no impact on grades, assessments or future studies.

Interested students must meet the inclusion criteria (as set out in the PIS), attend a pre-study health screening and provide parental consent.

### **What are the benefits of taking part?**

This research will help the development of future breakfast based interventions to help young people improve their health and wellbeing. Group data will be presented back to the school once the study is completed. We would also be happy to discuss the potential for future workshops or seminars that you think could be beneficial.

The researchers would work closely with the teachers and develop workshops that encouraged the participants to think objectively about the scientific process and stimulate ideas for future careers. Participants would be provided with a summary of their body composition (on request) as well as a £20 high street voucher on completion of all measures.

### **Are there any possible disadvantages or risks of taking part?**

Each measurement requires a few drops of blood from a small finger-prick. Some participants may experience a light bruising but this will not affect student's ability to

work. All researchers collecting blood samples are fully trained and follow International Standard Procedures for protocol and hygiene.

The research is not evaluating mental health or disordered eating behaviours however, we acknowledge on the information sheets that some students might be experiencing concerns in this area and have signposted some guidance for this. Therefore, it would be prudent for us to meet with the school counsellor to discuss the study, in the event they receive an increase in numbers of students requesting to meet with them (see attached letter).

To minimise disruption to the student's normal school day we will ask participants to begin the study before school (see PIS). For example, a start time of 7.30am would allow us to have almost half of measures collected (42 minutes worth) before 8.30am.

### **Who is organising and funding the research?**

This research is being conducted as part of a PhD research project and is funded by the Faculty of Health and Life Sciences at Oxford Brookes University.

If you are interested in taking part or require any further information please contact us:

Ms Sarah Kennedy  
PhD Researcher  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)  
Tel: 01865 483283

Dr Miriam Clegg  
Senior Lecturer in Nutrition  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)  
Tel: 01865 484365

This research has been approved by the University Research Ethics Committee, Oxford Brookes University. If you have any concerns about the way in which the study has been conducted, please contact [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet.

## Parent/Guardian Information sheet

Project title: The effect of a functional food based breakfast on blood glucose, insulin, satiety (fullness), mood and cognitive performance in adolescents.

Dear Parent(s)/Guardian(s),

Your child's school is supporting a new research project investigating the effect of a breakfast enriched with 'functional food' ingredients on measures of blood glucose and insulin response, satiety, mood and cognitive performance. Below is an outline of the research project and what your child's involvement would be should they decide to take part.

### **What is the purpose of the study?**

There is a large amount of scientific research that looks at the associations between functional foods and their role in protecting against chronic diseases including cancer, cardiovascular diseases and type 2 diabetes. A functional food gives health benefits in addition to the vitamins and minerals it contains. Popular functional foods include those rich in antioxidants (e.g. blueberries) and high in dietary fibre (e.g. oats). Research suggests that these foods help stabilise blood glucose levels and improve insulin efficiency (a hormone which controls glucose levels in the blood). These foods are also reported to increase feelings of fullness (satiety), improve mood and improve cognitive performance (including memory).

This research study aims to investigate the effect of a breakfast muffin enriched with functional food ingredients on the above factors when compared with a control breakfast muffin (without the functional ingredients).

### **Why has my child been invited to participate?**

Your child's school has agreed to support this research study. Everyone in year 9 has been invited to participate; however, it may be that your child is unable to take part in the research due to a pre-existing health condition. If your child meets **ALL** of the criteria below then they would be eligible to take part.

- ✓ Aged 13-17 years
- ✓ No known diabetes or impaired glucose tolerance
- ✓ No medical conditions or medications known to affect glucose, appetite or digestion
- ✓ No major medical or surgical event requiring hospitalisation within the preceding three months
- ✓ Fasting blood glucose <6.1 mmol/l (we will check this for them during screening)
- ✓ No allergies or intolerances to the breakfast ingredients (milk, eggs, chocolate, wheat, gluten)
- ✓ No diagnosed learning disorder
- ✓ Fluent in English language

### **Does my child have to take part?**

It is up to you and your child to decide whether or not they want to take part in the research. If your child does decide to take part they are free to withdraw at any time and without giving a reason. Their decision to participate or not participate in the

study is completely voluntary and will have no impact on their school marks, assessments or any future studies.

