Exploring the Role of Short-Chain Fatty Acids and Resistant Starch on Energy Balance, Appetite Control and Glycaemic Regulation

A thesis submitted for the degree of Doctor of Philosophy, Imperial College London

Jennifer E. Pugh

Nutrition Research Section

Department of Medicine

Imperial College London

2024

Copyright Declaration

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non-Commercial 4.0 International License (CC BY-NC). Under this license, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose. When reusing or sharing this work, ensure you make the license terms clear to others by naming the license and linking to the license text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes. Please seek permission from the copyright holder for uses of this work that are not included in this license or permitted under UK Copyright Law.

Abstract

Background

Obesity, a major public health issue, places individuals at increased risk of metabolic diseases, like type 2 diabetes (T2D). Increased intake of dietary fibre and non-digestible carbohydrates (NDCs) have been associated with improved energy balance and metabolic outcomes, such as fasting glucose, potentially mitigating chronic disease development. This thesis is formed of two parts:

Part 1

Short-chain fatty acids (SCFAs) are produced via bacterial fermentation of fibre in the colon. Evidence suggests that SCFAs may play a role in obesity and chronic disease prevention (**Chapter 1**). To address a gap in the literature, a systematic review and meta-analysis investigating the effects of SCFA on glycaemia in humans was conducted in **Chapter 2**. **Chapter 3** describes a clinical trial investigating the effects of inulin-propionate ester (IPE), a vehicle for colonic SCFA delivery, on weight gain prevention in younger adults at risk of weight gain. **Chapter 4** outlines the mechanistic sub-study of this trial, investigating the effects of IPE on appetite hormones, energy intake, and energy expenditure. **Chapter 5** discusses the findings of Part 1.

Part 2

Resistant starch (RS), a type of NDC that remains largely undigested in the upper gastrointestinal tract, has been associated with improved glycaemic response. **Chapter 6** outlines how increasing RS intake may modulate glycaemic responses. **Chapter 7** presents a systematic review and meta-analysis of the effects of RS types on glycaemia in individuals with T2D and pre-diabetes. **Chapter 8** investigates the effects of high-RS meals on glucose control in individuals with T2D. **Chapter 9** discusses the work presented in Part 2.

Summary

This thesis highlights the need for further research into the effects of NDCs and SCFAs in energy homeostasis and metabolic response. It also emphasises the importance of fibre intake for preventing weight gain and the management of chronic diseases, like T2D.

Declaration of Contributions

The majority of the work in this thesis was performed by the author. All collaboration and assistance are detailed below.

Chapter 2

The systematic review and meta-analysis was co-authored with Dr Anna Cherta-Murillo, with the assistance of Sumayya Alshehhi, Dana Hajjar and Professor Gary Frost.

Chapter 3

The study was conducted with assistance from Dr Katerina Petropoulou, Dr Martina Tashkova, Sumayya Alshehhi, Dr George Thom, Louise McCombie, Professor Douglas Morrison and Professor Gary Frost. Statistical analysis was primarily conducted by Joana C. Vasconcelos and A. Toby Prevost, the clinical trial statisticians.

Chapter 4

The study was conducted in collaboration with Dr Katerina Petropoulou, Dr Martina Tashkova, Sumayya Alshehhi, Dr George Thom, Louise McCombie, Professor Douglas Morrison and Professor Gary Frost. Gut hormone assays were conducted with assistance from Dr Katerina Petropoulou, Sumayya Alshehhi, Dr Mingzhu Cai and Professor Kevin Murphy. Glucose and insulin assays were conducted with assistance from Dr Katerina Petropoulou, Sumayya Alshehhi, Wenan Zhang and Kathy Li. Statistical analysis was primarily conducted by Joana C. Vasconcelos and A. Toby Prevost, the clinical trial statisticians.

Chapter 7

The systematic review and meta-analysis was co-authored with Dr Mingzhu Cai and Nunzia Altieri, with the oversight from Professor Gary Frost.

Chapter 8

This study was conducted with assistance from Dr Martina Tashkova, Nunzia Altieri, Valentine Deremiens and Kathy Lee. Sample analysis was conducted with assistance from David Xi and Yucen Wu.

Research studies

All the clinical study visits were performed with the help of the research nurses and administration staff at the NIHR Imperial Clinical Research Facility.

Acknowledgements

I would like to thank to my primary supervisor, Professor Gary Frost, for granting me the fantastic opportunity of working on a large clinical trial as part of my PhD. This experience has been invaluable and I have learned so much from it. I am also very grateful to my supervisor, Dr Edward Chambers, for his sound guidance, honesty and sense of humour over the past four years.

A special thank you to Dr Katerina Petropoulou, who mentored me from day one. Her support has been pivotal in my development as a researcher. Likewise, I am thankful to Dr Martina Tashkova for her help in conducting studies and caring for participants. I would also like to thank Drs Aygul, Aaron, Hannah, Marianna and Isabel Garcia-Perez for their words of wisdom and encouragement throughout.

Additionally, I would like to thank the staff at the Clinical Research Facility and Imperial Clinical Trials Unit, the participants who volunteered in the studies, the members of the Nutrition Research Section who have helped me along the way and the Master's students I have had the pleasure of supervising. Without their contributions, none of this work would have been possible and I am immensely grateful.

I would also like to thank my family and friends, who have celebrated my achievements, listened to my frustrations and endured many stool sample related stories.

Finally, Mam and Dad, thanks for making me eat my Five-a-Day, instilling a love of fibre in me from the beginning.

Publications, Conference Presentations and Talks

First Author Publications

- **Pugh, J. E**., Anjum, A., Petropoulou, K., Thom, G., Mccombie, L., Tashkova, M., Alaraj-Alshehhi, S., Babalis, D., Prechtl, C., Lean, M. J., Prevost, A. T., Vasconcelos, J. C., Preston, T., Morrison, D., & Frost, G. (2022). Increase in colonic PRopionate as a method of prEVENTing weight gain in adults aged 20–40 years (iPREVENT): Protocol of a multi-centre, double-blind, randomised, parallel-group trial to investigate the efficacy of inulin-propionate ester versus inulin (control) in the prevention of weight gain over 12 months (No. 11:1157). F1000Research. https://doi.org/10.12688/f1000research.125950.1
- **Pugh, J. E**., Cai, M., Altieri, N., & Frost, G. (2023). A comparison of the effects of resistant starch types on glycemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis. Frontiers in Nutrition, 10. https://doi.org/10.3389/fnut.2023.1118229
- **Pugh, J. E**., Petropoulou, K., Vasconcelos, J. C., Anjum, A., Thom, G., McCombie, L., Tashkova, M., Alshehhi, S., Babalis, D., Holroyd, L., Sadiq, B. A., Prechtl, C., Preston, T., Chambers, E., Lean, M. J., Dhillo, W., Prevost, A. T., Morrison, D., & Frost, G. (2024). Increase in colonic PRopionate as a method of prEVENTing weight gain over 12 months in adults aged 20–40 years (iPREVENT): A multi-centre, double-blind, randomised, parallel-group trial. eClinicalMedicine, 76. https://doi.org/10.1016/j.eclinm.2024.102844

Other Publications

- Cai, M., Dou, B., **Pugh, J. E**., Lett, A. M., & Frost, G. S. (2021). The impact of starchy food structure on postprandial glycemic response and appetite: A systematic review with meta-analysis of randomized crossover trials. The American Journal of Clinical Nutrition, 114(2), 472–487. https://doi.org/10.1093/ajcn/nqab098
- Cherta-Murillo, A., **Pugh, J. E**., Alaraj-Alshehhi, S., Hajjar, D., Chambers, E. S., & Frost, G. S. (2022). The effects of SCFAs on glycemic control in humans: A systematic review and meta-analysis. The American Journal of Clinical Nutrition, 116(2), 335–361. https://doi.org/10.1093/ajcn/nqac085
- Bobokhidze, E., Weech, M., Petropoulou, K., Wilson, T., **Pugh, J**., Fallaize, R., Garcia-Perez, I., Lo, F. P.-W., Solis, A. R., Vickar, J., Giannarou, S., Mylonas, G., Lo, B., Lloyd, A. J., Koulman, A., Beckmann, M., Draper, J., Frost, G., & Lovegrove, J. A.

(2024). Standardised and Objective Dietary Intake Assessment Tool (SODIAT): Protocol of a dual-site dietary intervention study to integrate dietary assessment methods (No. 13:1144). F1000Research. https://doi.org/10.12688/f1000research.155683.1

Conference presentations

- **Dietary Manipulations for Health and in the Prevention and Management of Disease** (Manchester Metropolitan University, UK) March 2024: Oral Communication
- **International Congress on Obesity** (Sao Paolo, Brazil), June 2024: Oral Communication

Talks

- The Reality of Endocrinology, Society for Endocrinology conference 2023: Running Clinical Studies in Human Nutrition

Abbreviations

AP	Area postrema
ARC	Arcuate nucleus
(i)AUC	(incremental) Area under the curve
BMI	Body mass index
BMR	Basal metabolic rate
BOLD	Blood oxygen level–dependent
CGM	Continuous glucose monitor
CNS	Central nervous system
CVD	Cardiovascular disease
DMV	Dorsal motor nucleus of vagus
EEC	Enteroendocrine cells
FFA	Free fatty acids
FFAR	Free fatty acid receptors
FMI	Fat mass index
GPCR	G protein-coupled receptors
GI	Glycaemic index
GIP	Gastric inhibitory peptide
GIPR	Gastric inhibitory peptide receptor
GL	Glycaemic load
GLP-1	Glucagon-like peptide 1
GLUT	Glucose transporter
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein
HOMA-%B	Homeostatic model assessment of β-cell function
HOMA-%S	Homeostatic model assessment of insulin sensitivity

HOMA-IR	Homeostatic model assessment of insulin resistance
IGN	Intestinal gluconeogenesis
IPE	Inulin-propionate ester
KO	Knock-out
LDL	Low-density lipoprotein
MET	Metabolic equivalent of tasks
α-MSH	α-Melanocyte-stimulating hormone
NDC	Non-digestible carbohydrate
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute of Health and Care Excellence
NPY	Neuropeptide Y
NSP	Non-starch polysaccharide
NTS	Nucleus of the solitary tract
POMC	Pro-opiomelanocortin
PPI	Patient and public involvement
PYY	Peptide YY
RCT	Randomised controlled trials
QUICKI	Quantitative insulin sensitivity check index
RDS	Rapidly digestible starch
RS	Resistant starch
SACN	Scientific Advisory Committee on Nutrition
SAP	Statistical analysis plan
SCFA	Short-chain fatty acid
SDS	Slowly digestible starch
SGLT2	Sodium-glucose co-transporter-2
T2D	Type 2 diabetes

VAS	Visual analogue scales
VLCD	Very low-calorie diets
WHO	World Health Organisation

Contents

Copyright Declaration 2
Abstract 3
Declaration of Contributions 4
Acknowledgements 5
Publications, Conference Presentations and Talks 6
First Author Publications 6
Other Publications 6
Conference presentations 7
Talks 7
Abbreviations 8
List of Tables 20
List of Figures 21
Part 1: The Role of Short-Chain Fatty Acids in Regulating Energy Balance, and Glucose Metabolism to Prevent Obesity and Chronic Disease 25
Chapter 1: Introduction 26
1.1 Overweight and obesity 27
1.1.1 Defining Obesity 27
1.1.2 The prevalence and burden of overweight and obesity 29
1.1.3 The Aetiology of Obesity 30
1.1.4 Evaluation of Current Obesity Treatments 30
1.1.5 The importance and current methods of weight gain prevention 31
1.2 Physiological Mechanisms Regulating Appetite and Energy Expenditure 33
1.2.1 Appetite Regulation in the central nervous system 33
1.2.2 Appetite Regulating Hormones 34
1.2.3 Appetite signalling within the gastrointestinal tract 35
1.2.4 Regulating Energy Expenditure 36

1.3 Dietary fibre 37
1.3.1 Defining dietary fibre 37
1.3.2 Dietary Fibre appetite regulation, energy intake and body weight 38
1.3.3 Dietary Fibre and markers of chronic disease risk 40
1.3.4 Dietary Fibre, the microbiota and fermentation in the colon 40
1.4 Short-chain Fatty Acids 42
1.4.1 Characteristics and microbial production of SCFA 42
1.4.2 SCFA Metabolism 44
1.4.3 SCFA Receptors 45
1.4.4 SCFA, appetite and energy intake 45
1.4.5 SCFA, energy expenditure and fat oxidation 47
1.4.6 SCFA and glucose homeostasis 48
1.4.7 SCFA and body weight 49
1.5 Rationale and general aims 50
1.5.1 Hypothesis 50
1.5.2 Thesis Aims 50
2 Chapter 2: The effect of short-chain fatty acids on glycaemic control in humans: Systematic Review and Meta-analysis 51
2.1 Summary of key findings 52
2.2 Introduction 53
2.3 Purpose of the Study 55
2.3.1 Hypothesis 55
2.3.2 Aims 55
2.3.3 Outcomes 55
2.4 Methods 56
2.4.1 Registration 56
2.4.2 Criteria 56
2.4.3 Search Strategy and Search Terms 57

2.4.4 Study selection 57
2.4.5 Data extraction 57
2.4.6 Data processing and analysis 58
2.4.7 Confounders of glycaemic control 58
2.4.8 Risk of Bias Assessment 59
2.4.9 Certainty of Evidence 59
2.5 Results 60
2.5.1 Study Search 60
2.5.2 Summary of identified studies 61
2.5.3 Study and study participant characteristics 61
2.5.4 Risk of Bias Assessment 72
2.5.1 The effect of acute SCFA interventions on glycaemic response 74
2.5.2 The effect of chronic SCFA interventions on glycaemic response 81
2.5.3 Certainty of evidence 86
2.5.4 Confounders of glycaemic control 86
2.5.5 Adverse events 87
2.5.6 Compliance and withdrawals 87
2.6 Discussion 89
2.6.1 Summarised discussion 89
2.6.2 Acute SCFAs and glycaemic response 89
2.6.3 Chronic SCFA and glycaemic response 93
2.6.4 Limitations 96
2.7 Summary 97
3 Chapter 3: The effect of elevated colonic propionate delivery on weight gain prevention in adults aged 20 to 40 98
3.1 Summary of key findings 99
3.2 Introduction 100
3.3 Purpose of the Study 102

3.3.1 Hypothesis 102
3.3.2 Aims 102
3.3.3 Outcomes 102
3.4 Methods 103
3.4.1 Study design 103
3.4.2 Ethical approval 103
3.4.3 Participants 103
3.4.4 Study visits 104
3.4.5 Data collection methods 105
3.4.6 Interventions 106
3.4.7 Safety reporting 109
3.4.8 Statistical analysis 109
3.5 Results 112
3.5.1 Recruitment and baseline characteristics 112
3.5.2 Primary Outcome Results 114
3.5.3 Secondary outcome results 115
3.5.4 Changes in lifestyle factors 117
3.5.5 Compliance and withdrawals 118
3.5.6 AEs and SAEs 120
3.6 Discussion 122
3.6.1 Summarised Discussion 122
3.6.2 Withdrawals, compliance and safety 122
3.6.3 Primary Outcome 122
3.6.4 Secondary Outcomes 123
3.6.5 Comparison with Prior Evidence 124
3.6.6 Limitations 124
3.7 Summary 126

4 Chapter 4: The effect of enhanced colonic propionate flux on weight gain prevention in adults aged 20 to 40 years: An exploratory mechanistic sub-study. 127
4.1 Summary of key findings 128
4.2 Introduction 129
4.3 Purpose of the Study 132
4.3.1 Hypothesis 132
4.3.2 Aims 132
4.3.3 Outcomes 132
4.4 Methods 133
4.4.1 Study design 133
4.4.2 Ethical approval 133
4.4.3 Participants 133
4.4.4 Study visits 134
4.4.5 Study interventions and measurements 135
4.4.6 Sample Processing and Analysis 140
4.4.7 Safety reporting 140
4.4.8 Statistical analysis 141
4.4.9 Sample Size Calculation 143
4.5 Results 144
4.5.1 Recruitment and Baseline Characteristics 144
4.5.1 Plasma GLP-1 and PYY 148
4.5.2 Subjective appetite scores 150
4.5.3 Ad-libitum Energy intake 152
4.5.4 Energy expenditure and substrate oxidation 153
4.5.5 Plasma glucose and serum insulin 155
4.5.6 Physical activity 157
4.5.7 Breath Hydrogen 158
4.6 Discussion 159

4.6.1 Summarised Discussion 159
4.6.2 Withdrawals 159
4.6.3 Primary Outcome 159
4.6.4 Secondary Outcomes 160
4.6.5 Allocation of study product 162
4.6.6 Limitations 162
4.7 Summary 164
5 Chapter 5: General Discussion 165
5.1 Summary of findings 166
5.2 Introduction 167
5.3 Detailed Discussion 168
5.4 Future work 172
5.5 Conclusions 173
6 Part 2: The Effect of Resistant Starch on Glycaemic Control in Individuals with Type 2 Diabetes 174
Chapter 6: Introduction 175
6.1 Type 2 Diabetes 176
6.1.1 Defining T2D 176
6.1.2 The Prevalence and burden of T2D 176
6.1.3 Evaluation of T2D management strategies 177
6.1.4 Dietary interventions for the management of T2D 177
6.1.5 Glycaemic index and T2D management 178
6.2 Physiological Mechanisms of Glucose Homeostasis 180
6.2.1 Glucose Regulating Hormones 180
6.2.2 Glucose transport 182
6.2.3 Organs involved in glucose homeostasis 182
6.2.4 Glucose dysregulation 183
6.3 Dietary carbohydrates, resistant starch and glycaemic control 184

6.3.1 Dietary Carbohydrates 184
6.3.2 Starch structure and digestion 186
6.3.3 Resistant Starch 187
6.3.4 Resistant starch as a fermentable carbohydrate 189
6.3.5 Resistant starch and glycaemic control 190
6.4 Rationale and general aims 194
6.4.1 Thesis Hypothesis 194
6.4.2 Thesis Aims 194
7 Chapter 7: The effects of resistant starch types on glycaemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis. 195
7.1 Summary of key findings 196
7.2 Introduction 197
7.3 Purpose of the Study 199
7.3.1 Hypothesis 199
7.3.2 Aims 199
7.3.3 Outcomes 199
7.4 Methods 200
7.4.1 Registration 200
7.4.2 Criteria 200
7.4.3 Search Strategy and Search Terms 200
7.4.4 Study selection 200
7.4.5 Data extraction 201
7.4.6 Data processing and analysis 201
7.4.7 Risk of Bias Assessment 201
7.5 Results 203
7.5.1 Study Search 203
7.5.2 Summary of identified studies 203
7.5.3 Study and study participant characteristics 204

7.5.4 Risk of Bias Assessment 211
7.5.5 The effect of acute RS interventions on glycaemic response 212
7.5.6 The effect of chronic RS interventions on glycaemic response 215
7.6 Discussion 223
7.6.1 Summarised discussion 223
7.6.2 Acute RS and glycaemic response 224
7.6.3 Chronic RS and glycaemic response 226
7.6.4 Limitations 228
7.6.5 Summary 230
8 Chapter 8: The effect of an 8-hour intervention of high RS and intact food structures on glycaemic response in individuals with type 2 diabetes 231
8.1 Summary of key findings 232
8.2 Introduction 233
8.3 Purpose of the Study 236
8.3.1 Hypothesis 236
8.3.2 Aims 236
8.3.3 Outcomes 236
8.4 Methods 237
8.4.1 Study design 237
8.4.2 Ethical approval 237
8.4.3 Participants 237
8.4.4 Study visits 238
8.4.5 Study interventions and measurements 241
8.4.6 Sample Processing and Analysis 247
8.4.7 Safety reporting 248
8.4.8 Statistical analysis 249
8.4.9 Sample Size Calculation 249
8.5 Results 250

8.5.1 Insights from the patient and public involvement panels 250
8.5.2 Recruitment and Baseline Characteristics 253
8.5.3 Interstitial fluid glucose and serum insulin responses 257
8.5.4 Subjective appetite scores 260
8.5.5 Breath Hydrogen 262
8.5.6 Meal acceptability 263
8.5.7 263
8.5.8 Gastrointestinal effects 264
8.5.9 Exploratory Analyses 265
8.6 PPI Discussion 266
8.7 Main Discussion 267
8.7.1 Summarised Discussion 267
8.7.2 Primary Outcome 267
8.7.3 Secondary Outcomes 270
8.7.4 Individual variability in response 271
8.7.5 Limitations 272
8.8 Summary 274
9 Chapter 9: General Discussion 275
9.1.1 Summary of findings 276
9.2 Introduction 277
9.3 Discussion 278
9.4 Future work 280
9.5 Conclusions 281
10 References 282
11 Appendices 372
Appendix 1: Database search strategy for systematic review (Chapter 2) 372
Appendix 2: Database search strategy for systematic review (Chapter 5) 374
Appendix 3: Meal preparation standard operating procedure for Chapter 8 study. ... 377

List of Tables

Table 1.1: Weight categories with BMI thresholds 29
Table 1.2 Mean concentrations of SCFA in the colon, portal vein, liver and peripheral circulation.....44
Table 2.1: Description of study participants characteristics, interventions and outcomes..61
Table 3.1: Baseline socio-demographic and clinical characteristics by arm.....112
Table 3.2: Clinical and Principal weight at baseline and the 12-month visit.....115
Table 3.3: Body composition and fasting biochemistry at baseline and the 12-month visit 116
Table 3.4: Changes in physical activity levels from Baseline to the 12-month visit.....117
Table 3.5: Changes in lifestyle factors from baseline to the 12-month visit 118
Table 3.6: Changes in Compliance at each study visit.....119
Table 3.7: Participant withdrawal rates at each study visit.....120
Table 3.8: Summary of adverse events by arm 120
Table 3.9: Serious adverse events 121
Table 4.1: Demographic characteristics by arm and timepoint 145
Table 4.2: Baseline characteristics by arm and timepoint.....146
Table 4.3: Medians (IQR) of the incremental iAUC of GLP-1 and PYY 148
Table 4.4: Medians (IQR) of the iAUC for Subjective appetite scores 150
Table 4.5: Intake (g) of ad-libitum meal (meal weight before – meal weight after).....152
Table 4.6: Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for energy expenditure and substrate oxidation 153
Table 4.7: Medians (IQR) of the iAUC of plasma glucose and serum insulin.....155
Table 4.8: Means (SD) of the different physical activity measurements 157
Table 7.1: Description of study participants characteristics, interventions and outcomes 204
Table 8.1: Macronutrient composition of meals for men 242
Table 8.2: Macronutrient composition of meals for women.....243
Table 8.3: PPI answers to questions relating to study themes and study methodology...251
Table 8.4: PPI comments on specific foods that may improve glycaemic response 252
Table 8.5: Demographic characteristics of participants.....254
Table 8.6: Baseline characteristics by visit 256

Table 8.7: Sequence of meals selected by participants separated into responders and non-responders.....258
Table 8.8: Student's t-tests to determine significant differences in glycaemic characteristics between responders and non-responders 265

List of Figures

Figure 2.1: A flow diagram of the study selection process adapted from the PRISMA 2020 statement. 61
Figure 2.2: Risk of bias assessment for included publications. 73
Figure 2.3: Forest plot of effect sizes for studies assessing the effect of acute acetate administration on postprandial glucose concentrations in healthy individuals. 74
Figure 2.4: Forest plot of effect sizes for studies assessing the effect of acute vinegar intake on postprandial glucose concentrations in healthy individuals. 75
Figure 2.5: Forest plot of effect sizes for studies assessing the effect of acute vinegar intake on postprandial glucose concentrations in individuals with prediabetes or T2D. 76
Figure 2.6: Forest plot of effect sizes for studies assessing the effect of acute propionate administration on postprandial glucose concentrations in healthy individuals. 77
Figure 2.7: Forest plot of effect sizes for studies assessing the effect of acute acetate administration on postprandial insulin concentrations. 78
Figure 2.8: Forest plot of effect sizes for studies assessing the effect of acute vinegar consumption on postprandial insulin concentrations in healthy individuals. 79
Figure 2.9: Forest plot of effect sizes for studies assessing the effect of acute vinegar consumption on postprandial insulin concentrations in individuals with prediabetes or T2D. 79
Figure 2.10: Forest plot of effect sizes for studies assessing the effect of acute propionate administration on postprandial insulin concentration. 80
Figure 2.11: Forest plot of effect sizes for studies assessing the effect of chronic vinegar supplementation administration on fasting blood glucose. 81
Figure 2.12: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on postprandial blood glucose. 82
Figure 2.13: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on fasting blood glucose. 82
Figure 2.14: Forest plot of effect sizes for studies assessing the effect of chronic vinegar supplementation administration on fasting insulin. 83

Figure 2.15: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on postprandial insulin response. 83
Figure 2.16: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on fasting insulin. 84
Figure 3.1: Study flow chart for iPREVENT 108
Figure 3.2: CONSORT Study Flow Diagram....113
Figure 3.3: Principal weight of IPE and inulin participants at each visit timepoint 114
Figure 4.1: Study flow chart for iPREVENT 138
Figure 4.2: Study meals for iPREVENT 139
Figure 4.3: CONSORT Study Flow Diagram....147
Figure 4.4: Gut hormone concentrations following intake of inulin or IPE at baseline and follow-up visit 149
Figure 4.5: Subjective appetite scores following intake of inulin or IPE at baseline and follow-up visit....151
Figure 4.6: *Ad-libitum* energy intake (kilocalories) following intake of inulin or IPE at baseline and follow-up 152
Figure 4.7: Energy expenditure, fat and carbohydrate oxidation following intake of inulin or IPE at baseline and 12 months visit 154
Figure 4.8: Glucose and insulin concentrations following intake of inulin or IPE at baseline and 12 months visit 156
Figure 4.9: Breath hydrogen concentrations following intake of inulin or IPE at baseline and 12 months visit 158
Figure 7.1: A flow diagram of the study selection process adapted from the PRISMA 2020 statement. 203
Figure 7.2: Risk of bias assessment for included publications 212
Figure 7.3: Forest plot of studies assessing the effect of acute RS intake on postprandial glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 213
Figure 7.4: Forest plot of studies assessing the effect of acute RS intake on postprandial glucose concentrations with subgroups for T2D versus prediabetes. 214
Figure 7.5: Forest plot of studies assessing the effect of acute RS intake on postprandial insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 215
Figure 7.6: Forest plot of studies assessing the effect of acute RS intake on postprandial insulin concentrations with subgroups for T2D versus prediabetes. 215

Figure 7.7: Forest plot of studies assessing the effect of chronic RS intake on postprandial glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 216
Figure 7.8: Forest plot of studies assessing the effect of chronic RS intake on fasting blood glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 217
Figure 7.9: Forest plot of studies assessing the effect of chronic RS intake on fasting blood glucose concentrations with subgroups for T2D versus prediabetes. 217
Figure 7.10: Forest plot of studies assessing the effect of chronic RS intake on postprandial insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 218
Figure 7.11: Forest plot of studies assessing the effect of chronic RS intake on fasting insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type. 219
Figure 7.12: Forest plot of studies assessing the effect of chronic RS intake on postprandial insulin concentrations with subgroups for T2D versus prediabetes. 219
Figure 7.13: Forest plot of studies assessing the effect of acute and chronic RS intake on GLP-1 concentrations with subgroups for length of study intervention. 220
Figure 7.14: Forest plot of studies assessing the effect of acute and chronic RS intake on GIP concentrations with subgroups for length of study intervention. 221
Figure 7.15: Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of insulin resistance (HOMA-IR). 222
Figure 7.16: Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of β-cell function (HOMA-%B). 222
Figure 7.17: Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of insulin sensitivity (HOMA-%S). 222
Figure 7.18: Forest plot of studies assessing the effect of chronic RS intake on glycated haemoglobin (HbA1c). 222
Figure 8.1: Study flow chart for this study 246
Figure 8.2: Study visit schedule for the type 2 diabetes food study 246
Figure 8.3: Ethnicity of patient and public involvement panel participants 250
Figure 8.4: Recruitment flow diagram 254
Figure 8.5: Interstitial fluid glucose and serum insulin concentrations following the consumption of the intervention and control meals 257

Figure 8.6: Subjective appetite scores following the consumption of the intervention and control meals 260
Figure 8.7: Breath hydrogen following the consumption of the intervention and control meals 262
Figure 8.8: Meal acceptability of Treatment Meals A and B 263
Figure 8.9: Gastrointestinal effects following the consumption of the intervention and control meals 264

Part 1: The Role of Short-Chain Fatty Acids in Regulating Energy Balance, and Glucose Metabolism to Prevent Obesity and Chronic Disease

Chapter 1: Introduction

1.1 Overweight and obesity

Despite growing public understanding of the importance of maintaining a healthy weight, obesity rates continue to rise. The following section defines obesity and its challenge to the public health system and evaluates current treatment methods.

1.1.1 **Defining Obesity**

Obesity is the accumulation of adiposity to the extent that it causes a detriment to health (World Health Organization, 2024b). Obesity is a chronic disease which increases the risk of numerous comorbidities including cardiovascular disease, type 2 diabetes (T2D) and cancer (Heymsfield & Wadden, 2017). Previously, an individual with obesity was defined as someone with a body mass index (BMI) of > 30. BMI is calculated by dividing weight in kilograms by height squared (m^2) and is the most common method of defining obesity. It is inexpensive, easy to calculate and does not require specialised equipment. **Table 1.1** outlines weight classifications according to BMI.

However, when using BMI, there is no way of distinguishing body fat from other tissues, such as muscle mass (Etchison et al., 2011). BMI alone should not be used to diagnose obesity and other assessments like skinfold thickness, waist circumference, fat mass index (FMI = fat mass [kg] / weight^2 [m^2]) and body fat percentage should be measured. Nonetheless, body fat percentage, FMI and central adiposity are highly correlated with BMI (Jeong et al., 2023). Furthermore, BMI is associated several cardiometabolic risk factors including insulin sensitivity and c-reactive protein (Lichtash et al., 2013).

Recently published guidance suggests that the use of BMI alone to diagnose obesity should solely be used at population level, during epidemiological studies and for health screening (Rubino et al., 2025).

The report also suggests that individuals with excess adiposity may remain free from illness for many years or potentially, indefinitely. Experts emphasise that this approach leads to ‘over-diagnosis’ of individuals with excess adiposity and the automatic assumption that obesity is a disease. Obesity, when considered a disease, is characterised by alterations in tissue or organ function, or an inability to perform essential daily tasks (Rubino et al., 2025). The recommendation is to categorise obesity as sub-clinical and clinical obesity. Once obesity is identified using BMI alongside another measure of adiposity, such as waist circumference or waist-hip ratio, the individual can be classified as having sub-clinical obesity. They should then be referred for further tests to assess whether any related illness is present. If such an illness is diagnosed, the individual will be identified to have clinical

obesity and can receive appropriate treatment and care on a case-by-case basis. This report aims to reduce stigma surrounding obesity and recognising that the excess deposition of adiposity *per se* is not a disease. However, some concerns have been raised. For example, new diagnostic criteria may complicate decision making for medical professionals and may delay disease diagnosis (EASO Secretariat, 2025).

Thresholds for BMI were developed within mostly Western populations and therefore are not always applicable to other ethnicities (Bodicoat et al., 2014). The National Institute of Health and Care Excellence (NICE) guidance from this year states that Asian, Chinese, Middle Eastern, Black African and African-Caribbean populations are at increased risk of metabolic disease and therefore the obese BMI threshold should be <27.5 kg/m^2 (Caleyachetty et al., 2021). This is to ensure action can be taken to treat obesity prior to the development of metabolic comorbidities such as cardiovascular disease (CVD) or T2D.

Table 1.1: Weight categories with BMI thresholds

Category	BMI range (kg/m^2)
Underweight	< 18.5
Healthy	18.5 – 24.9
Overweight	25 – 29.9
Obese (class1)	30 – 34.9
Obese (class 2)	35 – 39.9
Obese (class 3)	≥ 40

1.1.2 The prevalence and burden of overweight and obesity

As of 2020, 42% of the global population were overweight or obese and it is forecasted that half the world's population will fall into these categories by 2030 (World Obesity Federation, 2024). In the UK, obesity rates virtually doubled in men and women between 1997 to 2019 (Moody, 2020). The industrialisation and economic development of countries in the Global South has resulted in the emergence of the 'double burden' of malnutrition and obesity (Popkin et al., 2020). This phenomenon arises as fresh, nutrient-dense produce becomes increasingly expensive and scarce meaning cheaper, energy-dense and highly processed diets are adopted (Popkin et al., 2020). As rates of obesity continue to rise, so do associated comorbidities such as CVD, T2D, osteoarthritis and some cancers (Guh et al., 2009; Pantalone et al., 2017). The development of these non-communicable diseases is strongly associated with increased adiposity, with 19% of CVD, 42% of T2D and 17% of stroke deaths attributable to a BMI of over 25 kg/m^2 (World Obesity Federation, 2024). Concerns regarding the health risks associated with obesity escalated during the COVID-19 pandemic, as data indicated that individuals with obesity had higher rates of severe illness and mortality than their healthy weight counterparts (M. Gao et al., 2021).

A collaborative analysis of 900,000 adults indicated that class 1 and class 3 obesity reduced life expectancy by 2 to 4 years and 8 to 10 years, respectively (Prospective Studies Collaboration, 2009). Individuals with obesity are not only dying sooner; they have longer periods of ill-health (Chong et al., 2023). This harms quality of life; a recent population-based cohort study reported that individuals with obesity scored 4.9 to 11.3 percentage points lower on a standardised quality of life scale than those with a healthy weight

(Stephenson et al., 2021). Individuals with a higher BMI, especially women, are also more likely to suffer from depression and anxiety than those with BMI ≤ 25 kg/m^2 (K. M. Scott et al., 2008). Further to the burden of obesity upon the individual, there is a large economic and social cost. The annual healthcare cost of obesity within the NHS is estimated to be £19 billion, mostly driven by the cost of treatments for comorbidities (Frontier Economics, 2023). The total cost to the UK economy was £98 billion (Frontier Economics, 2023).

1.1.3 The Aetiology of Obesity

The risk factors for obesity are complex and many environmental and genetic influences drive weight gain. Over the past 60 years, there has been a dramatic rise in obesity rates, associated with a consistent positive energy balance. A positive energy balance is when energy input outweighs energy output, driven by high energy intakes or low physical activity levels (Hill et al., 2012). Since human physiology has not changed over the last century, environmental changes have driven the obesity epidemic. Advancements in processing methods and ingredients have enabled the production of hyper-palatable, energy dense foods that promote overconsumption (Hall et al., 2019). Analyses of the National Health and Nutrition Examination Survey (NHANES) in the US detected an average increase in daily energy intake of 240 kcal from 1970 to 2010 (Ford & Dietz, 2013). Evidence indicates that a consistent excess of as little as 50 to 100 kcal per day drives body weight gain (Hill et al., 2003; Zhai et al., 2008). In addition, the reduction in manual labour from domestic tasks with the advent of household appliances, alongside the transition towards sedentary desk jobs has lowered overall energy expenditure (Church et al., 2011; Ng & Popkin, 2012). Despite extensive homeostatic mechanisms to regulate energy balance, the current environment, often referred to as “obesogenic” is driving the increased incidence of obesity and metabolic disease worldwide. To combat the effects this chronic positive energy balance, population-level interventions need to be implemented urgently to slow rates of obesity development.

1.1.4 Evaluation of Current Obesity Treatments

Obesity requires ongoing management. Although weight loss is achievable, many find it challenging, with one in 210 men and one in 124 women returning to a healthy weight after gaining fat mass (Fildes et al., 2015). Consequently, healthcare professionals use the loss of 5-10% of body weight reduction as a clinically significant and achievable target (Tak & Lee, 2021). Primary approaches to weight loss are bariatric surgery, pharmacotherapy and lifestyle modification. Recent advancements in pharmacological treatments, particularly glucagon-like peptide-1 (GLP-1) agonists have had promising outcomes. A systematic review assessing GLP-1 agonist use in individuals with T2D reported weight loss of 8 to 14

kg (Yao et al., 2024). However, these treatments may cause side effects like gastrointestinal symptoms or hypoglycaemia and are still inaccessible to many (Moll et al., 2024). Orlistat, a lipase inhibitor, also improves weight loss outcomes (Davidson et al., 2019). However, Orlistat may alter stool frequency or consistency and lower nutrient absorption (Nicolai et al., 2018).

Lifestyle interventions or behavioural therapy to increase physical activity and lower energy intake remain the most common approaches for obesity treatment at a population level. However, the success of these interventions is limited with one study reporting 18.8% of control group, 38.2% of remote support group, and 41.4% of in-person support group members lost ≥ 5% body weight over two years (Appel et al., 2011). High-intensity interventions, such as very low-calorie diets (VLCD), have proven effective, at times leading to remission of comorbidities like T2D. However VLCD are not intended to be sustained for long periods and the weight is regained (Lean et al., 2024).

Bariatric surgery is the most intensive option. Yielding losses of 60% of excess weight 12 months post-surgery (Chang et al., 2014), improved glycaemic control (Sandoval & Patti, 2023) and alterations in anorectic gut hormone secretion (Dimitriadis et al., 2017). However, these surgical interventions are costly and carry risks, including post-surgery complications. further, treatment is currently limited to individuals with a BMI > 40 kg/m^2 or a BMI 35 – 40 kg/m^2 with one or more obesity-related conditions (Kassir et al., 2016).

Despite the current potential of weight loss interventions, weight regain remains common. Strategies focusing on obesity prevention and weight management may be more effective, however this area lacks sufficient research.

1.1.5 **The importance and current methods of weight gain prevention**

Weight gain prevention is considered a more sustainable and effective approach to lowering chronic disease risk compared with weight loss treatments (World Health Organization, 2011). Weight gain prevention strategies could also assist in preventing weight regain in individuals who have lost weight, as 50% of individuals who underwent a lifestyle modification weight loss intervention reportedly regained the weight within one year (Blomain et al., 2013). Young adulthood is a period of rapid weight gain but also a time where lifestyle and dietary habits are formed that may determine health outcomes in later life (Nelson et al., 2008; Reas et al., 2007). Individuals who gained weight in young adulthood had worsened fasting glucose, systolic and diastolic blood pressure, total cholesterol, low density lipoprotein (LDL) -cholesterol, high-density lipoprotein (HDL) -

cholesterol and triglycerides (Truesdale et al., 2006). Furthermore, Weight management in early adulthood is associated with a reduced risk of CVD development (Ohlsson et al., 2016).

Current strategies for preventing weight gain focus on controlling energy intake and adhering to healthy eating and physical activity guidelines. A systematic review reported that 25% of lifestyle interventions effectively maintained weight for over two years (mean difference in weight change -0.5 to -11.5 kg) (T. Brown et al., 2009). However, the limited evidence in this research area makes it difficult to design guidelines or interventions for weight gain prevention (T. Gill et al., 2005).

As rates of obesity continue to rise, current prevention methods appear inadequate. Broader, population-level nutrition strategies are needed to reduce appetite, limit energy intake and enhance energy expenditure to achieve long-term energy balance.

1.2 Physiological Mechanisms Regulating Appetite and Energy Expenditure

Energy balance refers to the relationship between energy intake from food and energy output from resting energy expenditure, thermogenesis, the thermic effect of food and physical activity (Hill et al., 2012). The body strives to maintain a neutral energy balance to stabilise body weight, preserve energy stores and ensure nutrient availability for physiological functions and survival.

The regulation of energy balance is complex, involving neural and hormonal signals to modulate energy requirements and energy intake. However, additional factors including food availability, hedonic preferences and social or cultural influences also impact energy intake (De Silva & Bloom, 2012). Modern food environments frequently disrupt these regulatory mechanisms and lead to elevated food intake and lower physical activity contributing to a positive energy balance and elevated adiposity (Hall, 2018). This section examines the physiological mechanisms involved in appetite regulation and energy balance to highlight potential therapeutic targets.

1.2.1 **Appetite Regulation in the central nervous system**

The hypothalamus and the caudal brain stem are central in modulating appetite and energy expenditure. They integrate mechanical (e.g. gastric distension), nutrient, hormonal and adipose changes with signals from the vagus nerve, spinal cord and circulation (Morton et al., 2006).

Within the hypothalamus, the arcuate nucleus (ARC) and ventromedial nucleus are central to appetite regulation (Morton et al., 2006). The ARC is located near the median eminence which has a permeable blood brain barrier enabling the detection of circulating nutrient and hormone (e.g. glucose, insulin, leptin) concentrations (Cone et al., 2001). The ARC neurons produce the neuropeptides cocaine and amphetamine regulated transcript (CART), pro-opiomelanocortin (POMC) and subsequently α-Melanocyte-stimulating hormone (α-MSH) to signal satiety, suppress energy intake and enhance energy expenditure (Broberger et al., 1998). The ARC also has neurons producing agouti-related peptide and neuropeptide Y (NPY), neuropeptides responsible for stimulating feeding and lowering energy expenditure (Hahn et al., 1998).