### **When will the research study run?**

On two Wednesday mornings (one per week) as an alternative to your child's food technology lesson. In-between test measures we will run fun, interactive workshops that will cover different themes (for example: careers, life at university, study skills and health and nutrition). We will work closely with your child's teachers to ensure that the workshops are tailored to complement their learning and career choice. Additionally, your child's teacher may provide work for them from the food technology lesson.

### **How long will the study last?**

Your child will be required to attend three separate sessions, a pre-study screening and two testing sessions. They will spend three and a half hours with the researchers on each of the two test days. During this time, for one and a half hours they will have measures collected (see attached protocol). The remaining two hours will be spent participating in workshop activities or completing school work. To minimise disruption to your child's normal school day we would begin the study before school starts (see coloured time chart below). Please note that the study can only run if the minimum number of participants required are recruited.

### **What are the benefits of taking part?**

- Your child will gain an insight into their personal lifestyle habits
- If your child fully completes all measures they will receive a £20 voucher redeemable at many high street stores. <http://www.highstreetvouchers.com/gift-vouchers/redeemers/love2shop-gift-cards-retailers>

### **Are there any possible disadvantages or risks of taking part?**

- Each measurement requires a few drops of blood from a small finger-prick. A light bruising may occur in some people but this will disappear within a couple of days and will not affect your child's ability to work. Trained researchers and a registered nurse will collect blood samples following International Standard Procedures for protocol and hygiene.
- During screening, in the unlikely event that your child's fasting blood glucose values are outside of the expected range the researchers will send a letter to you recommending you visit your GP to discuss this further. Please note, a reading outside of the range does not necessarily indicate a problem.
- This research is comparing differences between breakfasts so provides no diagnostic information on your child's performance on the memory test. Additionally, we are not evaluating mental health or disordered eating behaviours. However, we acknowledge that some children may be experiencing concerns in these areas or might be worried about someone close to them. Your child has received an information sheet, similar to this, on which we have stressed that if they feel uneasy completing the mood questionnaire then they are free to stop at any time. We have also provided websites and helpline numbers of dedicated charities who offer support to young people. Alternatively, they can discuss any issues with the school counsellor who will also be able to provide them with support and guidance.

<http://www.youngminds.org.uk>

<http://www.getconnected.org.uk> : Tel 0808 808 4994 or text 80849

<http://www.b-eat.co.uk> : Tel 0845 634 7650 or text 07786 20 18 20

### **Will what my child says in this study be kept confidential?**

- All information collected will be owned by Oxford Brookes University and will be kept strictly confidential (subject to legal limitations and apart from notifying you of a high blood glucose value). During screening your child will be allocated a private code which the researchers will use for all data collection, handling, storage as well for presentation and publication of research material. Only researchers working on this study will have passwords to access the data. Data generated by the study must be retained for a period of ten years in accordance with the University's policy on Academic Integrity.
- As participation would result in absence from usual lessons, and due to the small sample size of each group, it will not be possible for your child to be anonymous to other participants however, all sensitive measures (i.e. height, weight, blood samples) will be collected in private.

### **My child would like to take part**

- Please sign the consent form and health questionnaire and place them in a sealed envelope. Your child also needs to sign the consent form. Your child can bring the sealed envelope into school and drop it into the sealed box provided. Alternatively, you can post or email the forms to us.
- Contact the researchers using the details below to register your interest

### **What will happen to the results of the study?**

They will be included in the write up of a PhD thesis and may form the basis of future breakfast based interventions to help young people to improve their health and wellbeing. We may also present the group data back to the school however we will ensure that your child is not identifiable from this.

If you require any further information please contact us:

Ms Sarah Kennedy  
PhD Researcher  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)  
Tel: 01865 483283

Dr Miriam Clegg  
Senior Lecturer in Nutrition  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)  
Tel: 01865 484365

This research has been approved by the University Research Ethics Committee, Oxford Brookes University. If you have any concerns about the way in which the study has been conducted, please contact [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet.

**STUDY PROTOCOL** - The effect of a functional food breakfast on blood glucose, insulin, satiety (fullness), mood and memory in adolescents. (example as per your child's information sheet)

You need to meet **ALL** of the inclusion criteria below to be eligible to take part.