In the brainstem the dorsal vagal complex, comprising of the nucleus of the solitary tract (NTS), dorsal motor nucleus of vagus (DMV) and the area postrema (AP), plays a significant role in appetite regulation. The AP and NTS receive signals from the gastrointestinal tract via the vagus nerve along with hormonal or nutrient signals via the circulation (Yu & Kim,

2012). These inputs trigger the release of NPY and POMC from the NTS, relaying information to the hypothalamus for further neuropeptide release. The bidirectionality of signalling between the hypothalamus and the brainstem ensures precise and continuous regulation of energy homeostasis.

1.2.2 **Appetite Regulating Hormones**

Current identified anorexigenic gut hormones include GLP-1, peptide YY (PYY), pancreatic polypeptide, gastric inhibitory peptide (GIP), oxyntomodulin and cholecystokinin (K. G. Murphy & Bloom, 2006). Peripheral signals like insulin, produced by pancreatic β-cells and leptin from adipocytes also facilitate satiety signalling. At present, ghrelin, released from the stomach in the absence of food intake, is the only known hormone to stimulate feelings of hunger and increase food intake (K. G. Murphy & Bloom, 2006). This subsection will define and explore the effects of GLP-1 and PYY, the two satiety hormones explored in this section of the thesis.

GLP-1 is a 30-amino acid protein synthesised by enteroendocrine L-cells in the small intestine and the colon, as well as by pancreatic α-cells and cells in the hypothalamus, pituitary, and brainstem. GLP-1 is released in response to lipids, peptides and glucose in the intestinal lumen (Campbell & Drucker, 2013; Chaudhri et al., 2006; Marchetti et al., 2012; van der Klaauw et al., 2013). GLP-1 enters the circulation via the hepatic portal vein, targeting sites of metabolic and appetite regulation, indicated by the presence of GLP-1 receptors (GLP1R) in the hypothalamus, NTS, pancreatic β-cells, stomach, myenteric plexus neurons, small intestine, colon, lung, kidney and heart (P. J. Larsen et al., 1997; Pyke et al., 2014; Turton et al., 1996). GLP-1 infusions lower energy intake in both animals and humans, via GLP1R activation in the hypothalamus and brainstem (Flint et al., 1998; Navarro et al., 1996; Tang-Christensen et al., 1996; Turton et al., 1996). Antagonism of the GLP1R induces hyperphagia and weight gain in rodents (Barrera et al., 2011). GLP-1 slows gastric emptying and acts as an incretin, binding to GLP1R and stimulating the release of insulin from pancreatic β-cells (Baggio & Drucker, 2007; Drucker, 2006).

PYY, a 36-amino acid peptide is released in response to energy intake and is also produced by enteroendocrine L-cells in the distal small intestine and colon (El-Salhy et al., 1983; Lundberg et al., 1982). Initially secreted as PYY_{1-36}, it is cleaved by dipeptidyl peptidase-4 (DPP-4) and its more bioactive form, PYY_{3-36} found in higher concentrations in the circulation. PYY secretion is positively associated with meal calorie content. PYY (Pedersen-Bjergaard et al., 1996; Zwirska-Korczala et al., 2007). PYY concentrations peak

60-120 minutes post-meal dependent on energy and macronutrient composition (Pedersen-Bjergaard et al., 1996; Zwirska-Korczala et al., 2007). PYY binds to Y-receptors in the brainstem and hypothalamus and infusions have lowered energy intake by 33% over 24 hours in rodents, effects which may be mediated by NPY Y2 receptor located on NPY neurons (Batterham et al., 2002). Combined PYY and GLP-1 administration in humans significantly reduced energy intake and brain activity similar to what is seen after a meal (De Silva et al., 2011). However, as PYY administration alone does not induce the same reduction in energy expenditure, co-secretion of GLP-1 and PYY appears necessary for a strong satiety signalling (Petersen & Moran, 2013; Sloth et al., 2007).

1.2.3 **Appetite signalling within the gastrointestinal tract**

Food intake initiates signalling throughout the gastrointestinal tract, beginning with nutrient sensing in the oral phase. Taste receptors on the tongue detect qualities of a food, like sweetness or bitterness (Margolskee, 2002).

As the ingested bolus moves through the stomach and intestines, it encounters a large network of mechanical and chemical receptors that sense changes in nutrient quantities, pH, gut hormone concentrations and gastrointestinal distension (Williams et al., 2016). These signals are then relayed to appetite centres in the brainstem via the vagus nerve.

Enteroendocrine cells (EECs) account for approximately 1% of the intestinal epithelium and play a key role in nutrient sensing along the gastrointestinal tract (Hooper, 2015). The detection of amino acids, monosaccharides, fatty acids and bile acids trigger release of GLP-1 and PYY from enteroendocrine L-cells (Psichas, Reimann, et al., 2015). Although most nutrients are digested higher up in the gastrointestinal tract, enteroendocrine L-cells are found in the highest concentrations in the ileum and colon (Suzuki et al., 2018). Consequently, EECs in the large intestine likely respond to metabolites from bacterial fermentation or receive neural inputs from other nutrient-sensing regions (Garcia-Gutierrez & Sayavedra, 2022).

Another mechanism for nutrient sensing and appetite regulation is the detection of glucose influx from the gastrointestinal tract in the hepatic portal vein, neural afferents signal to appetite centres in the hypothalamus and brainstem (Bacharach et al., 2023). Modulating intestinal gluconeogenesis (IGN) has been shown to regulate energy intake and improve glucose homeostasis (Mithieux et al., 2009; Troy et al., 2008). Furthermore, studies show that glucose infusions in the portal vein reduce energy intake to subsequent meals,

suggesting that portal vein glucose concentration is a key indicator of satiety (Bacharach et al., 2023).

1.2.4 **Regulating Energy Expenditure**

As mentioned, energy expenditure is the sum of resting energy expenditure, thermogenesis, the thermic effect of food and physical activity. Resting energy expenditure accounts for approximately 70% of daily energy expenditure and reflects the energy necessary to sustain physiological processes required for survival (Tran et al., 2022). Resting energy expenditure is primarily determined by lean mass but fat mass, age and thyroxine concentrations also contribute to its variability (Johnstone et al., 2005).

Adaptive thermogenesis refers to the dynamic alterations in energy expenditure triggered by environmental factors such as cold exposure or food intake. Under these conditions, the body expends energy for thermoregulation and digestive processes (Tran et al., 2022). The World Health Organisation (WHO) defines physical activity as “any bodily movement produced by skeletal muscles that requires energy expenditure” (World Health Organization, 2024c).

The hypothalamus plays a role in energy expenditure alongside its role in appetite regulation. Activation of the VMN by GLP-1 is associated with enhanced thermogenesis and adipose tissue ‘browning’ (Beiroa et al., 2014). Brown adipose tissue has long been identified in rodents but not human adults (Kozak, 2010). However, emerging evidence indicates that brown adipose tissue is a metabolically active and thermogenic organ in adults. Though brown adipose tissue appears to be retained to varying degrees but is associated with a metabolically healthy phenotype, with one study detecting higher concentrations of brown adipose tissue in ‘metabolically healthy’ individuals who were obese than those who were metabolically unhealthy (Jurado-Fasoli et al., 2024). Therefore, brown adipose tissue may be a potential therapeutic target for enhancing energy expenditure to prevent a positive energy balance. Brown adipose tissue is activated by sympathetic signals from the autonomic nervous system which is mediated partly by POMC and α-MSH release from the ARC (Leitner et al., 2017).

Research suggests that weight loss induces compensatory mechanisms that lower energy expenditure, promoting weight regain as the body attempts to return to a predetermined set point (Rosenbaum & Leibel, 2010). This underscores the need for therapeutic approaches for weight gain prevention that target both appetite regulation and energy expenditure.

1.3 Dietary fibre

The type of carbohydrates being consumed over the last century has shifted dramatically, transitioning from fibre-rich, plant-based diets to a diet comprised of highly processed, rapidly digestible carbohydrates. Whilst the development of obesity is multi-etiological, this shift in carbohydrate quality plays a potential role in disease development. Therefore, it is necessary to assess whether enhancing dietary fibre intake could be a realistic population-level intervention for obesity and chronic disease prevention. This section examines the physiological effects of dietary fibre on appetite, energy balance and glycaemic control.

1.3.1 **Defining dietary fibre**

Many definitions of dietary fibre exist, although the most accepted definition was established by CODEX Alimentarius in 2009, which highlights the conferred health benefit and defines dietary fibre as:

"'Carbohydrate polymers with ≥10 monomeric units (decision on whether to include carbohydrates with DP 3-9 was left to national authorities), which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: 1) edible carbohydrate polymers or lignin naturally occurring in the food consumed 2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities 3) synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities."

Since the 1970s, dietary fibre has been associated with reduced chronic disease risk (Trowell, 1972). Initially, fibre was recognised primarily for its role in increasing faecal bulk and alleviating constipation. However, recent research has highlighted its broader health benefits, including its role in the reduction in risk of cancer and coronary heart disease (Hu et al., 2023; D. J. . Jenkins et al., 1998). Considering these findings, the Scientific Advisory Committee on Nutrition (SACN) increased dietary fibre recommendations to 30 grams per day in 2015. While estimates suggest that Palaeolithic humans consumed 100 grams of dietary fibre per day (Eaton, 2006), current UK intakes fall around 18g, with little change in recent years (Gressier & Frost, 2022; Public Health England & Food Standards Agency, 2021), with less than 10% of the population meet SACN recommendations (Public Health England, 2020).

Dietary fibre can also be further categorised based on several physical and functional characteristics, including solubility, viscosity and fermentability. Soluble fibre is associated with slowing gastric emptying and reducing cholesterol (L. Brown et al., 1999; D. J. Jenkins et al., 1978). Whereas fermentable fibres can be metabolised by colonic bacteria and play a role in modulating the gut microbiome, which can play an additional role in metabolic health (Carmody et al., 2015; Filippo et al., 2010). The distinct characteristics of different types of dietary fibre contribute to its effects on digestion and metabolism, underscoring its key role in overall health.

1.3.2 **Dietary Fibre appetite regulation, energy intake and body weight**

Fructooligosaccharides like inulin and oligofructose, have been shown to mitigate weight gain in rodents. High cecal and circulating GLP-1 and PYY, lower circulating ghrelin and a subsequent reduced energy intake have been reported after oligosaccharide supplementation (Cani et al., 2004, 2005; Delmée et al., 2006). In some cases, these fructo-oligosaccharides also protected against high-fat diet-induced weight gain and adipose accumulation (Cani et al., 2004, 2005; Delmée et al., 2006; Delzenne et al., 2005). In rats, pectin supplementation over 4 weeks led to a decrease in energy intake, enhanced plasma GLP-1 and PYY and reduced leptin concentrations (Adam et al., 2015, 2016). This reduced body weight in obese rats (Adam et al., 2016) and protected against weight gain and adipose deposition in rats fed a high-fat diet (Adam et al., 2015). Beta-glucan supplementation was shown to reduce activity in brain regions linked to appetite (ARC, VMN, NTS), reduce NPY expression, lower energy intake and increase PYY concentrations in several rodent studies (Anastasovska et al., 2012; Arora et al., 2012, 2012; X.-F. Huang et al., 2011; N. Lin et al., 2013). Furthermore, rodents weaned on high- fibre diet were more resistant to weight gain and had greater circulating PYY and GLP-1 than those weaned on the control diet (Reimer et al., 2012).

Epidemiological studies consistently demonstrate that higher fibre intake is associated with lower body weight and waist circumference in adults. In Belgium, a study of 3038 adults indicated that fibre intakes were inversely associated with waist circumference (Y. Lin et al., 2011). Similarly, a prospective cohort study on 89,432 European participants demonstrated that an additional 10g total fibre and cereal fibre per day, respectively, reduced body weight gain by 39g and 77g and waist circumference by 0.08 and 0.1 cm per year (Du et al., 2010). The CARDIA study also indicated that those in the highest quintile (>10.5 g/1000 kcal/day) for fibre intake weighed on average 3.65 kg less than those in the lowest quintile (<5.9g/1000 kcal/day) (Ludwig et al., 1999).

These observational findings are reflected in intervention studies in humans. A WHO-commissioned systematic review of 27 studies comparing high and low fibre intakes found a mean difference in body weight of -0.37 kg (Reynolds et al., 2019). Further, a systematic review of randomised controlled trials (RCTs) involving adults who were overweight or obese reported that soluble fibre supplementation reduced BMI by 0.84 kg/m^2 and body weight by 2.52 kg (S. V. Thompson et al., 2017). A later review of 27 RCTs in participants who were overweight or obese found a mean reduction of 1.25 kg of body weight (Huwiler et al., 2022). Similarly, the effect of insoluble fibre found in legumes, fruit and vegetables has been investigated. Systematic reviews of RCTs examining legumes or fruit and vegetables reported a mean difference in body weight of -0.37 kg and -0.68 kg, respectively (S. J. Kim et al., 2015; Mytton et al., 2014).

These effects may be mediated by reduced energy intake, potentially via energy dilution, or increased satiety. For example, in a study of participants with pre-diabetes exhibited lower energy intake from an *ad libitum* meal after consuming inulin for 18 weeks (Guess et al., 2015). Healthy weight individuals who consumed high-fibre bread also demonstrated lower energy intakes during the intervention day compared with those consuming the low-fibre control (Santaliestra-Pasías et al., 2016). Additionally, participants with obesity who consumed pea fibre products for 12 weeks consumed less energy at an *ad libitum* meal (Lambert et al., 2017). A similar cohort who consumed inulin had lower subjective hunger responses after 6 weeks (Daud et al., 2014). Additionally, other studies investigating the effects of cellulose (Guess et al., 2015) or inulin (Calikoglu et al., 2021; Daud et al., 2014) fibres reported higher postprandial circulating PYY and GLP-1.

Dietary fibre may promote satiation and satiety via several mechanisms (J. Slavin & Green, 2007). Firstly, it replaces available calories with indigestible starch, displacing energy within food and lowering energy density and potentially, the palatability of the food as well (Baer et al., 1997; Drewnowski, 1998). High-fibre foods also tend to require more chewing resulting in an extended oral processing time, which is associated with lower energy intake (Zijlstra et al., 2009). Fibre further contributes to satiety by increasing meal bulk and enhancing viscosity of gastrointestinal contents leading to greater gastric distension, slower gastric emptying and the inhibition ghrelin secretion (Dikeman & Fahey Jr., 2006; Janssen et al., 2011; Y. Zhao et al., 2024). Viscosity has also been negatively correlated with energy intake (Dikeman & Fahey Jr., 2006; Zijlstra et al., 2008). By slowing gastrointestinal transit, fibre extends nutrient exposure to EECs, which may enhance the release of GLP-1 and PYY from L-cells; in turn, these hormones further delay gastric motility (Spreckley & Murphy, 2015).

These responses prolong the postprandial phase and increase length of satiety signalling, potentially lowering energy intake over this period.

1.3.3 Dietary Fibre and markers of chronic disease risk

The major indicators of chronic disease risk include blood glucose and cholesterol concentrations (World Health Organization, 2023b). Dietary fibre is a recognised modifiable factor for reducing risk of CVD and T2D. Ludwig and colleagues reported that higher fibre intake was associated with healthier blood pressure and fasting insulin, LDL and HDL cholesterol levels (Ludwig et al., 1999). A systematic review which included 4,635 participants showed that higher intakes of dietary fibre at 25 to 35g per day resulted in standard mean differences of -0.15 for total cholesterol, -0.09 for LDL cholesterol and -0.09 fasting glucose compared with lower fibre intakes, translating to 15-31% reduced risk of coronary heart disease, stroke and T2D incidence (Reynolds et al., 2019). An umbrella review of meta-analyses of RCTs further supports this, indicating that high-fibre interventions improved fasting plasma glucose, fasting insulin, total cholesterol and LDL cholesterol levels in a pooled analysis involving 47,197 healthy participants and individuals with T2D and pre-diabetes (Fu et al., 2022). Despite these benefits to long-term cardiometabolic health, fibre intakes within the population remain inadequate.

1.3.4 Dietary Fibre, the microbiota and fermentation in the colon

Dietary fibres are resistant to hydrolysis in the upper digestive tract and some reach the colon intact (Cummings & Macfarlane, 1991). The gut microbiota comprises of a rich community, including bacteria, fungi, viruses and other unicellular organisms which inhabit the length of the gastrointestinal tract but are most highly concentrated in the distal small intestine (10^8/mL) and colon (10^{11}/mL) (Sender et al., 2016). The gut microbiota is unique to the individual and remains relatively stable in adulthood, however, environmental factors such as diet, antibiotic use, smoking, alcohol intake and physical activity can modulate microbial composition (Capurso & Lahner, 2017; Ramirez et al., 2020; Varghese et al., 2024; Zmora et al., 2019).

A growing body of evidence has shown that the composition of the gut microbiota may serve as an indicator of chronic disease risk, as individuals with T2D and obesity exhibit distinct microbial compositions to healthy individuals, though the direction of this relationship remains unclear (Pascale et al., 2018; Sonnenburg & Bäckhed, 2016).

Dietary carbohydrates, particularly dietary fibre, serve as a key substrates for anaerobic microbial fermentation in the colon (Cummings & Macfarlane, 1991). The colonic bacteria

can break down plant cell material which forms a major portion of dietary fibre, which human digestive enzymes cannot degrade (Walker et al., 2008). This anaerobic fermentation enables gut bacteria to metabolise these non-digestible carbohydrates yielding both energy for the gut bacteria as well as beneficial metabolites and signalling molecules. The type of dietary fibre consumed also modulates the composition of the gut microbiota and the ratios of the metabolites produced (K. P. Scott et al., 2008). This anaerobic fermentation enables gut bacteria to metabolise these non-digestible carbohydrates yielding both energy for the gut bacteria alongside beneficial metabolites and signalling molecules for the host.

The main end-products of saccharolytic fermentation in the ileum and colon are short-chain fatty acids (SCFAs), methane (CH_4), hydrogen (H_2), carbon dioxide (CO_2) and heat (G. T. Macfarlane et al., 1992). SCFAs appear to play a role in appetite, energy balance and chronic disease risk (Byrne et al., 2015; Chambers, Morrison, et al., 2015; Morrison & Preston, 2016; A. H. Sukkar et al., 2019). The evidence for this will be examined in **Section 1.4**.

1.4 Short-chain Fatty Acids

SCFA are increasingly recognised as the link between dietary fibre intake and its effects on energy balance, appetite regulation and improved metabolic outcomes. However, exploring the effects of SCFA in human trials has proven challenging. Due to their volatility and rapid absorption in the upper gastrointestinal tract, SCFAs are difficult to deliver to the distal small intestine and colon where they are naturally produced in higher quantities and tend to have the greatest effect. Furthermore, measurement of SCFA in the distal gastrointestinal tract is difficult as it is largely inaccessible *in vivo*. Methods of increasing SCFA production, for example, via enhanced intake of dietary fibre is complex. The production of SCFA is largely driven by the gut microbial composition, limiting control over SCFA concentrations and relative ratios. The following section will explore the evidence, mostly from animal studies, for the role of SCFA in appetite regulation, energy homeostasis, lipid metabolism and glycaemic control.

1.4.1 **Characteristics and microbial production of SCFA**

SCFAs, carboxylic acids with chain lengths of less than 6 carbon atoms, are generated as metabolites of microbial fermentation of undigested carbohydrates in the colon (Cummings, 1981). The principle SCFAs, acetate, propionate and butyrate, are produced at a ratio of 57:22:21, respectively, constituting of more than 85% of total SCFA generated by the microbiota (Cummings et al., 1987). *In vitro* fermentation studies indicate that the gut microbiota yields 400-600 mmol of SCFA per day (Bergman, 1990; den Besten et al., 2013). SCFA production is influenced by gastrointestinal transit time, dietary fibre content, gut microbiota interspecific interactions and composition (den Besten et al., 2013; S. Macfarlane & Macfarlane, 2003). SCFAs can also come from sources within the diet, particularly fermented foods or vinegar, a natural source of acetic acid, providing up to 1000 mg SCFA in a standard serving (P. A. Gill et al., 2021). These food sources of SCFA have been explored and described elsewhere (Sieuwerts et al., 2008; Wolfe & Dutton, 2015).

The energy harvest hypothesis suggests that SCFAs generated from anaerobic fermentation would have been a significant source of energy for early humans and potentially provided a similar energy contribution to that observed in modern gorillas, i.e. approximately 50% of total energy intake (G. S. Frost et al., 2014; Popovich et al., 1997). In contrast, contemporary humans receive 5-10% of the total energy requirement from SCFA (Bergman, 1990; McNeil, 1984). Bacteria hydrolyse the fibres and other non-digestible carbohydrates into oligosaccharides and monosaccharides before they undergo glycolysis

via the Embden-Meyerhof-Parnas pathway or are converted to phosphoenolpyruvate via the pentose-phosphate pathway (den Besten et al., 2013). This suggests that SCFA have the potential to modulate energy balance, particularly during periods of low energy availability.

Acetate ($C_2H_3O_2$) is produced by the colonic microbiota at an estimated rate of 0.2 mmol kg bodyweight^{-1} h^{-1} (Pouteau et al., 1998). Acetate is produced from acetogenic fibres via two metabolic pathways: the Acetyl-CoA pathway and the Wood-Ljungdahl pathway. In the Acetyl-CoA pathway, pyruvate undergoes decarboxylation to form Acetyl-CoA, which is then converted into acetate. The Wood-Ljungdahl pathway synthesises acetate from Acetyl-CoA and CO_2 (Drake, 1994). Acetate-producing bacteria species include *Bifidobacterium* and *Prevotella* (Koh et al., 2016). Acetate is the only SCFA to mostly bypass metabolism by colonocytes and hepatocytes and reach the peripheral circulation in higher quantities, at rates of approximately 17.3 (7.3) µmol kg bodyweight^{-1} h^{-1}(Bloemen et al., 2009), giving it potentially influencing peripheral metabolic processes

Propionate ($C_3H_5o_2$) is produced at an estimated rate 0.04 mmol kg bodyweight^{-1} h^{-1}(G. N. Thompson et al., 1990), by certain species of bacteria, including *Eubacterium* and *Roseburia* (Reichardt et al., 2014). Propionate biosynthesis occurs via three metabolic pathways. The most common pathway is the conversion of succinate into pyruvate and CO_2 which is then decarboxylated to form propionate (Al-Lahham et al., 2010). In the acrylate pathway, lactate is converted to the intermediate, acrylate and then transformed into propionate (Al-Lahham et al., 2010). The less common propanediol pathway involves the degradation of deoxy sugars to 1,2-propanediol which are then converted into propionate (Reichardt et al., 2014). Approximately 80% of propionate is subject to hepatic metabolism by the liver, with only approximately 1.2 (0.4) µmol kg bodyweight^{-1} h^{-1} reaching the peripheral circulation (Bloemen et al., 2009). Unlike acetate and butyrate, propionate is an end-product of fermentation meaning that it does not undergo SCFA interconversion (Boets et al., 2017).

Butyrate ($C_4H_7o_2$) is produced in similar rates to propionate, however exact estimates have not been established in humans (den Besten et al., 2013). Examples of butyrogenic bacteria species are *Anaerostipes* and *Roseburia* (Koh et al., 2016). Butyrate is produced from Acetyl-CoA, with acetate and butyryl-CoA serving as intermediates (Bach Knudsen et al., 2018) and approximately 24% of acetate is converted to butyrate (Boets et al., 2017). Butyrate has the principal role of maintaining gut barrier integrity by whilst acting as a primary energy source forcolonocytes and there is an association between butyrate production and gastrointestinal diseases (Hodgkinson et al., 2023; Roediger, 2008). Therefore, it is found

in smaller concentrations in the peripheral circulation due to this important role as a primary energy source for colonocytes (Hamer et al., 2008).

1.4.2 **SCFA Metabolism**

SCFAs are absorbed by colonic epithelial cells via the apical membrane through active transport and passive diffusion (den Besten et al., 2013). Active transport for SCFA absorption involves three mechanisms: cotransport with bicarbonate ions (HCO_3^-), proton-coupled transport via monocarboxylate transporter 1 and sodium-coupled transport via sodium-coupled monocarboxylate transporter 1 (Rechkemmer et al., 1988).

SCFA that escape colonic metabolism enter the hepatic portal vein and are transported to the liver. Approximately 90% butyrate is oxidised by colonocytes, and 95% propionate is metabolised in the liver (Bloemen et al., 2009; Neis et al., 2019). Propionate is a gluconeogenic substrate and some have argued that it contributes to metabolic dysregulation (G. K. Adler et al., 2021). However, a recent study using ^{13}C-labelled propionate indicated that only 6% propionate administered was incorporated into glucose molecules, suggesting a minimal contribution (Boets et al., 2017). Although this study was conducted in postprandial conditions where rates of gluconeogenesis are low, different effects may be seen in a fasted state. Similarly, acetate is a substrate for hepatic cholesterol synthesis but appears to play a minimal contribution, with 1% of acetate administered detected in circulating cholesterol (Boets et al., 2017).

Specific SCFA transport proteins for absorption across different tissues are not well characterised, though organic anion transporters 2 and 7 may facilitate SCFA transport into hepatic and renal tissues (den Besten et al., 2013). Studies using ^{13}C-labelled SCFAs indicate that the majority of SCFAs are absorbed and oxidised by the body, with most ^{13}C excreted in CO_2 from the lungs and 0.05% found in urine (Boets et al., 2017). **Table 1.2** summarises concentrations of SCFA in the colon, portal vein, liver and circulation.

Table 1.2 Mean concentrations of SCFA in the colon, portal vein, liver and peripheral circulation

	Acetate (μmoL/L)	Propionate (μmoL/L)	Butyrate (μmoL/L)
Descending colon*	43.5	14.2	14.7
Sigmoid colon*	50.1	19.5	17.9
Portal vein**	174	41	23
Hepatic**	108	8	7

Peripheral arterial**	107	2	3
Peripheral venous**	69	5	4

*(Cummings et al., 1987); **(Frampton, 2022) who calculated averages from studies in individuals undergoing abdominal surgery (Bloemen et al., 2009; Neis et al., 2019; Peters et al., 1992; van der Beek et al., 2015) and sudden death victims (Cummings et al., 1987).

1.4.3 **SCFA Receptors**

SCFAs interact with specific receptors on cell surfaces, namely the G protein-coupled receptors (GPCRs) also known as free fatty acid receptors (FFARs) 2 and 3 and olfactory G protein-coupled receptor-78 (Olfr-78) (A. J. Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). FFAR 2 exhibit a higher affinity for acetate and propionate and are expressed in the pancreas, spleen, heart, adipose tissue, muscle tissue and immune cells (A. J. Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). FFAR 3 show a higher affinity for propionate and butyrate and are primarily located in adipose tissue, spleen, pancreas, bone marrow, lymph nodes and sympathetic ganglia (A. J. Brown et al., 2003; Kimura et al., 2011; Le Poul et al., 2003; G. Li et al., 2014; Tang et al., 2015). Both FFAR 2 and 3 are expressed throughout the gastrointestinal tract and located on enteroendocrine L-cells responsible for the secretion of GLP-1 and PYY (A. J. Brown et al., 2003; G. Li et al., 2014; Nøhr et al., 2013). Although SCFA receptors are present in many metabolically active tissues, circulating concentrations of SCFA may be below the threshold for GPCR activation, limiting their capacity to initiate signalling cascades in peripheral tissues under normal physiological concentrations (Z. Ang & Ding, 2016).

1.4.4 **SCFA, appetite and energy intake**

In *in vitro* studies, propionate and acetate, at a concentration of 1 mmol/L, enhanced GLP-1 secretion in rodent colon cells, primarily through activation of the FFAR 2 receptor, with FFAR 2 knockout (KO) causing a 70% reduction in GLP-1 release (Tolhurst et al., 2012). In human cells, the administration of propionate enhanced GLP-1 and PYY secretion (Chambers, Viardot, et al., 2015) but FFAR 2 KO only partially reduced PYY secretion in response to butyrate and propionate (Larraufie et al., 2018). Potentially suggesting that FFAR 3 activation also contributes to propionate- and butyrate-induced PYY secretion (Samuel et al., 2008).

In murine models, intracolonic propionate administration increased PYY and GLP-1 via FFAR 2 activation, however, GLP-1 secretion reduced after FFAR 2 and 3 KO mice (Psichas, Sleeth, et al., 2015). In contrast, acetate and butyrate, but not propionate increased GLP-1 after colonic or intravenous infusions (Christiansen et al., 2018). A SCFA colonic infusion enhanced release of PYY and slowed gastric motility in rodents. In the same study, similar, but less pronounced effects were seen when PYY was administered directly, indicating the SCFA action on motility in the colon is partly independent of PYY release (Cherbut et al., 1998). Additionally, an eight-day administration of propionate and butyrate lowered energy intake and butyrate administration enhanced circulating GLP-1 and PYY concentrations in mice (H. V. Lin et al., 2012), whilst acetate and propionate binding olfr78 enhanced PYY secretion from enteroendocrine L-cells (Nishida et al., 2021). Oral sodium SCFA supplementation lowered food intake compared with the control in rodents, without affecting body weight (Jiao et al., 2021). Among the SCFAs, sodium acetate and sodium propionate significantly increased GLP-1 and PYY secretion (Jiao et al., 2021).

Beyond PYY and GLP-1 signalling, SCFAs may influence appetite centrally by crossing the blood brain barrier. Acetate has been detected in human cerebrospinal fluid and appears to be an energy source for neuroglia (G. Frost et al., 2014; Nagashima et al., 2010). Frost *et al* reported that intraperitoneal, but not colonic, acetate infusions lowered energy intake and enhanced expression of POMC in the ARC, without altering GLP-1 and PYY concentrations in murine models (G. Frost et al., 2014). Butyrate has been shown to suppress expression of orectic neuropeptide NPY and lowered brain activity in dorsal vagal complex, subsequently reducing energy intake in rodents, this effect was abolished following a vagotomy (Z. Li et al., 2018). Furthermore, De Vadder and colleagues have demonstrated that propionate and butyrate can stimulate IGN which is believed to influence satiety and glucose homeostasis (De Vadder et al., 2014). IGN-enhanced glucose concentrations are sensed by portal vein afferents and signal satiety to appetite centres in the hypothalamus and brain stem (Mithieux & Gautier-Stein, 2014). However, in the study using IGN deficient and wild type mice fed a propionate-enriched diet, no differences in body weight were observed (De Vadder et al., 2014).

In human studies, the compound inulin-propionate ester (IPE) has been developed to deliver propionate to sites of action in the colon, resulting in enhanced PYY secretion comparable to concentrations after a 1000 kcal meal (Chambers, Viardot, et al., 2015). Additionally, rectal and distal colonic acetate administration elevated PYY secretion in both normal weight and overweight or obese subjects (Freeland & Wolever, 2010; Van der Beek et al., 2014,

2016). Rectal SCFA infusions increased PYY secretion under both fed and fasted conditions whereas GLP-1 secretion was unaffected (Canfora et al., 2017). However, other studies investigating propionate in the form of sodium propionate and IPE have observed no effect on anorectic gut hormone secretion (Byrne et al., 2016, 2019; Chambers et al., 2018; Malkova et al., 2020; Pingitore et al., 2017; Polyviou et al., 2016). However, IPE has been shown to lower *ad libitum* energy intake in humans (Alhabeeb et al., 2014; Byrne et al., 2016, 2019; Chambers, Viardot, et al., 2015; Polyviou et al., 2016), potentially suggesting that enhanced satiety signalling may involve other mechanisms. For example, IPE demonstrably lowered blood oxygen level–dependent (BOLD) signalling was in brain regions involved in reward processing and slowed responses to images of energy dense foods in participants with obesity (Byrne et al., 2016). These findings could suggest that propionate may influence eating behaviour via appetite or food reward pathways in the brain.

This evidence suggests that SCFAs may influence energy intake and appetite regulation via enhanced gut hormone secretion, neural signalling, exerting effects on the brain and peripheral tissues. Their impact may vary depending on the type of SCFA, mode of administration, and metabolic context.

1.4.5 **SCFA, energy expenditure and fat oxidation**

In rodent models, acetate and butyrate administration has been shown to increase energy expenditure (den Besten et al., 2015; Z. Gao et al., 2009; Hattori et al., 2010; Sahuri-Arisoylu et al., 2016; Yamashita et al., 2009) and enhance lipid oxidation (den Besten et al., 2015; Z. Gao et al., 2009; Hattori et al., 2010). Chronic administration of acetate and butyrate may also protect against high-fat diet induced weight gain (den Besten et al., 2015; Z. Gao et al., 2009; Sahuri-Arisoylu et al., 2016). Propionate may enhance resting energy expenditure in rodents via FFAR3 activation on the sympathetic ganglia, an effect that was abolished in FFAR3 KO mice (Kimura et al., 2011). Further, the same group reported that FFAR2 deficient mice were obese on a normal diet, whilst FFAR2 overexpression in adipose tissue protected mice from weight gain on a high-fat diet (Kimura et al., 2013). The activation of the sympathetic nervous system via FFAR3 (Kimura et al., 2011; Nøhr et al., 2013) and enhanced thermogenesis, associated with increased expression of uncoupling protein 1 or 2 in the liver, muscle or brown adipose tissue, appear to drive the observed effects on energy expenditure and fat oxidation (den Besten et al., 2015; Hu et al., 2016; Z. Li et al., 2018, 2018; Murakami et al., 2015; Sahuri-Arisoylu et al., 2016; Yamashita et al., 2009).

Human studies have shown similar results where acetate administration to the distal colon was associated with enhanced lipid oxidation in the fasted state (Canfora et al., 2017; Van der Beek et al., 2014, 2016), whilst orally ingested propionate increased fasting lipid oxidation (Chambers et al., 2018). Additionally, both IPE and sodium propionate elevated postprandial (Byrne et al., 2019) and fasting (Chambers et al., 2018) energy expenditure, respectively. Canfora *et al* reported that a colonic infusion of all SCFAs enhanced fat oxidation (Canfora et al., 2017). Whereas acetate and propionate increased fasting energy expenditure but only acetate concentrations in the large intestine were significantly correlated with this effect (Canfora et al., 2017).

The activation of the sympathetic nervous system via FFAR3 (Kimura et al., 2011; Nøhr et al., 2013) and enhanced thermogenesis may drive the observed effects on energy expenditure and fat oxidation (den Besten et al., 2015; Hu et al., 2016; Z. Li et al., 2018, 2018; Murakami et al., 2015; Sahuri-Arisoylu et al., 2016; Yamashita et al., 2009). These findings suggest that SCFAs may enhance energy expenditure and fat oxidation to facilitate energy homeostasis and protect against diet-induced obesity.

1.4.6 **SCFA and glucose homeostasis**

In vitro, SCFA improve β-cell function in the pancreas, contributing towards insulin secretion and potential improvements in glucose tolerance. Acetate, propionate and butyrate were shown to stimulate GLP-1 release *in vitro* (Dumoulin et al., 1998; Tolhurst et al., 2012), likely via FFAR2 and 3 activation on enteroendocrine L-cells (Chambers, Viardot, et al., 2015; Kaji et al., 2014). GLP-1 is insulinotropic, delays gastric emptying rate, inhibits the production of glucagon and enhances glucose disposal in peripheral tissues (D'Alessio et al., 1994; Todd & Bloom, 2007). Propionate may also facilitate β-cell proliferation, prevent apoptosis and improve glucose-stimulated insulin release, independent of GLP-1, as shown *in vitro* (Pingitore et al., 2017). Furthermore, direct activation of FFAR2 on pancreatic β-cells stimulates insulin secretion and FFAR2 KO rodent models have disturbed glucose tolerance and insulin secretion (McNelis et al., 2015; Priyadarshini et al., 2015). Acetate and propionate prevented apoptosis in pancreatic islets, a response associated with FFAR2 activation (Pingitore et al., 2017, 2019). This evidence suggests that FFAR2 play a significant role in insulin secretion

In animal studies, SCFAs lowered serum glucose in rodents over 35 days (Jiao et al., 2021) and SCFA activation of FFAR2 enhanced insulin sensitivity and glucose utilisation within tissues (Kimura et al., 2013). All three SCFA improved whole body insulin sensitivity in

murine models (Z. Gao et al., 2009; Hong et al., 2005). Butyrate and propionate improved glucose tolerance, lowered postprandial glucose and fasting insulin (De Vadder et al., 2014; Han et al., 2014; H. V. Lin et al., 2012), an effect that may be partially driven by IGN, (De Vadder et al., 2014; Mithieux & Gautier-Stein, 2014). Acetate upregulates GLUT-4 in myocytes, doubling glucose uptake in T2D rodent models and enhancing glycogen storage to promote glucose disposal (Fushimi et al., 2001, 2002; Yamashita et al., 2009). Propionate may also improve insulin-stimulated glucose uptake into adipocytes and basal glucose uptake in myocytes *in-vitro,* an effect which may be partially modulated by FFAR3 (Han et al., 2014). Furthermore, plasma glucose and insulin levels were significantly lower in acetate-administered Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Yamashita et al., 2007). Conversely however, another study of rodent models with T2D, observed improved insulin secretion and glycaemic outcomes after FFAR2 and 3 KO (Tang et al., 2015).

In vitro and rodent studies show SCFAs stimulate GLP-1 and insulin secretion, improve glucose uptake, glycogen storage, and insulin sensitivity. Whilst evidence from animal models looks promising, results from human studies is limited and contradictory. Differences in part, may be due to the supraphysiological dose sizes utilised in animal studies. The evidence in humans is explored in greater detail in **Chapter 2**.

1.4.7 **SCFA and body weight**

In murine models, acetate, propionate and butyrate have been shown to prevent high-fat diet induced weight gain (den Besten et al., 2015; De Vadder et al., 2014; Z. Gao et al., 2009; Kimura et al., 2011; H. V. Lin et al., 2012; Yamashita et al., 2007). However, the few studies in humans have yielded limited results, potentially due to the difficulty of delivering SCFA to targeted sites in the colon over a chronic intervention. The few human studies exploring these effects are described in **Chapter 3** and the potential mechanisms are investigated in **Chapter 4.**

1.5 Rationale and general aims

Part one of this thesis aims to explore novel approaches for the prevention of obesity and chronic disease using metabolites of fibre fermentation, SCFA. The objectives of this section are:

To explore the role of SCFAs in glucose metabolism, exploring their effects in healthy individuals and those with disrupted glucose homeostasis in **Chapter 2** . This investigation addresses a gap in the literature regarding the impact of SCFAs on glycaemic response, emphasising the scarcity of high-quality human intervention studies in this area.

To understand the effects of colonic propionate delivery via IPE on weight gain prevention in younger adults at heightened risk of weight gain in **Chapter 3**

To investigate the effects of colonic propionate delivery via IPE on appetite regulation andenergy expenditure, on the same cohort of younger adults, in **Chapter 4**

1.5.1 **Hypothesis**

I hypothesised that increasing SCFA in the colon would facilitate weight gain prevention and improve glycaemic regulation.

1.5.2 **Thesis Aims**

The aims of this section of this thesis are to:

- Assess the effects of acute and chronic SCFA administration on postprandial and fasting glycaemia.
- Investigate whether increased colonic delivery of propionate via the vehicle, IPE, could prevent weight gain in overweight, young adults over 12 months.
- Explore the mechanisms by which increased colonic delivery of propionate may affect energy balance when compared to the inulin control, from baseline to 12 months.

Chapter 2: The effect of short-chain fatty acids on glycaemic control in humans: Systematic Review and Meta-analysis

Research outputs from this chapter:

Cherta-Murillo, A., **Pugh, J. E.,** Alaraj-Alshehhi, S., Hajjar, D., Chambers, E. S., & Frost, G. S. (2022). The effects of SCFAs on glycemic control in humans: A systematic review and meta-analysis. The American Journal of Clinical Nutrition, 116(2), 335–361. https://doi.org/10.1093/ajcn/nqac085

2.1 Summary of key findings

- Studies investigating the effects of the SCFAs acetate, propionate and butyrate or vinegar as a dietary form of acetic acid on glycaemic response are limited. GRADE scoring indicated a low certainty of evidence and 91% of studies had a high risk of bias or some concerns
- Vinegar, as a dietary source of acetic acid, was the only intervention detected to significantly lower postprandial glucose response. No effect was observed in other SCFAs
- The lack of effect seen was partially driven by the small sample of studies, which is partially due to the difficulty in delivering SCFAs to sites of action in the distal colon. Further studies in human participants, using homogenous methodologies of SCFA delivery are required to determine the effect and mechanisms of SCFA on glycaemic response

2.2 Introduction

Individuals with a BMI of >30 kg/m^2 or > 35 kg/m^2 have a 2.5 or 3.6 times greater relative risk of developing T2D, respectively (Ganz et al., 2014). T2D is defined as a state of chronic hyperglycaemia driven by insulin resistance that prevents glucose disposal in peripheral tissues. This subsequently leads to impaired insulin production in pancreatic β-cells, and the upregulation of endogenous glucose production (Cersosimo et al., 2000). Diagnoses of T2D account for 90% of diabetes cases and have increased dramatically in the last 40 years from 108 million to 422 million (World Health Organization, 2023a). Obesity is a determinant factor in the development of non-communicable diseases, specifically T2D (Wesolowska-Andersen et al., 2022).