- ✓ Aged 13-17 years
- ✓ No known diabetes or impaired glucose tolerance
- ✓ No medical conditions or medications known to affect glucose, appetite or digestion
- ✓ No major medical or surgical event requiring hospitalisation within the preceding three months
- ✓ Fasting blood glucose <6.1 mmol/l (we will check this for you during screening)
- ✓ No allergies or intolerances to the breakfast ingredients (milk, eggs, chocolate, wheat, gluten)
- ✓ No diagnosed learning disorder
- ✓ Fluent in English language

What happens at the first visit?

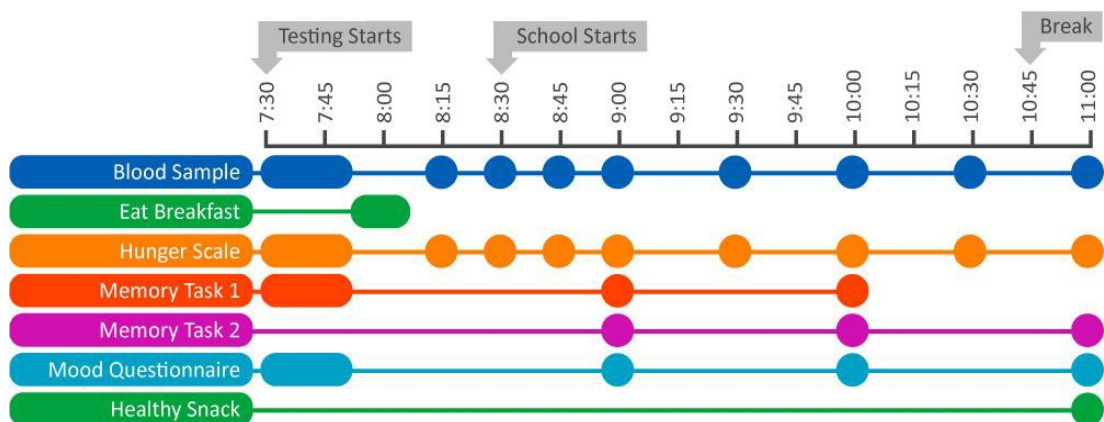
This session is a private screening. It will take place in the morning before school starts and the following measures will be taken:

- Height and weight
- Resting metabolic rate (see picture 1)
- Fasting blood glucose levels (see picture 2)
- We will provide an accelerometer which you will wear on your upper arm for four days (this estimates your physical activity levels). On these days we will also ask you to keep a food diary.

What happens on a testing day? (2 sessions)

On each testing day we will take the following measures at the timings shown in the time chart below:

- Finger prick blood samples
- Hunger ratings (a scale which you mark to show how hungry you are)
- Mood questionnaire (a list of questions to determine how you are feeling at that moment)
- Memory tests (a task in which you recall the location of words on a map and a list of words)



Time chart: Study schedule for session 2 and 3



### How do I prepare for the study?

- You need to fast overnight from 9pm – this means **no food or drink**, although you can drink water
- On the day before a test:
  - ✓ limit your caffeine intake (maximum of 2-3 cups of coffee/tea, no caffeine in the evening)
  - ✓ no alcohol intake
  - ✓ restrict participating in unusually intense physical activity (e.g. long runs)
  - ✓ consume the same dinner the night before each test
  - ✓ do not smoke on the morning of the test

## **EXPLANATION OF STUDY MEASURES**

### Resting metabolic rate (RMR)

Resting metabolic rate is an estimate of the total amount of energy (calories) used by your body to sustain vital functions (organs, metabolism etc) when you are at rest. Depending on how physically active you are, this represents around 60-70% of the total energy you expend. This is calculated by measuring the amount of oxygen you inhale and the amount of carbon dioxide you exhale.

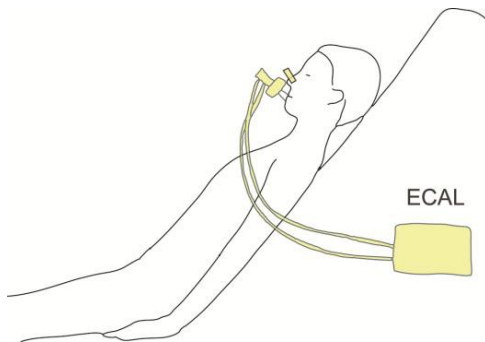
### When will we measure RMR?