Glucose homeostasis refers to the body's capacity to maintain blood glucose concentrations to meet the physical demands of the current metabolic state via hormonal (e.g. insulin and glucagon), and neuropeptide control (Röder et al., 2016). Individuals with T2D are often in a state of hyperglycaemia, defined as blood glucose concentrations of greater than 7 mmol/L when fasted (Luitse et al., 2012). Hyperglycaemia causes damage to key tissues resulting in microvascular and macrovascular complications, such as cardiovascular and kidney disease (A. I. Adler et al., 2000). Common pharmacological interventions for T2D are metformin, sulphonylureas and meglitinides, which have anti-hyperglycaemic effects (British Nutrition Foundation, n.d.). However, as metabolic syndrome, prediabetes and T2D become more prevalent, there is a rising demand for non-pharmacological interventions to improve glucose homeostasis.

As mentioned in **Chapter 1**, data from epidemiological and intervention studies suggest that dietary fibre and wholegrains influence glycaemic outcomes and the risk of developing T2D (Reynolds et al., 2019). One way in which they may exert these effects is via the production of SCFA (**Chapter 1, Section 1.4**) (Payne et al., 2012).

Evidence from *in vitro* and animal studies suggests that SCFA could influence glycaemic control through several mechanisms, which have been explored in greater detail in **Chapter 1, Section 1.4.7**. *In vitro* and animal studies have associated SCFA with improved glucose homeostasis and insulin sensitivity via multiple mechanisms. In summary, acetate, propionate and butyrate stimulate GLP-1 release, potentially via FFAR2 and 3 activation on enteroendocrine L-cells in the gastrointestinal tract, a hypothesis reinforced by studies demonstrating that effects were reduced or lost after FFAR2 and 3 KO (Chambers, Viardot, et al., 2015; Dumoulin et al., 1998; Kaji et al., 2014; Tolhurst et al., 2012). GLP-1 is

insulinotropic and inhibits the production of glucagon promoting glucose tolerance (D'Alessio et al., 1994; Todd & Bloom, 2007). However, SCFA may also directly stimulate insulin secretion by activation of FFAR on pancreatic β-cells (Pingitore et al., 2017). SCFA administration in rodents enhanced fat oxidation in adipocytes and hepatocytes associated with modulation of PPAR- γ (den Besten et al., 2015). Whilst acetate and propionate may suppress lipolysis in adipocytes lowering concentrations of free fatty acids (FFA) and improving insulin sensitivity (Z. Gao et al., 2009; Morrison & Preston, 2016).

The activation of AMPK by SCFA may upregulate glucose transporter (GLUT) -4 receptor expression in myocytes, potentially enhancing glucose disposal in muscle tissues (Maruta et al., 2016; Yamashita et al., 2007). Furthermore, acetate-feeding in rodents promoted glycogen storage in liver and muscle tissues (Fushimi et al., 2001). SCFA also improved insulin-stimulated glucose uptake into adipocytes and basal glucose uptake in myocytes *in-vitro* (Han et al., 2014). Additionally, propionate- and butyrate-stimulated IGN may play a role in insulin sensitivity through glucose-sensing in the portal vein and signalling via the vagus nerve (De Vadder et al., 2014; Mithieux et al., 2009).

In humans, the current evidence of the effect of SCFAs on glycaemia is limited and contradictory, its overall effect has yet to be systematically reviewed. This is except for vinegar, a dietary form of acetate, which appears to affect glucose response (Shishehbor et al., 2017). Acetic acid within vinegar is found in concentrations between 6 to 25% depending on production methods (Mas et al., 2014).

The current literature suggests that SCFAs affect glucose metabolism, however, animal and *in vitro* models have their limitations and do not necessarily reflect observations in human studies. The aim of this Chapter is to evaluate the quality of current human studies investigating SCFA and glycaemia and identify any effects of acute and chronic SCFA administration on postprandial and fasting glucose and insulin along with insulin sensitivity in humans.

2.3 Purpose of the Study

2.3.1 **Hypothesis**

I hypothesised that acute and chronic SCFA administration would affect measurements of glycaemia, indicated by fasting and/or postprandial glucose and insulin, glycated haemoglobin and measures of insulin resistance.

2.3.2 **Aims**

The primary aim of this systematic review and meta-analysis was to determine whether acute and chronic SCFA administration has any effect on postprandial or fasting glycaemia.

2.3.3 **Outcomes**

The outcomes investigated in this systematic review and meta-analysis were:

- Postprandial glucose and insulin
- Fasting glucose and insulin
- Homeostatic measurement of insulin resistance (HOMA-IR), Homeostatic measurement of β-cell function (HOMA-%B), Homeostatic measurement of insulin sensitivity (HOMA-%S)
- Glycated haemoglobin (HbA1c)
- Hormones: GLP-1, GIP, PYY

2.4 Methods

2.4.1 **Registration**

This systematic review and meta-analysis was registered to PROSPERO on 14th January 2021, reference number CRD42021231115.

2.4.2 **Criteria**

This population, intervention, comparator, outcome and study design (PICOS) criteria were predefined before conducting the literature search to conceptualise the intention and strategy of the systematic review.

Population: Healthy weight (BMI 18 – 24.99), overweight (BMI 25 – 29.99 kg/m^2) or obese (BMI ≥ 30 kg/m^2) adults (18-65 years old). Could be healthy or diagnosed with metabolic syndrome, prediabetes or type 2 diabetes. Studies involving humans with other diseases (e.g., coronary heart disease) and animal studies were not included. Additionally, studies that did not represent natural metabolic responses, e.g., clamping studies, were also excluded.

Intervention: Any acute (<24-hour duration) or chronic (>24-hour duration) studies which used acetate, vinegar (a dietary form of acetic acid), propionate, butyrate or mixed SCFA. Any studies which used drugs or hormones that contained SCFA were not deemed suitable for inclusion.

Comparator: Any suitable placebo, for example, inulin as a placebo for inulin-propionate ester. Studies where different populations were compared, e.g., those with impaired glycaemic control compared to healthy populations or if different doses of SCFA were used, were not included.

Outcome: Any quantifiable measures of glycaemia, such as fasting or postprandial glucose and insulin, HbA1c, insulin sensitivity indexes (i.e., HOMA-IR, HOMA-%S). Studies which did not include quantifiable outcomes of interest were excluded.

Study design: Controlled intervention studies and randomised controlled trials were included. Reviews, conference abstracts, dissertation abstracts, lectures, information pieces, study registers and *corrigendum*s were excluded. Studies published before 1980 and in languages other than English were also excluded.

The exclusion criteria were chosen to ensure the outcomes of interest were not biased by participant characteristics (e.g. disease state), other components of the intervention, an unsuitable comparator or an unsuitable study design.

2.4.3 **Search Strategy and Search Terms**

PubMed (Medline), Cochrane CENTRAL, EMBASE, Web of Science, and Scopus were used to search for records published up until 7 December 2021. Full details of search strategies can be found in **Appendix 1**.

2.4.4 **Study selection**

The study selection was performed using the Covidence systematic review software (Veritas Health Innovation; www.covidence.org), which allowed researchers to select 'no', 'maybe' or 'yes' for inclusion in the systematic review. The first stage was title and abstract screening by at least two of the reviewers independently via the Covidence software. Then a full-text screen was conducted by the three reviewers independently to determine whether studies met the PICOS criteria. Any conflicts that arose during study selection process were discussed between the reviewers until a consensus was reached. On the rare occasion that an agreement could not be reached, the issue was discussed with a researcher uninvolved in the review process.

2.4.5 **Data extraction**

The data from the eligible papers were extracted by the four reviewers independently and the information was added to an Excel spreadsheet (Excel 2016, Microsoft Corporation, USA). The following information was extracted; first author, publication date, type of SCFA intervention, acute or chronic study, study duration, study design, sample size, population type, BMI, age, administration method, concentration and/or volume of SCFA, primary and secondary outcomes collected. Sometimes multiple studies were identified within a single eligible publication, these were extracted and analysed separately. In the case where there were multiple interventions within one study, the intervention that most reflected real life was selected. For example, when there was vinegar given in pill and liquid form, liquid form was selected. When the data was contained within figures, means and variances were extracted using WebPlotDigitizer 4.4 (Ankit Rohatgi, USA) (https://apps.automeris.io/wpd/). In the

case of postprandial glucose and insulin, data was extracted from figures for each time point and the incremental area under the curve (AUC) was calculated using the trapezoidal rule (Wolever, 2006). When measurements for outcomes of interest were reported to have been taken but data were not found in the results section, the researchers were contacted to gain access to the raw data for incorporation into the meta-analysis.

2.4.6 **Data processing and analysis**

Descriptive data (e.g., age, BMI) was converted into mean ± standard deviation (SD). If standard error or 95% Confidence intervals were reported in the paper, these were converted to SD Measurements of glycaemic control included postprandial glucose and insulin area under the curve or incremental area under the curve, fasting blood glucose or insulin, HbA1c and measurements of insulin sensitivity. When there were fewer than 3 studies for any outcome category a narrative review was conducted (Deeks et al., n.d.). When there were more than three studies for the outcome, a meta-analysis was conducted. The outcomes which were investigated by more than three studies were acute interventions using acetate, vinegar and propionate and chronic studies which used propionate. Meta-analyses using the Sidik-Jonkman random effects model produced standard mean differences (SMDs) and 95% confidence intervals (Cis) for postprandial blood glucose and insulin after acute acetate, propionate and vinegar intake, and postprandial blood glucose and insulin, fasting glucose and insulin, and HbA1c after chronic propionate administration. The meta-analysis model used was selected due to the heterogeneity between studies, allowing for a wider 95% CI to better reflect the uncertainty. For crossover studies, the SMD was calculated by assuming a parallel design for a more conservative analysis. Heterogeneity was assessed using the I^2 statistic. I^2 values ranged from 0% to 100%, with values of 25% to 49%, 50% to 74%, and ≥75% classified as low, moderate, and high, respectively (Higgins et al., 2003).

2.4.7 **Confounders of glycaemic control**

Eligible studies were also assessed for whether researchers had accounted for confounders of glycaemic response. Reviewers assessed whether actions had been taken to minimise or control variability in glycaemia caused by actions or characteristics of participants. Acute confounders of glycaemic control are physical activity, the length of the fasting period, and fibre or alcohol intake. Chronic confounders include changes in the body weight and body fat percentage throughout the study.

2.4.8 **Risk of Bias Assessment**

Risk of bias was assessed by three reviewers and any discrepancies between categorisation were discussed with an independent party. The Cochrane revised risk of bias tool (v2.0) was used to assess the risk that aspects of the methodology or measurement of outcomes will result in spurious results (Higgins et al., 2021). The risk of bias was assessed in the following domains: randomisation process, assignment to intervention, intervention adherence, effect of missing outcome data, measurement of outcome and selection of reported result. Using the algorithms provided in the risk of bias tool, the risk of bias for each domain was assessed to be low, of some concerns or high. If the study had any high risk of bias domains or more than three domains with some concerns, the study was deemed to have a high risk of bias.

2.4.9 **Certainty of Evidence**

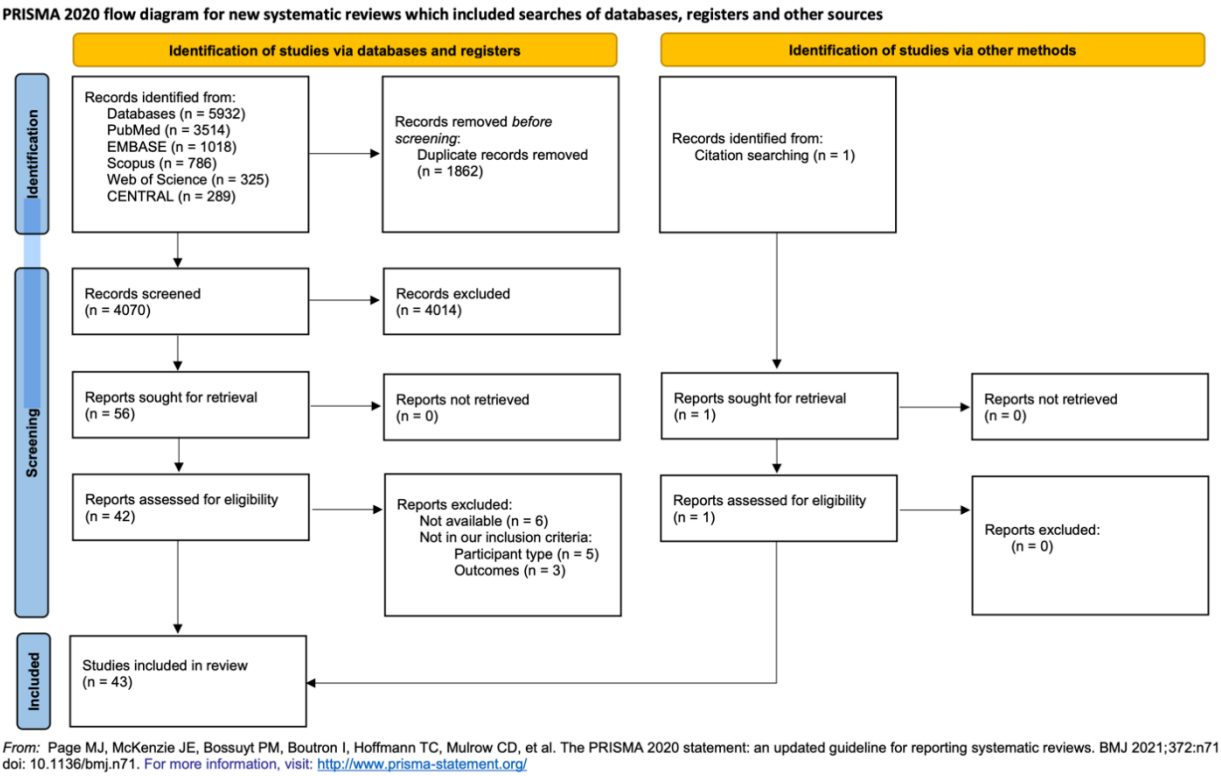
Certainty of evidence was assessed using Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) criteria. This framework is an easy and accessible method for assessing the strength of evidence for clinical recommendations. Table summarizing the findings was constructed using GRADEpro software (McMaster University and Evidence Prime Inc.) (http://gradepro.org, accessed 10 December 2021). Evidence for outcomes could be ranked as certainty of evidence was assessed using the following domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. The estimated effect for each outcome was then classified as very low (true effect is probably markedly different from the estimated effect), low (true effect might be markedly different from the estimated effect), moderate (true effect is probably close to the estimated effect) or high quality (true effect is similar to the estimated effect) (Siemieniuk & Guyatt, n.d.). Strong recommendations may be made if GRADE criteria deem evidence for outcome as high quality.

2.5 Results

2.5.1 **Study Search**

The literature searches identified 5932 references after the database search and any papers that were identified by researchers via manual search. PubMed yielded the most, with 3514 publications and 1018, 786, 325 and 289 publications were identified from EMBASE, Scopus, Web of Science and Cochrane CENTRAL, respectively. Reviewers also identified one paper by manually searching the bibliographies of included publications. Duplicates were subsequently removed (n=1862), and after title and abstract screening, 4014 publications were excluded. After the full-text screening of 56 publications against the predefined PICOS criteria, a further 14 studies were excluded. This meant 43 publications with a total of 53 studies were eligible for this review and meta-analysis. Some studies had more than two comparators per study or more than two studies per publication. For acetate, there was one publication with two studies (Scheppach, Cummings, et al., 1988). For the vinegar interventions, there were two publications with multiple studies (Darzi et al., 2014; C. S. Johnston et al., 2010) and two studies with multiple interventions within one study (Brighenti et al., 1995; Feise & Johnston, 2020; C. S. Johnston & Buller, 2005; Liatis et al., 2010). Not all the studies (n=44) from these publications or interventions within studies were meta-analysed and the comparisons selected are identified in the footnotes of the meta-analysis forest plots. A summary of this process can be found in **figure 2.1.**

Figure 2.1: A flow diagram of the study selection process adapted from the PRISMA 2020 statement.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

2.5.2 Summary of identified studies

Five publications, including 44 participants, investigated acetate (all acute interventions). Acute vinegar was investigated in 25 studies with 240 participants and chronic vinegar intake was explored in 6 interventions in 275 participants. Two chronic studies, with 30 participants, investigated butyrate (both reporting the same publication). Acute propionate administration was investigated in eight studies with 120 participants and chronic propionate interventions were explored in 6 studies with 157 participants. Five publications investigated acute mixed SCFA administration in36 participants.

2.5.3 Study and study participant characteristics

The study and study participant characteristics are described in the table below (**table 2.1**).

Table 2.1: Description of study participants characteristics, interventions and outcomes

Study	Participant characteristics	Intervention characteristics	Outcomes Analysed
Acute Acetate			
Freeland and Wolever 2010	6 participants with hyperinsulinemia	RCT, crossover Duration: 60 mins	glucose, insulin

	Age: 44 BMI: 31	Intervention 20 mmol/L Route of administration: intravenous and rectal Control: saline	
Laurent et al. 1995	3 males, 3 females healthy volunteers Age: 22 BMI: 21.1	RCT, crossover Duration: 300 mins Intervention: 12 mmol/h Route of administration: gastric Control: saline	glucose, insulin
Scheppach et al. 1988 (study 1)	5 healthy volunteers Age: 33 BMI: 22.4	RCT, crossover Duration: 360 mins Intervention: acetate drink (195 mmol) Route of administration: oral Control: chloride	glucose, insulin
Van der Beek et al. 2016	6 males healthy volunteers Age: 35 BMI: 31	RCT, crossover Duration: 300 mins Intervention: 100-180 mmol/L Route of administration: colonic Control: saline	glucose, insulin
Acute Vinegar			
Brighenti et al. 1995	4 males, 1 female healthy volunteers Age: 37 BMI: NR	RCT, crossover Duration: 95 min Intervention: 20 mL white vinegar Route of administration: oral Control: white bread	glucose
Darzi et al. 2014 (study 1)	13 males, 3 females healthy volunteers Age: 22.2	RCT, crossover Duration: 30g white wine vinegar Intervention: 180 mins	glucose

	BMI: 22.1	Route of administration: oral Control: 75g sugar-free squash	
Darzi et al. 2014 (study 2)	6 male, 8 female healthy volunteers Age: 27.5 BMI: 22.7	RCT, crossover Duration: 30g white wine vinegar Intervention: 180 mins Route of administration: oral Control: water	glucose
Fiese and Johnston 2020; A	5 males, 7 females healthy volunteers Age: 22.6 BMI: 21.2	RCT Duration: 60 mins Intervention: 25g liquid vinegar Route of administration: oral Control: water	glucose
Fiese and Johnston 2020; B	5 males, 7 females healthy volunteers Age: 22.6 BMI: 21.2	RCT Duration: 60 mins Intervention: 25g vinegar pills Route of administration: oral Control: water	glucose
Fiese and Johnston 2020; C	5 males, 7 females healthy volunteers Age: 22.6 BMI: 21.2	RCT Duration: 60 mins Intervention: 25g crushed vinegar pills Route of administration: oral Control: water	glucose
Hlebowicz et al. 2007	6 males, 7 females healthy volunteers Age: 25 BMI: 22.8	RCT, crossover Duration: 120 minutes Intervention: 28g white wine vinegar Route of administration: oral Control: white bread	glucose

Johnston et al. 2004; A	11 healthy volunteers Age: NI BMI: NI	RCT, crossover Duration: 60 minutes Intervention: 20g apple cider vinegar Route of administration: oral Control: placebo drink (unspecified)	glucose, insulin
Johnston et al. 2004; B	10 participants with T2D Age: NI BMI: NI	RCT, crossover Duration: 60 minutes Intervention: 20g apple cider vinegar Route of administration: oral Control: placebo drink (unspecified)	glucose, insulin
Johnston and Buller 2005; A	1 male, 10 female, healthy volunteers Age: 27.9 BMI: 22.7	RCT, crossover Duration: 60 minutes Intervention: 20g apple cider vinegar Route of administration: oral Control: sweetened water	glucose, insulin
Johnston and Buller 2005; B	1 male, 10 female, healthy volunteers Age: 27.9 BMI: 22.7	RCT, crossover Duration: 60 minutes Intervention: 20g apple cider vinegar Route of administration: oral Control: sweetened water	glucose, insulin
Johnston et al. 2010 (study 1)	4 male, 6 female, healthy volunteers Age: 35 BMI: 27.5	RCT, crossover Duration: 120 minutes Intervention:2-20g apple cider vinegar Route of administration: oral Control: unspecified placebo	glucose
Johnston et al. 2010 (study 2A)	2 male, 7 female, healthy volunteers	RCT, crossover Duration: 120 mins	glucose

	Age: 50 BMI: 33.7	Intervention: 20g raspberry vinegar Route of administration: oral Control: unspecified placebo	
Johnston et al. 2010 (study 3)	2 males, 8 females, healthy volunteers Age: 38 BMI: 26.3	RCT, crossover Duration: 120 mins Intervention: 20g apple cider vinegar Route of administration: oral Control: unspecified placebo	glucose
Johnston et al. 2010 (study 4)	4 males, 5 females, healthy volunteers Age: 69 BMI: 31.4	RCT, crossover Duration: 120 mins Intervention: 20g apple cider vinegar Route of administration: oral Control: unspecified placebo	glucose
Leeman et al. 2005	3 males, 10 females, healthy volunteers Age: 19-32 BMI: 22.5	RCT, crossover Duration: 120 mins Intervention: 28g white vinegar Route of administration: oral Control: boiled potatoes	glucose, insulin
Liatis et al. 2010; A	3 males, 5 females with T2D Age: 57.4 BMI: 29.8	RCT, crossover Duration: 120 mins Intervention: 20g wine vinegar (high GI 86/100) Route of administration: oral Control: high GI meal	glucose, insulin
Liatis et al. 2010; B	4 males, 4 females with T2D Age: 61.4 BMI: 30.1	RCT, crossover Duration: 120 mins Intervention: 20g wine vinegar (low GI 38/100) Route of administration: oral Control: low GI meal	glucose, insulin

Östman et al. 2005	10 male, 2 female, healthy volunteers Age: 22.9 BMI: 21.5	RCT, crossover Duration: 120 mins Intervention: 20g white vinegar Route of administration: oral Control: white bread	glucose, insulin
Mettler et al. 2009	9 male, 18 female healthy volunteers Age: 26 BMI: 22.5	RCT, crossover Duration: 120 minutes Intervention: unspecified vinegar type Route of administration: oral Control: 75g vanilla milk rice + cinnamon	glucose
Mitrou et al. 2015a	4 males, 4 females with impaired glucose tolerance Age: 46 BMI: 30	RCT, crossover Duration: 300 minutes Intervention: 30 mL wine vinegar Route of administration: oral Control: water	glucose, insulin, skeletal muscle glucose uptake
Mitrou et al. 2015b	4 males, 7 females with T2D Age: 53 BMI: 25	RCT, crossover Duration: 300 minutes Intervention: 30 mL unspecified vinegar Route of administration: oral Control: water	glucose, insulin, skeletal muscle glucose uptake
Zhao et al. 2020	15 male healthy volunteers Age: 23.6 BMI: 20.3	RCT, crossover Duration: 200 minutes Intervention: 30g black rice vinegar Route of administration: oral Control: white rice + dried apple	glucose
Chronic Vinegar			

Ali et al. 2019	26 males, 29 females with T2D Age: 30-60 years BMI: NI	RCT, parallel Duration: 10 weeks Intervention: 20 mL date vinegar Route of administration: oral Control: usual diet, honey in water	fasting glucose, HbA1c
Derakhshandeh-Rishehri et al. 2014	32 male, 40 female healthy volunteers Age: 31.6 BMI: 25.3	RCT, parallel Duration: 4 weeks Intervention: unspecified vinegar Route of administration: oral Control: honey, normal diet	fasting glucose, fasting insulin, HOMA-IR
Gheflati et al. 2019	20 males, 42 females with T2D Age: 50.8 BMI: 29	RCT, parallel Duration: 8 weeks Intervention: 20 mL apple vinegar Route of administration: oral Control: normal diet	Fasting glucose, fasting insulin, HOMA-IR, QUICKI
Hosseini et al. 2011	15 males, 15 females with T2D Age: 30-60 BMI: NR	Parallel Duration: 4 weeks Intervention: 15 mL unspecified vinegar Route of administration: oral Control: usual diet, water	fasting glucose, HbA1c
Jasbi et al. 2019	41 male, 4 female healthy volunteers Age: 29.9 BMI: 28.2	RCT, parallel Duration: 8 weeks Intervention: 60 mL red wine vinegar Route of administration: oral Control: apple cider vinegar tablet	Fasting glucose, fasting insulin, HOMA-IR
White and Johnston 2007	4 males, 7 females with T2D	Crossover	Fasting glucose

	Age: 40-72 BMI: 29.1	Duration: 30mL apple cider vinegar Intervention: 2 days Route of administration: oral Control: usual diet, water	
Chronic Butyrate			
Roshanravan et al. 2017	10 males, 20 females with T2D Age: 49 BMI: 30.3	RCT, parallel Duration: 45 days Intervention: 600 mg sodium butyrate, 10g inulin Route of administration: oral Control: unspecified placebo	Fasting glucose, fasting insulin, HOMA-IR, HbA1c
Roshanravan et al. 2018	10 males, 20 females with T2D Age: 49 BMI: 30.3	RCT, parallel Duration: 45 days Intervention: 600 mg sodium butyrate, 10g inulin Route of administration: oral Control: unspecified placebo	QUICKI
Acute Propionate			
Adler et al. 2021	12 male, 15 female healthy volunteers Age: 30 BMI: 26.7	RCT, crossover Duration: 240 minutes Intervention: 1.5g calcium propionate Route of administration: oral Control: calcium carbonate	glucose
Byrne et al. 2016	20 male healthy volunteers Age: 52 BMI: 25.2	RCT, crossover Duration: 360 minutes Intervention: 10g inulin propionate ester Route of administration: oral Control: inulin	Glucose, insulin

Chambers et al. 2018	9 male, 9 female healthy volunteers Age: 50 BMI: 30.5	RCT, crossover Duration: 180 minutes Intervention: sodium propionate tablet Route of administration: oral Control: sodium chloride tablet	Glucose, insulin
Darwiche et al. 2001	5 male, 4 female healthy volunteers Age: 32 BMI: 23.6	RCT, crossover Duration: 125 minutes Intervention: 1.85g propionate within bread Route of administration: oral Control: bread	Glucose, insulin
Darzi et al. 2012	9 male, 11 female healthy volunteers Age: 25 BMI: 23.1	RCT, crossover Duration: 180 minutes Intervention: 43.8g sourdough bread Route of administration: oral Control: bread	Glucose, insulin
Laurent et al. 1995	3 male, 3 female healthy volunteers Age: 22 BMI: 21.2	RCT Duration: 300 mins Intervention: 4 mmol/h Route of administration: intragastric infusion Control: saline	Glucose, insulin
Tirosh et al. 2019	9 male, 5 female healthy volunteers Age: 41 BMI: 23.7	RCT, crossover Duration: 240 mins Intervention: 1g calcium propionate Route of administration: oral Control: unspecified placebo	Glucose, insulin
Todesco et al. 1991	3 male, 3 female healthy volunteers Age: 32	Duration: 180 minutes Intervention: 3.3g sodium propionate	glucose

	BMI: 22.5	Route of administration: oral Control: bread	
Chronic Propionate			
Byrne et al. 2019	9 male, 12 female healthy volunteers Age: 18-65 BMI: NR	RCT, crossover Duration: 1 week Intervention: 10g inulin propionate ester Route of administration: GIT Control: bread and smoothie	Glucose, insulin
Chambers et al. 2015	19 female, 30 male healthy volunteers Age: 54 BMI: 32.5	RCT, parallel Duration: 24 weeks Intervention: 10g inulin-propionate ester Route of administration: GIT Control: inulin	Fasting insulin, HOMA-IR, HbA1c
Chambers et al. 2019	3 male, 6 female healthy volunteers Age: 60 BMI: 29.8	RCT, crossover Duration: 6 weeks Intervention: 20g inulin propionate ester Route of administration: GIT Control: cellulose	Glucose, insulin, HOMA-IR
Pingitore et al. 2017	19 male, 30 female healthy volunteers Age: 53 BMI: 32.5	RCT, parallel Duration: 24 weeks Intervention: 10g inulin-propionate ester Route of administration: GIT Control: inulin	Glucose, insulin
Todesco et al. 1991	3 male, 3 female healthy volunteers Age: 32 BMI: 22.5	RCT, crossover Duration: 1 week Intervention: 9.9g sodium propionate bread Route of administration: oral Control: white bread	glucose

Venter et al. 1990	20 female healthy volunteers Age: 20-21 BMI: 18-22.5	Paired comparison Duration: 7 weeks Intervention: 7.5g sodium propionate Route of administration: oral Control: calcium phosphate	Glucose, insulin
Acute mixed SCFAs			
Alamowitch et al. 1996	6 healthy volunteers Age: 26 BMI: 20.9	RCT, crossover Duration: 18 hours Intervention: Acetate: 60, propionate: 25, butyrate: 15 mmol/L Route of administration: ileal infusion Control: saline	Basal hepatic glucose production, insulin sensitivity
Canfora et al. 2017	12 male, healthy volunteers Age: 36 BMI: 25 - 35	RCT, crossover Duration: 300 minutes Intervention: 200 mmol/L (high acetate: 24, 8, 8 mmol/L) (high butyrate: 8, 8, 24 mmol/L) (high propionate: 8, 24, 8 mmol/L) Route of administration: rectal infusion Control: saline	Glucose, insulin, carbohydrate oxidation
Laurent et al. 1995	3 male, 3 female healthy volunteers Age: 22 BMI: 21.2	RCT, crossover Duration: 300 mins Intervention: Acetate 12 mmol/h Propionate 4 mmol/h Route of administration: gastric infusion Control: saline	Glucose, insulin
Wolever et al. 1989	3 male, 3 female healthy volunteers Age: 33	RCT, crossover Duration: 120 minutes	Glucose, insulin

	BMI: NR	Intervention: Acetate 90 mmol/L + 30 mmol/L Propionate or Acetate 180 mmol/L + 60 mmol/L Propionate Route of administration: rectal infusion Control: saline	
Wolever et al. 1991	6 healthy volunteers Age: 29 BMI: 24.1	RCT, crossover Duration: 120 minutes Intervention: Acetate (180 mmol/L) + propionate (60 mmol/L) Route of administration: rectal infusion Control: saline	Glucose, insulin
BMI, body mass index; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; NR, not reported; RCT, randomised controlled trial; QUICKI, quantitative insulin-sensitivity check index			

2.5.4 **Risk of Bias Assessment**

The risk of bias assessment indicated that most included studies had a high risk of bias (47%) or some concerns over risk of bias (44%). Only 9% of included studies had a low risk of bias. Domains where bias was more prevalent were risk of bias arising from deviations from intended interventions (D2.2), in measurements of the outcome (D4) and the selection of the reported results (D5). The risk of bias assessment for each publication can be found in **Figure 2.2.**

Figure 2.2: Risk of bias assessment for included publications.

Study name	D1	D2.1	D2.2	D3	D4	D5	Overall
ACETATE							
Freeland 2010	-	+	x	+	+	+	x
Johnston 2010	+	+	+	+	-	+	-
Laurent 1995	-	+	+	+	+	-	-
Scheppach 1988	-	-	x	x	-	-	x
Van der Beek 2016	+	+	+	+	+	+	+
BUTYRATE							
Roshanravan 2017	+	-	+	+	-	-	x
Roshanravan 2018	+	-	+	+	-	-	x
PROPIONATE							
Adler 2021	+	+	+	-	+	-	-
Byrne 2016	+	+	+	+	+	-	-
Byrne 2019	+	+	+	+	+	+	+
Chambers 2015	+	+	-	-	+	+	-
Chambers 2018	-	+	+	+	+	+	-
Chambers 2019	+	-	x	+	+	+	x
Darwiche 2001	+	+	+	+	x	-	x
Darzi 2012	+	-	+	+	-	+	-
Laurent 1995	-	+	+	+	+	-	-
Pingitore 2017	+	+	-	-	+	+	-
Tirosh 2019	-	+	+	+	+	-	-
Todesco 1991	-	+	+	+	x	-	x
Venter 1990	+	+	-	+	+	-	-
MIXED							
Alamowitch 1996	+	+	+	+	+	+	+
Canfora 2017	+	+	+	+	+	+	+
Laurent 1995	-	+	+	+	+	-	-
Wolever 1988	-	-	+	+	+	+	-
Wolever 1991	-	+	-	+	-	+	x
VINEGAR							
Ali 2019	+	-	+	+	-	+	-
Brighenti 1995	-	-	-	+	-	+	x
Darzi 2014	+	+	+	+	x	+	x
Derakhshandeh-Rishehri 2014	-	x	x	-	+	+	x
Feise 2020	-	-	+	+	-	+	x
Gheflati 2019	-	+	+	+	-	+	-
Hlebowicz 2008	-	+	+	+	x	+	x
Hosseini 2011	-	-	x	+	x	+	x
Jasbi 2019	-	+	-	+	-	+	x
Johnston 2010	+	+	+	+	-	+	-
Johnston 2004	-	-	-	+	-	-	x
Johnston 2005	-	-	-	+	-	+	x
Leeman 2005	-	+	-	+	+	+	-
Liatis 2010	-	+	-	-	+	+	x
Mettler 2009	+	-	+	+	-	-	x
Mitrou 2015a	-	+	+	+	+	+	-
Mitrou 2015b	-	+	-	+	+	+	-
Ostman 2005	-	+	+	+	+	+	-
White 2007	-	+	-	+	x	+	x
Zhao 2020	+	+	+	+	x	+	x

Judgement	
+	Low
-	Some concerns
x	High

Risk of Bias...	
D1	...arising from randomization process
D2.1	...due to deviations from the intended interventions (asssignment to the intervention)
D2.2	...due to deviations from the intended interventions (effect of adhering to intervention)
D3	...due to missing outcome data
D4	...in measurement of outcome
D5	...in selection of reported result

2.5.1 **The effect of acute SCFA interventions on glycaemic response**

The effect of acute SCFA administration on postprandial glucose and insulin was investigated using acetate, vinegar, propionate and mixed SCFA.

2.5.1.1 The effect of acute SCFA interventions on postprandial glucose

Seven studies (n=44), were included in the acute acetate meta-analysis (Freeland & Wolever, 2010; C. S. Johnston et al., 2010; Laurent et al., 1995; Scheppach, Cummings, et al., 1988; Van der Beek et al., 2016). Acetate administered orally or via intravenous, gastric, colonic (proximal and distal) or rectal infusions, had no significant effect on postprandial glucose [SMD (95% CI) = 0.09 (–0.26, 0.44)] as shown in **Figure 2.3**.

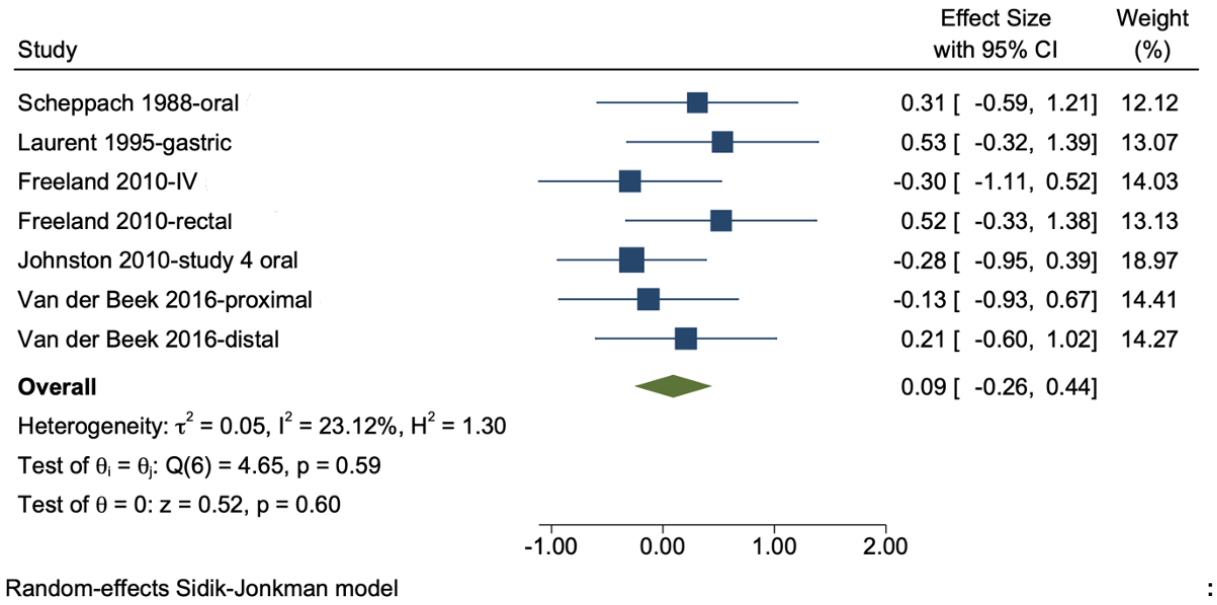


Figure 2.3: Forest plot of effect sizes for studies assessing the effect of acute acetate administration on postprandial glucose concentrations in healthy individuals.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Method of administration denoted as -oral, -gastric, -IV, -rectal, -proximal/distal (colon). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. IV, intravenous.