- First thing in the morning (before any food or drink is consumed)
- During the screening session

### How RMR is measured?

- Firstly you will rest for 5 minutes, during this time you can practice using the mouthpiece
- We will then ask you to put on the nose clip
- The machine will be connected and we will collect your data for around 10 minutes

There are no known risks associated with measuring RMR. We maintain hygiene at all times by sterilising mouthpieces between participants and cleaning the nose clip using a sterile wipe.

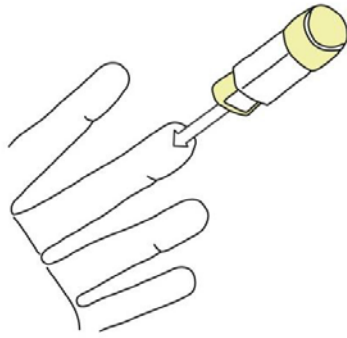


Picture 1. ECAL machine to collect resting metabolic rate



## Blood glucose measures

We measure blood glucose by using a 'one click' lancet to make a small finger prick. We then collect a drop of blood onto a cuvette and analyse this using a blood glucose monitor (picture 3). On testing days will take a few extra drops of blood (from the same finger prick) so we can also measure the levels of insulin in your blood.



*Picture 2. Finger prick using a 'one click' lancet monitoring machine*



*Picture 3: Cuvette and blood glucose*

Consent Form

The effect of a functional food based breakfast on blood glucose, insulin, satiety (fullness), mood and cognitive performance in adolescents.

Ms Sarah Kennedy, PhD Research Student  
 Dr Miriam Clegg, Senior Lecturer in Nutrition  
 Functional Food Centre, Oxford Brookes University, Gipsy Lane Campus, Oxford OX3  
 0BP  
 Tel: 01865 483283  
 Email: [sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk) / [mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)

Parent(s)/Guardian(s) to **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	<input type="checkbox"/>	<input type="checkbox"/>
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	<input type="checkbox"/>	<input type="checkbox"/>
3. I understand that my child's participation is voluntary and that they are free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	<input type="checkbox"/>	<input type="checkbox"/>
4. I understand that confidentiality of information provided can only be protected within the limits of the law and due to the small sample size of this study there may be implications for maintaining full anonymity of my child's participation in the study.	<input type="checkbox"/>	<input type="checkbox"/>
5. I agree for my child to take part in the above research.	<input type="checkbox"/>	<input type="checkbox"/>

Name of child: ..... Date .....

Name of parent/guardian:..... Date .....

Address:.....  
 .....

Contact tel no: .....

Email address: .....

Signature of parent .....

Signature of child .....

Participant Information Sheet

Project title: The effect of a functional food breakfast on blood glucose, insulin, satiety (fullness), mood and memory in adolescents.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it involves. Please take your time to read the following information and discuss it with your parent(s) or guardian(s).

**What is the purpose of the study?**

A functional food gives health benefits in addition to the vitamins and minerals it contains. Popular functional foods include those rich in antioxidants (e.g. blueberries) and high in dietary fibre (e.g. oats). Research suggests that functional foods can:

- Help stabilise the body's response to glucose (the energy released from eating carbohydrates).
- Improve the efficiency of insulin (a hormone which controls glucose levels in the blood).
- Keep you feeling fuller for longer
- Improve mood and cognitive performance (including memory).

This research study aims to investigate the effect of a breakfast muffin enriched with functional food ingredients on the above factors when compared with a control breakfast muffin (without the functional ingredients).

**Why have I been invited to take part? Do I have to take part?**

Everyone in year 9 has been invited to participate however, it may be that you are unable to take part due to a pre-existing health condition. If you meet **ALL** of the criteria set out in the study protocol (see attached) then you are eligible to take part.

It is up to you, and your parent(s)/guardian(s) to decide whether or not to take part in the research. If you do decide to take part you are free to withdraw at any time and without giving a reason. Your decision to participate, or not participate, in the study is completely voluntary and will have no impact on your school marks, assessments or any future studies.