15 studies (n=186), investigated the effect of vinegar on acute postprandial glucose response in healthy individuals (Brighenti et al., 1995; Darzi et al., 2014; Feise & Johnston, 2020; Hlebowicz et al., 2007; C. S. Johnston et al., 2004, 2010; C. S. Johnston & Buller, 2005; Leeman et al., 2005; Mettler et al., 2009; Östman et al., 2005; W. Zhao et al., 2020). Orally consumed vinegar, a dietary form of acetate, had a significant effect on postprandial glucose in healthy individuals [-0.27 (-0.54, 0.00)]. Interstudy heterogeneity was significant (I^2= 66.21%; p=0.00). Seven studies from five publications (n=67) investigated the effect of vinegar on acute postprandial glucose response in individuals with prediabetes or T2D (C. S. Johnston et al., 2004, 2010; Liatis et al., 2010; Mitrou et al., 2015a, 2015b). Orally

administered vinegar appears to have a significant effect on postprandial glucose in participants with pred or T2D [-0.53 (-0.92, -0.14)]. Results are presented in forest plots in **Figures 2.4 and 2.5.**

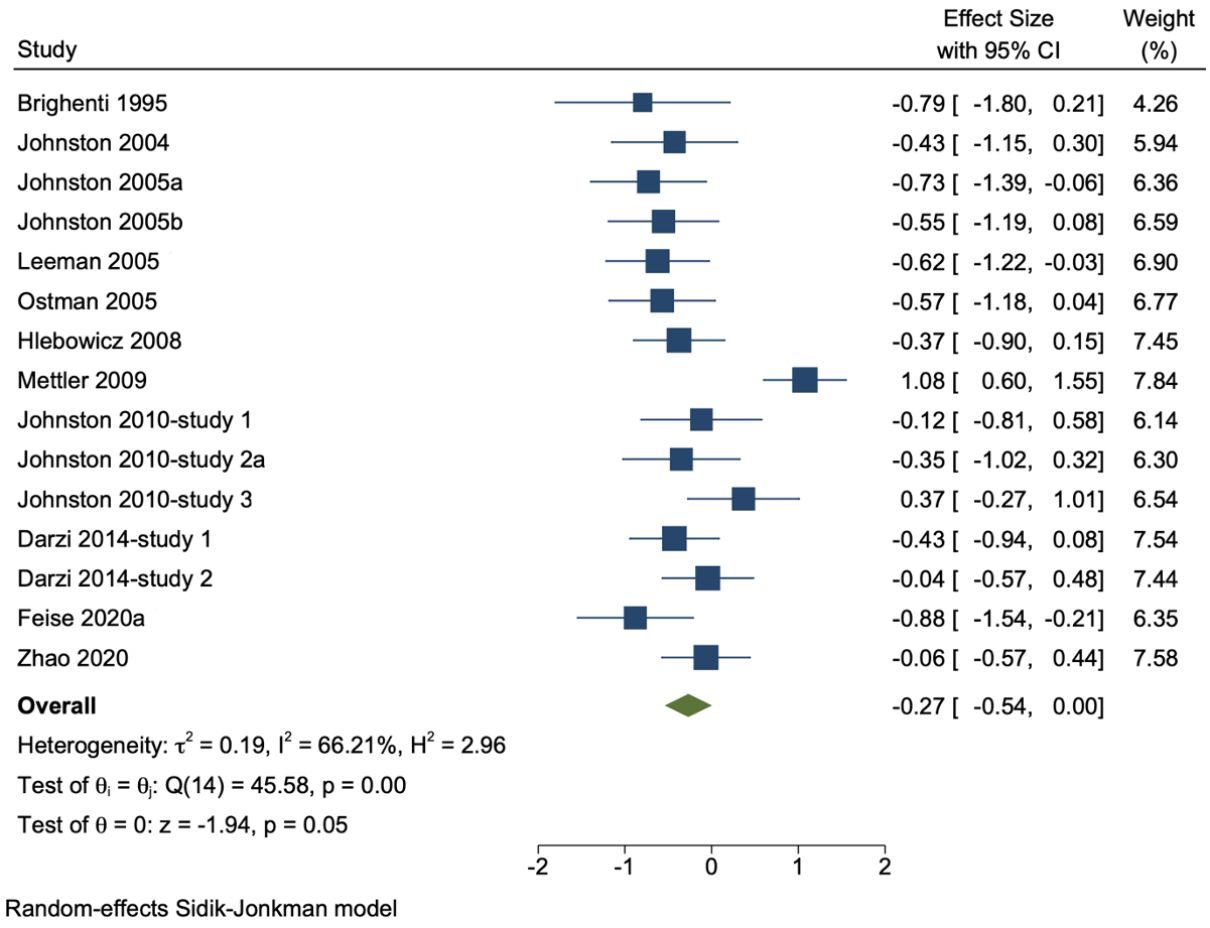


Figure 2.4: Forest plot of effect sizes for studies assessing the effect of acute vinegar intake on postprandial glucose concentrations in healthy individuals.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. A and b denote two studies conducted in the same year. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

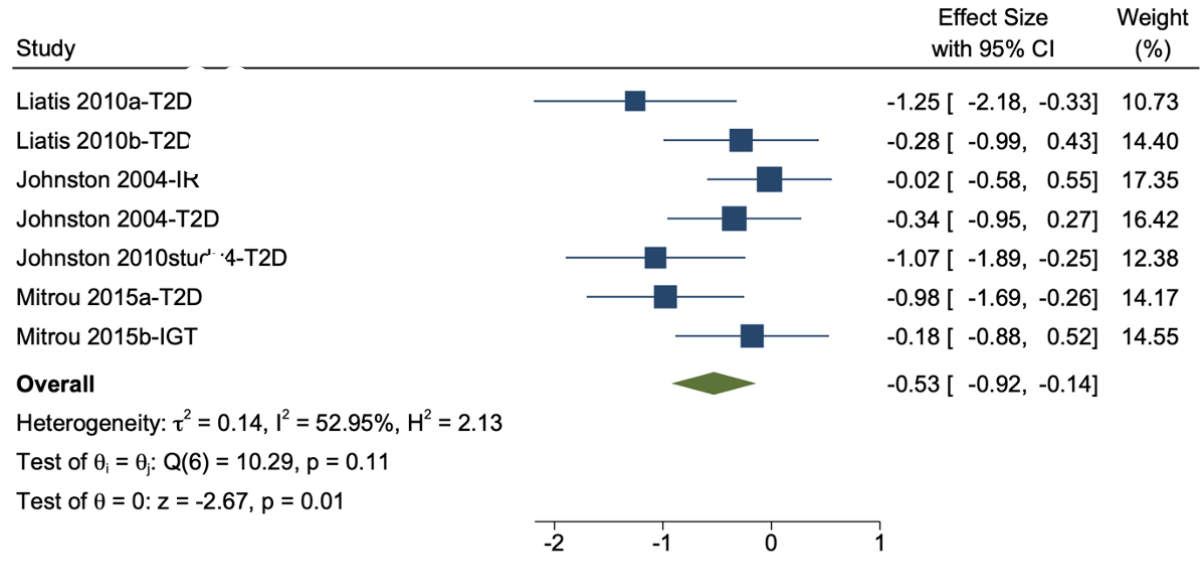


Figure 2.5: Forest plot of effect sizes for studies assessing the effect of acute vinegar intake on postprandial glucose concentrations in individuals with prediabetes or T2D.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. A and b denote sub-studies. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. IGT, impaired glucose tolerance; IR, insulin resistance; T2D, type 2 diabetes.

Eight studies (n=123) explored the acute effect of propionate on postprandial glucose response (G. K. Adler et al., 2021; Byrne et al., 2016; Chambers et al., 2018; Darwiche et al., 2001; Darzi et al., 2012; Laurent et al., 1995; Tirosh et al., 2019; Todesco et al., 1991). Propionate does not appear to have a significant effect on postprandial glucose response [0.07, (-0.32, 0.47)] as seen in **Figure 2.6**.

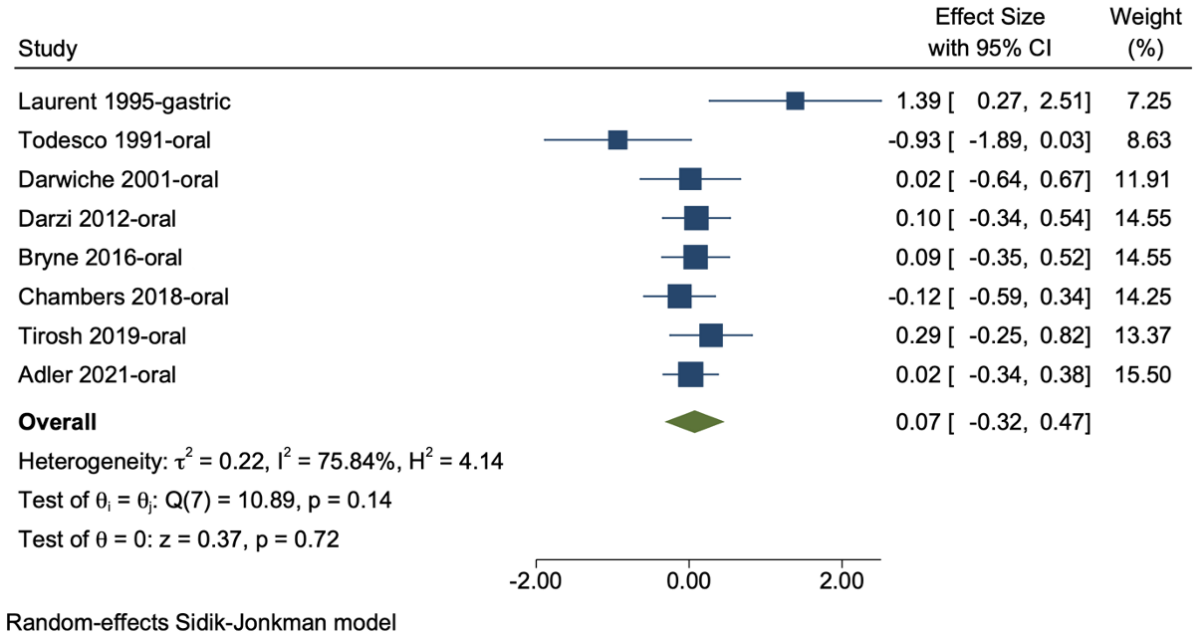


Figure 2.6: Forest plot of effect sizes for studies assessing the effect of acute propionate administration on postprandial glucose concentrations in healthy individuals.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Methods of administration denoted as -oral or -gastric. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

Five studies investigated the effects of mixed SCFA infusions on postprandial glucose (Alamowitch et al., 1996; Canfora et al., 2017; Laurent et al., 1995; Wolever et al., 1989, 1991). Wolever and colleagues rectally infused different ratios of SCFA mixtures: acetate at 180 mmol/L and propionate at 60 mmol/L or acetate at 90 mmol/L and propionate at 30 mmol/L into six healthy participants, with saline, used as a control (Wolever et al., 1989). Neither SCFA combination had a significant effect on blood glucose, compared to the control. In a separate study, the same team combined acetate (180 mmol/L) and propionate (60 mmol/L) and compared this condition to propionate, acetate or saline infusions alone (Wolever et al., 1991). Similarly, Laurent and colleagues compared the effects of acetate and propionate to acetate, propionate or saline solution administered via gastric infusion (Laurent et al., 1995). No significant changes in blood glucose were observed. In another study, healthy-weight males received an 18-hour ileal perfusion of SCFAs (acetate; 60 mmol/L, propionate; 25 mmol/L and butyrate; 15 mmol/L) compared to an ileal perfusion of saline and observed no differences basal hepatic glucose production (Alamowitch et al., 1996). Canfora and colleagues rectally infused 200 mmol/L mixtures of SCFA which were higher in either acetate, propionate or butyrate (Canfora et al., 2017). No significant differences were seen between any interventions or compared with the placebo.

2.5.1.2 The effect of acute SCFA interventions on postprandial insulin

Six studies (n=35), from four publications explored the effect of acetate administration via oral, intravenous, gastric, colonic or rectal infusions on postprandial insulin response (Freeland & Wolever, 2010; Laurent et al., 1995; Scheppach, Cummings, et al., 1988; Van der Beek et al., 2016). Acetate was found to have no significant effect on postprandial insulin [0.35 (-0.07, 0.77)] as shown in the forest plot in **Figure 2.7**.

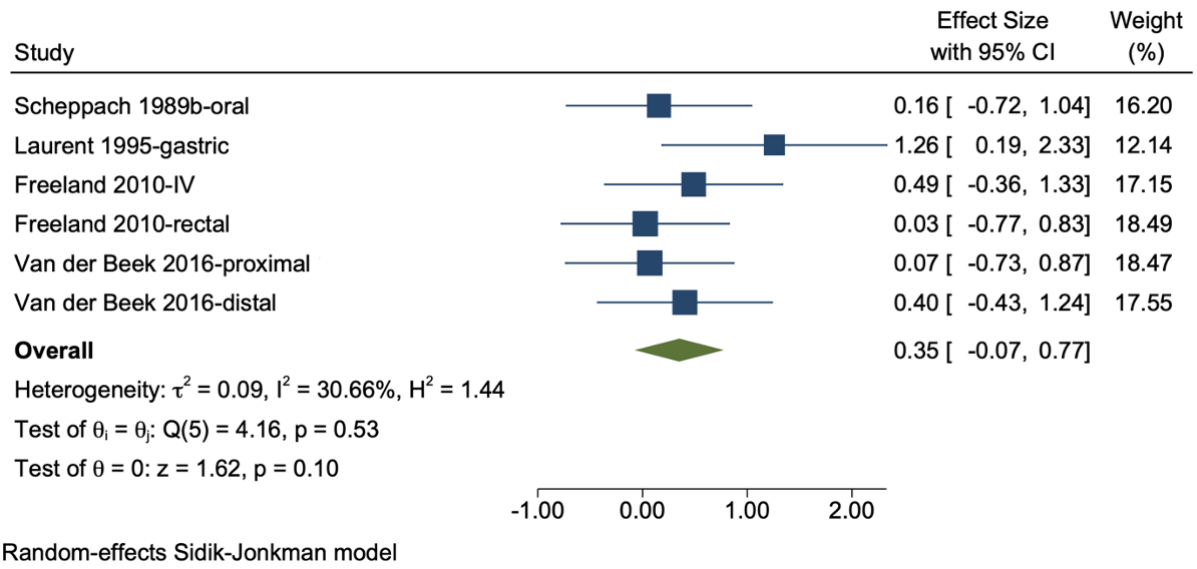


Figure 2.7: Forest plot of effect sizes for studies assessing the effect of acute acetate administration on postprandial insulin concentrations.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Method of administration denoted as -oral, -gastric, -IV, -rectal, -proximal/distal (colon). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

The intake of vinegar and its effect on postprandial insulin response in healthy volunteers (n=55), was explored in five studies (C. S. Johnston et al., 2004; C. S. Johnston & Buller, 2005; Leeman et al., 2005; Östman et al., 2005). Oral supplementation of vinegar does not affect postprandial insulin in healthy individuals [-0.29 (-0.66, 0.08)]. Vinegar consumption was investigated in six studies (n=58) on participants with T2D and prediabetes (C. S. Johnston et al., 2010; Liatis et al., 2010; Mitrou et al., 2015a, 2015b). The meta-analysis found no significant effects on postprandial insulin [-0.16 (-0.75, 0.44)]. Interstudy heterogeneity was significant in the meta-analysis on vinegar supplementation in participants with T2D and prediabetes (I^2=77.03%; p=0.00) as seen in the forest plots below (**Figures 2.8, 2.9)**.

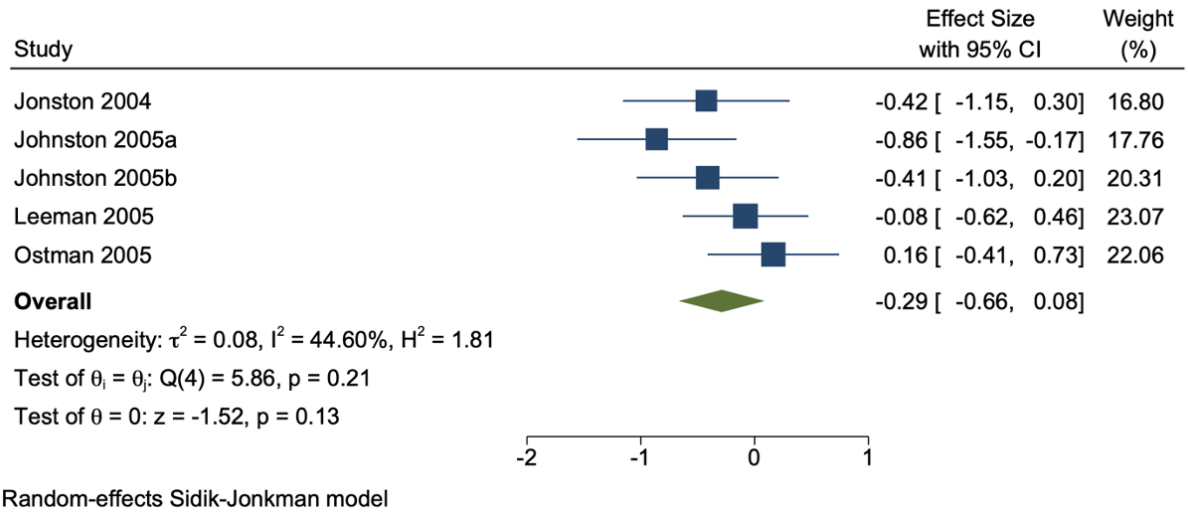


Figure 2.8: Forest plot of effect sizes for studies assessing the effect of acute vinegar consumption on postprandial insulin concentrations in healthy individuals.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. A and b denote sub-studies. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

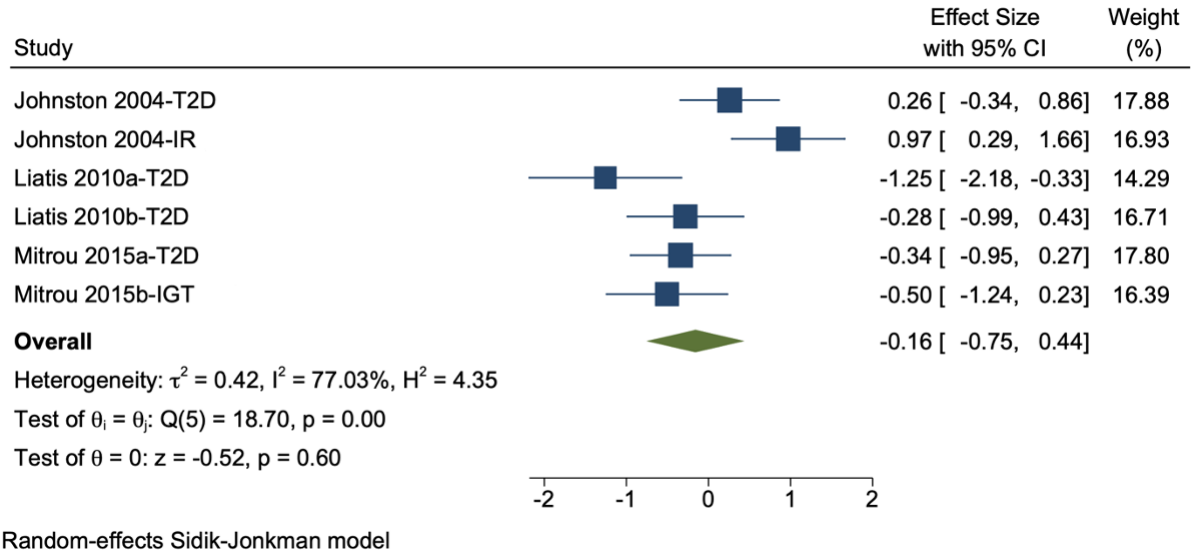


Figure 2.9: Forest plot of effect sizes for studies assessing the effect of acute vinegar consumption on postprandial insulin concentrations in individuals with prediabetes or T2D.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. A and b denote sub-studies. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. IGT, impaired glucose tolerance; IR, insulin resistance; T2D, type 2 diabetes

Oral and gastric administration of propionate and its effect on postprandial insulin was explored in seven studies (n=117) (G. K. Adler et al., 2021; Byrne et al., 2016; Chambers et al., 2018; Darwiche et al., 2001; Darzi et al., 2012; Laurent et al., 1995; Tirosh et al., 2019). The meta-analysis indicates that there is no significant effect on postprandial insulin after acute propionate administration [0.24 (-0.30, 0.78)] as seen in **Figure 2.10**.

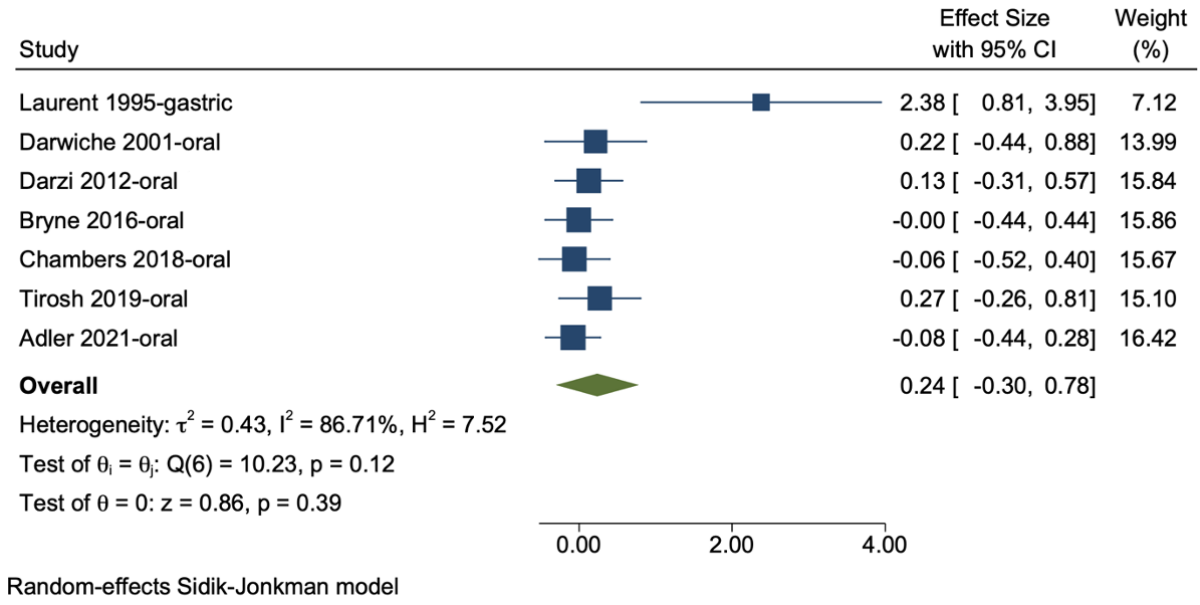


Figure 2.10: Forest plot of effect sizes for studies assessing the effect of acute propionate administration on postprandial insulin concentration.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Methods of administration denoted as -oral or -gastric. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

Five studies investigated the effects of mixed SCFA infusions on postprandial insulin (Alamowitch et al., 1996; Canfora et al., 2017; Laurent et al., 1995; Wolever et al., 1989, 1991). Concentrations and administration methods have been described in **Section 2.5.4.1**. Three studies reported no significant differences between interventions of mixed SCFA infusions compared to saline (Alamowitch et al., 1996; Laurent et al., 1995; Wolever et al., 1989) or unknown placebo control (Canfora et al., 2017). However, Wolever and colleagues reported lowered insulin secretion after administrating acetate (180 mmol/L) and propionate (60 mmol/L) compared to propionate, acetate or saline infusions alone (Wolever et al., 1991).