**When will the research study run?**

On two Wednesday mornings (one per week) as an alternative to your food technology lesson. In-between test measures we will run fun, interactive workshops that will cover different themes (for example: careers, life at university, study skills and health and nutrition). We will work closely with your teachers to ensure that the workshops are tailored to complement your learning and career choice. Additionally, your teacher will provide work for you from the food technology lesson.

**How long will the study last?**

You will be required to attend three separate sessions, a pre-study screening and two testing sessions. You will spend three and a half hours with the researchers on each of the two test sessions. During this time, for one and a half hours you will have measures collected (see attached protocol). The remaining two hours will be spent participating in workshop activities or completing school work.

### **What are the benefits of taking part?**

- You will gain an insight into your personal lifestyle habits
- If you complete all measures you will receive a £20 voucher redeemable at many high street stores. <http://www.highstreetvouchers.com/gift-vouchers/redeemers/love2shop-gift-cards-etailers>

### **Are there any possible disadvantages or risks of taking part?**

- Each blood sample measurement requires a few drops of blood from a small finger-prick. A light bruising may occur but this will disappear within a couple of days and will not affect your ability to work. Trained researchers and a registered nurse will collect blood following International Standard Procedures for protocol and hygiene. It is not always possible to get all the blood we require from one finger prick. On these rare occasions we may ask to prick your finger again.
- During screening, in the unlikely event your fasting blood glucose values are outside of the expected range we will send a letter to your parent/guardian recommending a visit to your GP to discuss this further. Please note, a reading outside of the range does not necessarily indicate a problem.
- This research is comparing differences between breakfasts so provides no diagnostic information on your performance on the memory test. Additionally, we are not evaluating mental health or disordered eating behaviours. However, if you are experiencing concerns in these areas or you are worried about someone close to you, then you may feel uneasy completing the mood questionnaire and may want to stop, which you are free to do at any time. Dedicated charities offer support to young people and we have provided some websites and helpline numbers below. Alternatively, you can speak in confidence to the school counsellor who will also be able to provide you with support and guidance.

<http://www.youngminds.org.uk>

<http://www.getconnected.org.uk> : Tel 0808 808 4994 or text 80849

<http://www.b-eat.co.uk> : Tel 0845 634 7650 or text 07786 201820

### **Will what I say in this study be kept confidential?**

- All information collected will be owned by Oxford Brookes University and will be kept strictly confidential (subject to legal limitations and apart from notifying your parents of a high fasting blood glucose value). During screening you will be allocated a private code which the researchers will use for all data collection, handling, storage as well for presentation and publication of research material. Only researchers working on this study will have passwords to access the data. Data generated by the study must be retained for a period of ten years in accordance with the University's policy on Academic Integrity.
- As participation would result in absence from usual lessons, and due to the small sample size of each group, it will not be possible for you to be anonymous to other participants however, all sensitive measures (i.e. height, weight, blood samples) will be collected in private.

### **What will happen to the results of the study?**

They will be included in the write up of a PhD thesis and may form the basis of future breakfast based interventions to help young people to improve their health and

wellbeing. We may also present the group data back to the school however we will ensure that you are not identifiable from this.

**Yes! I would like to take part**

- ✓ Ask your parent/guardian to sign the consent form and health questionnaire and then place it in a sealed envelope. You also need to sign this. Bring the envelope to school and drop it into the sealed box, alternatively you can post it or email it to us.
- ✓ Contact the researchers using the details below to register your interest.

Ms Sarah Kennedy  
PhD Researcher  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[sarah.kennedy-2013a@brookes.ac.uk](mailto:sarah.kennedy-2013a@brookes.ac.uk)  
Tel: 01865 483283

Dr Miriam Clegg  
Senior Lecturer in Nutrition  
Functional Food Centre  
Oxford Brookes University,  
Gipsy Lane,  
Oxford, OX3 0BP  
[mclegg@brookes.ac.uk](mailto:mclegg@brookes.ac.uk)  
Tel: 01865 484365

This research has been approved by the University Research Ethics Committee, Oxford Brookes University. If you have any concerns about the way in which the study has been conducted, please contact [ethics@brookes.ac.uk](mailto:ethics@brookes.ac.uk).

Thank you for taking the time to read this information sheet.

**STUDY PROTOCOL:** The effect of a functional food breakfast on blood glucose, insulin, satiety (fullness), mood and memory in adolescents.

You need to meet **ALL** of the inclusion criteria below to be eligible to take part.

- ✓ Aged 13-17 years
- ✓ No known diabetes or impaired glucose tolerance
- ✓ No medical conditions or medications known to affect glucose, appetite or digestion
- ✓ No major medical or surgical event requiring hospitalisation within the preceding three months
- ✓ Fasting blood glucose <6.1 mmol/l (we will check this for you during screening)
- ✓ No allergies or intolerances to the breakfast ingredients (milk, eggs, chocolate, wheat, gluten)
- ✓ No diagnosed learning disorder
- ✓ Fluent in English language

What happens at the first visit?

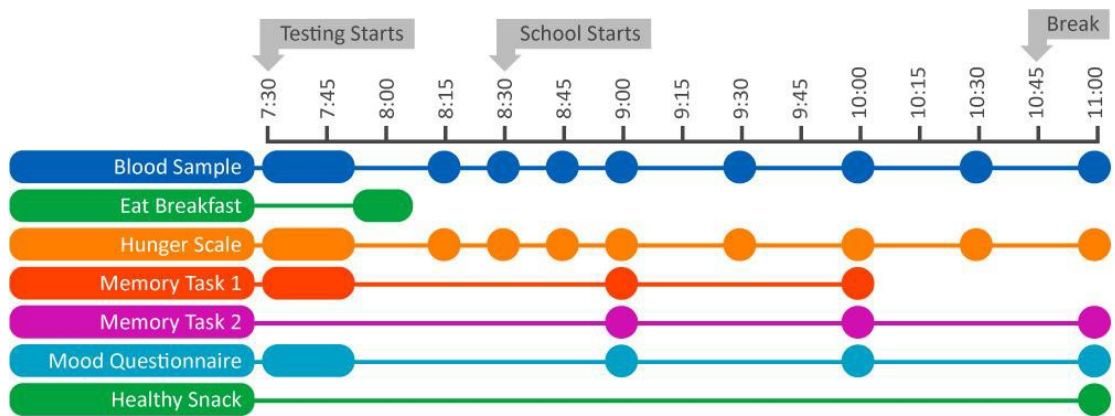
This session is a private screening. It will take place in the morning before school starts and the following measures will be taken:

- Height and weight
- Resting metabolic rate (*see picture 1*)
- Fasting blood glucose levels (*see picture 2*)
- We will provide an accelerometer which you will wear on your upper arm for four days (this estimates your physical activity levels). On these days we will also ask you to keep a food diary.

What happens on a testing day? (2 sessions)

On each testing day we will take the following measures at the timings shown in the time chart below:

- Finger prick blood samples
- Hunger ratings (a scale which you mark to show how hungry you are)
- Mood questionnaire (a list of questions to determine how you are feeling at that moment)
- Memory tests (a task in which you recall the location of words on a map and a list of words)



Time chart: Study schedule for session 2 and 3

### How do I prepare for the study?

- You need to fast overnight from 9pm – this means **no food or drink**, although you can drink water
- On the day before a test:
  - ✓ limit your caffeine intake (maximum of 2-3 cups of coffee/tea, no caffeine in the evening)
  - ✓ no alcohol intake
  - ✓ restrict participating in unusually intense physical activity (e.g. long runs)
  - ✓ consume the same dinner the night before each test
  - ✓ do not smoke on the morning of the test

## **EXPLANATION OF STUDY MEASURES**

### Resting metabolic rate (RMR)

Resting metabolic rate is an estimate of the total amount of energy (calories) used by your body to sustain vital functions (organs, metabolism etc) when you are at rest. Depending on how physically active you are, this represents around 60-70% of the total energy you expend. This is calculated by measuring the amount of oxygen you inhale and the amount of carbon dioxide you exhale.

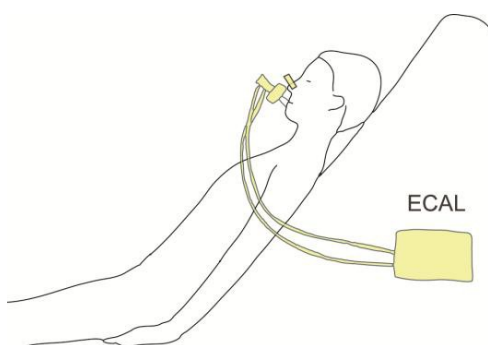
### When will we measure RMR?

- First thing in the morning (before any food or drink is consumed)
- During the screening session

### How RMR is measured?

- Firstly you will rest for 5 minutes, during this time you can practice using the mouthpiece
- We will then ask you to put on the nose clip
- The machine will be connected and we will collect your data for around 10 minutes

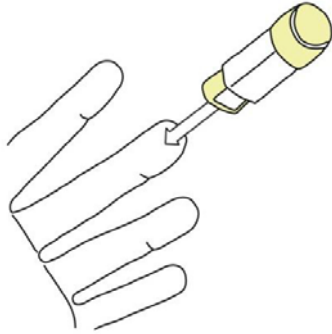
There are no known risks associated with measuring RMR. We maintain hygiene at all times by sterilising mouthpieces between participants and cleaning the nose clip using a sterile wipe.



Picture 1. ECAL machine to collect resting metabolic rate

## Blood glucose measures

We measure blood glucose by using a 'one click' lancet to make a small finger prick. We then collect a drop of blood onto a cuvette and analyse this using a blood glucose monitor (picture 3). On testing days will take a few extra drops of blood (from the same finger prick) so we can also measure the levels of insulin in your blood.



Picture 2. Finger prick using a 'one click' lancet monitoring machine



Picture 3: Cuvette and blood glucose



### Child Health Questionnaire

All of the information provided here will be kept strictly confidential and will be available only to the researchers. This form must be completed by the parent or guardian.

(Please circle as appropriate)

- Is your child allergic to any foods? Yes    No  
If yes, which food(s)? .....  
.....
  
- Does your child have any intolerance to foods? Yes    No  
If yes, which food(s)? .....  
.....
  
- Does your child have any medical conditions that might affect glucose regulation or appetite? Yes    No  
If yes, provide detail .....  
.....
  
- Has your child had any surgery requiring hospitalisation within the last 3 months? Yes    No  
If yes, provide detail .....  
.....
  
- Is your child taking any medication which might affect mood, glucose regulation or appetite? Yes    No  
If yes, provide detail .....  
.....
  
- Does your child have a diagnosed learning disorder? Yes    No  
If yes, provide detail .....  
.....



## Appendix 4f Mood questionnaire

Each of the words below describes feelings or mood. Please use the rating scale next to each word to describe your feelings at this moment. For e.g. If you definitely do not feel active at the moment then you would **tick the box** in column 1 against the word 'active'. Work rapidly, but please mark all the words. Your first reaction is best.

0:00 - baseline					
How do you feel at the moment?	1 Definitely do not feel	2 Feel slightly	3 Unsure	4 Feel moderately	5 Definitely feel
active	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
sleepy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
energetic	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
at-rest	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
anxious	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
laid back	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
fatigued	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
tense	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
alert	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
nervous	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
calm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
lively	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
worried	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
wide-awake	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
tired	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
drowsy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
exhausted	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
restful	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
fearful	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
quiet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1:00

<b>How do you feel at the moment?</b>	1 <b>Definitely do not feel</b>	2 <b>Feel slightly</b>	3 <b>Unsure</b>	4 <b>Feel moderately</b>	5 <b>Definitely feel</b>
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2:00

How do you feel at the moment?	1 Definitely do not feel	2 Feel slightly	3 Unsure	4 Feel moderately	5 Definitely feel
active	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
sleepy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
energetic	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
at-rest	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
anxious	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
laid back	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
fatigued	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
tense	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
alert	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
nervous	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
calm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
lively	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
worried	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
wide-awake	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
tired	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
drowsy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
exhausted	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
restful	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
fearful	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
quiet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3:00

<b>How do you feel at the moment?</b>	1 <b>Definitely do not feel</b>	2 <b>Feel slightly</b>	3 <b>Unsure</b>	4 <b>Feel moderately</b>	5 <b>Definitely feel</b>
<b>active</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>sleepy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>energetic</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>at-rest</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>anxious</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>laid back</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fatigued</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tense</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>alert</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>nervous</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>calm</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>lively</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>worried</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>wide-awake</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>tired</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>drowsy</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>exhausted</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>restful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>fearful</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>quiet</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>