2.5.2 **The effect of chronic SCFA interventions on glycaemic response**

The effect of chronic SCFA administration on postprandial and fasting glucose and insulin was investigated using vinegar, propionate and butyrate.

2.5.2.1 The effect of chronic SCFA interventions on glucose response

The effect of chronic effect of vinegar supplementation on fasting blood glucose was investigated in six studies (n=143) (Ali et al., 2019; Derakhshandeh-Rishehri et al., 2014; Gheflati et al., 2019; Hosseini et al., 2011; Jasbi et al., 2019; A. M. White & Johnston, 2007). The meta-analysis indicated that chronic vinegar supplementation has no significant effect on fasting blood glucose [-1.60 (-4.30, 1.09)] and substantial heterogeneity (I^2=99.04%; p=0.00) as seen in **Figure 2.11**.

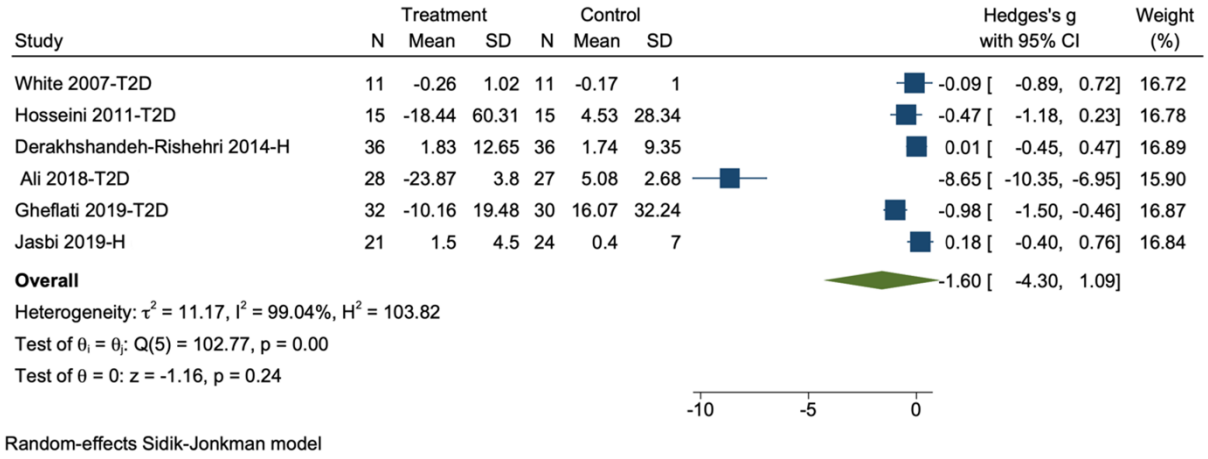


Figure 2.11: Forest plot of effect sizes for studies assessing the effect of chronic vinegar supplementation administration on fasting blood glucose.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. H, healthy volunteers; T2D, type 2 diabetes.

The effect of chronic propionate supplementation on postprandial and fasting blood glucose was investigated via oral supplementation and the delivery of propionate to the lower gastrointestinal tract via ingestion of the compound inulin-propionate ester (IPE). The effect on postprandial glucose response was reported in five studies (n=73) (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Todesco et al., 1991; Venter et al., 1990) and four of these studies (n=67), reported fasting glucose response (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Venter et al., 1990). No overall effects were seen for postprandial glucose [-0.08 (-0.43, 0.27)] a shown in **figure 2.12** nor fasting blood glucose [-0.14 (-0.47, 0.19)] seen in **figure 2.13**.

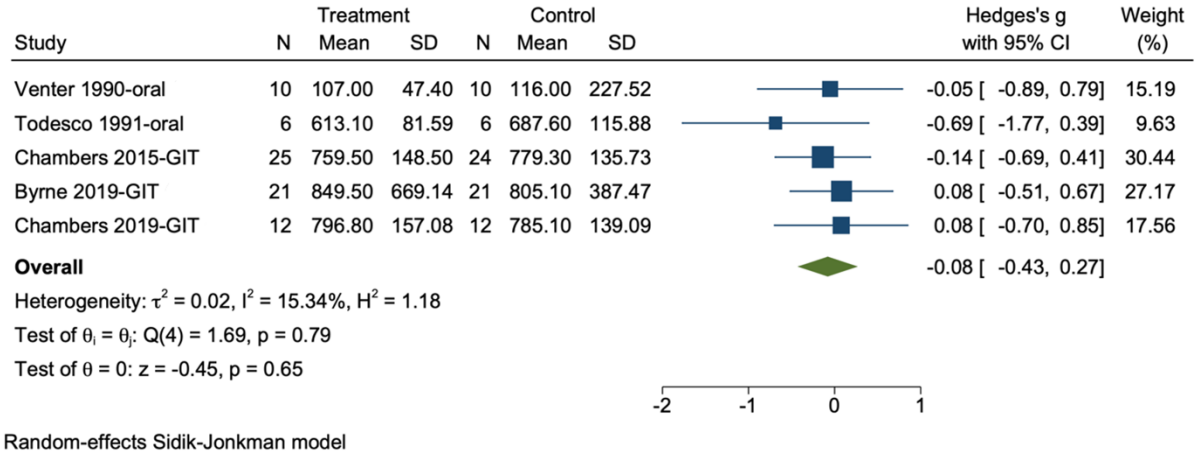


Figure 2.12: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on postprandial blood glucose.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. GIT, gastrointestinal tract.

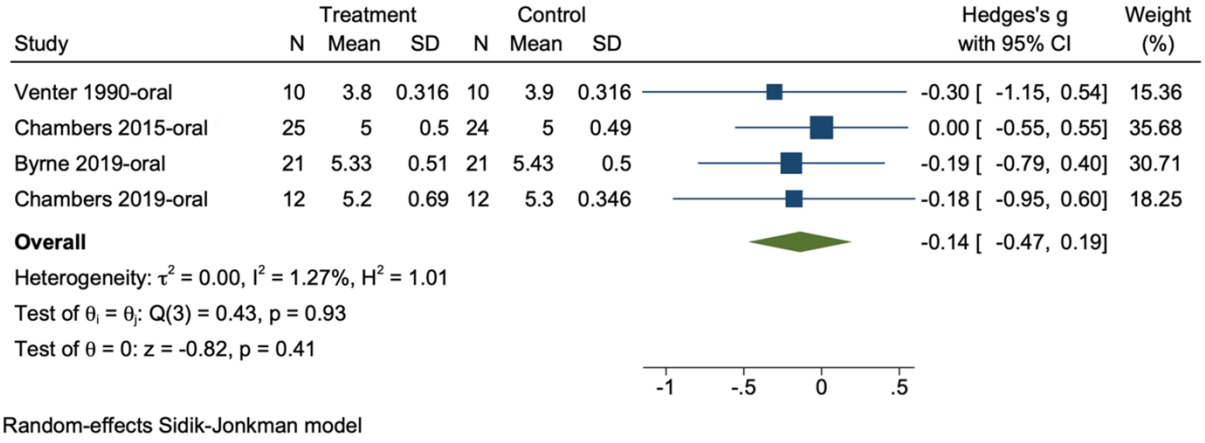


Figure 2.13: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on fasting blood glucose.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Only oral method of administration. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

The effect of chronic butyrate supplementation on glucose response was explored in one study (Roshanravan et al., 2017). Out of the three intervention groups, sodium butyrate, inulin or sodium butyrate and inulin, only a combination inulin and sodium butyrate supplementation significantly lowered fasting blood glucose compared with the starch placebo.

2.5.2.2 The effect of chronic SCFA interventions on insulin response

The effect of chronic vinegar supplementation on fasting insulin was explored in three studies (n=89) (Derakhshandeh-Rishehri et al., 2014; Gheflati et al., 2019; Jasbi et al.,

2019). No significant effect [0.06 (-0.50, 0.62)] and significant heterogeneity (I^2=72.24%; p=0.03), were seen in the meta-analysis, shown in the forest plot below (**figure 2.14**).

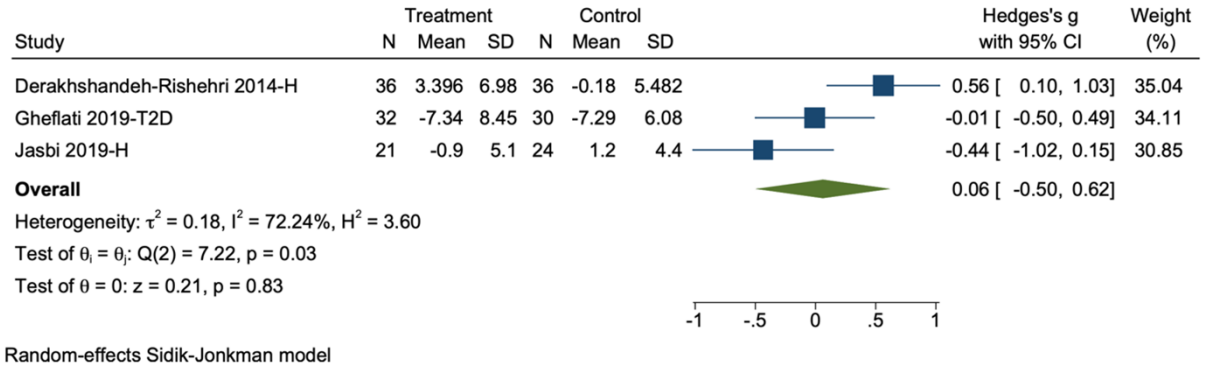


Figure 2.14: Forest plot of effect sizes for studies assessing the effect of chronic vinegar supplementation administration on fasting insulin.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. A and b denote sub-studies. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. H, healthy; T2D, type 2 diabetes.

Postprandial and fasting insulin after chronic propionate supplementation were both investigated in the same four studies (n=67) (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Venter et al., 1990). The meta-analyses identified no significant overall effects for postprandial [-0.06 (-0.39, 0.287)] or fasting [-0.22 (-0.65, 0.21)] insulin, as shown in **Figures 2.15** and **2.16**, respectively.

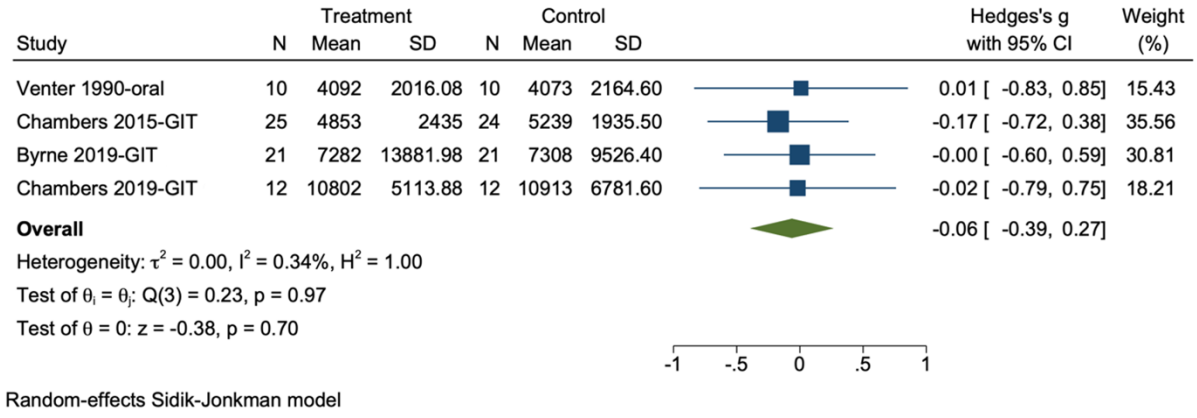


Figure 2.15: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on postprandial insulin response.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Methods of administration denoted as -oral or -GIT. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. GIT, gastrointestinal tract.

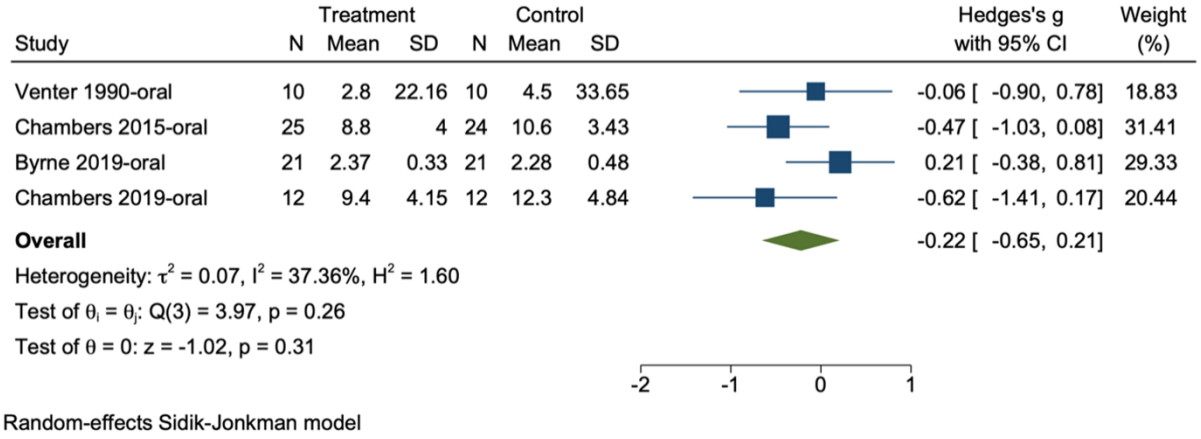


Figure 2.16: Forest plot of effect sizes for studies assessing the effect of chronic propionate supplementation administration on fasting insulin.

Data are presented as SMD ± 95% CI. Random-effects Sidik-Jonkman model. Only oral method of administration. The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

In the one study that investigated chronic butyrate supplementation, no significant changes in insulin were reported compared with the starch control (Roshanravan et al., 2017).

2.5.2.3 Chronic SCFA supplementation and measures of insulin sensitivity

Three studies explored the effects of vinegar on HOMA-IR. Two studies found that after chronic vinegar supplementation, HOMA-IR significantly decreased (Gheflati et al., 2019; Jasbi et al., 2019) and one study found that HOMA-IR significantly increased (Derakhshandeh-Rishehri et al., 2014). One study also reported a significant reduction in HOMA-B after vinegar intake (Gheflati et al., 2019).

Two studies explored the effect of chronic propionate supplementation on HOMA-IR, one reported a significantly lower HOMA-IR in the propionate intervention compared with the cellulose control (Chambers, Byrne, Morrison, et al., 2019) whilst the other reported no significant difference (Chambers, Viardot, et al., 2015).

One study investigated the effect of butyrate on HOMA-IR and found no significant change (Roshanravan et al., 2017).

Two studies investigated insulin sensitivity using Quantitative insulin sensitivity check index (QUICKI). Chronic vinegar supplementation was reported to induce a significant reduction in QUICKI (Gheflati et al., 2019), whereas butyrate was reported to have no significant effect (Roshanravan et al., 2017).

Two studies reported either a trend towards a reduction (Ali et al., 2019) or a significant decrease (Hosseini et al., 2011) in HbA1c post-vinegar supplementation. Two studies

reported no significant difference in HbA1c after chronic propionate (Chambers, Viardot, et al., 2015), or chronic butyrate intake (Roshanravan et al., 2017).

2.5.2.4 SCFA supplementation and gut hormone secretion

Three studies investigated the effect of acetate on gut hormone secretion (Freeland & Wolever, 2010; Scheppach, Cummings, et al., 1988; Van der Beek et al., 2016). In one study, a significant increase in GLP-1 and PYY was reported compared to the saline control, which was greater when acetate was administered rectally (Freeland & Wolever, 2010). Van der Beek and colleagues reported a significant increase in PYY after distal colonic infusions of acetate, but no significant difference in GLP-1. One study reported that acetate had no effect GIP excursion (Scheppach, Cummings, et al., 1988).

One study reported that oral supplementation of sodium butyrate and sodium butyrate with inulin induced an increase in GLP-1 concentration (Roshanravan et al., 2017).

The effect of propionate and gut hormones was investigated in five studies (Byrne et al., 2016, 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017). Four studies investigated the effect of IPE supplementation on gut hormone release and found no significant difference in PYY and/or GLP-1 compared to the control (Byrne et al., 2016, 2019; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017). One study found a significant increase in GLP-1 and PYY excursion after acute IPE intake, however no significant effect was identified after chronic supplementation of IPE (Chambers, Viardot, et al., 2015).

Canfora and colleagues reported significant increases in PYY secretion after acute infusions of high acetate, propionate and butyrate mixed SCFA infusions, no effect was seen for GLP-1 (Canfora et al., 2017).

2.5.2.5 Studies identified post-publication

Since the publication of this systematic review and meta-analysis, two new studies have been published investigating SCFA and glycaemic response in humans. The results of these publications are summarised below.

Khosravi and colleagues reported that supplementation with sodium butyrate over six weeks lowered postprandial glucose response compared with starch in individuals with T2D (Khosravi et al., 2022) . However, a significant increase in fasting insulin concentrations and HOMA-IR was also reported.

Another study investigated the effects of apple cider vinegar over eight weeks in individuals with T2D and healthy blood glucose control reported significant reductions in HbA1c in the group with T2D and no changes in individuals with normal glycaemic control (Khalid et al., 2022). This may have been associated with reductions in BMI which were also observed.

2.5.3 **Certainty of evidence**

The certainty of evidence was very low for all outcomes excluding propionate chronic postprandial glucose and mixed SCFAs. Very low certainty indicates very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect. The very low certainty arose from many studies having a high risk of bias, some publication bias, small sample sizes and high heterogeneity, which raises the question of the research integrity and comparability. Additionally, there was substantial variation in dosage, form of SCFA, study population and administration method between studies. Furthermore, intragastric, rectal and colonic infusions of SCFA are not very applicable, replicable or tolerable which means that the studies have low transferability. Inconsistencies were identified as the results had very wide 95% CIs and for many of the studies, glycaemia was not the primary outcome.

2.5.4 **Confounders of glycaemic control**

Confounders of glycaemic control should be accounted for to ensure any differences in glucose and insulin responses during the study visit are due to the interventions and to reduce inter- and intraindividual variability in glycaemic response. Confounders of glycaemic control for acute studies include fibre intake, strenuous physical activity, alcohol consumption, an overnight fast and standard meal consumption before the study day.

Five acute studies requested participants consume a low-fibre meal the day before the study visit (Alamowitch et al., 1996; Darzi et al., 2014; Laurent et al., 1995; Scheppach, Cummings, et al., 1988; Van der Beek et al., 2016). Six studies prevented participants from participating in strenuous exercise before the study visit (Darzi et al., 2012, 2014; Feise & Johnston, 2020; C. S. Johnston et al., 2010; Todesco et al., 1991; W. Zhao et al., 2020). Alcohol intake was discouraged the day before the study visit in seven studies (Byrne et al., 2016; Chambers et al., 2018; Darzi et al., 2012, 2014; Feise & Johnston, 2020; Scheppach, Cummings, et al., 1988; W. Zhao et al., 2020). Participants in all studies were requested to fast for ≥ 6 hours before the study visit, except for the study conducted by Brighenti and colleagues which provided no information (Brighenti et al., 1995).

Confounders for chronic studies include changes in body fat and total body weight and intake of a standard meal, participation in strenuous activity and alcohol intake. Of the included studies, 50% controlled for changes in body composition and weight change (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017; Roshanravan et al., 2017, 2018; Todesco et al., 1991; Venter et al., 1990). Three studies instructed participants to consume a standard meal before their visit (Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019). Four studies prevented participants from participating in strenuous activity (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017), and five studies required participants to abstain from alcohol before their visit (Ali et al., 2019; Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017).

2.5.5 **Adverse events**

To determine the safety and applicability of the intervention to a free-living population, any adverse events should have been reported and assessed for severity and expectedness. Some studies failed to report any adverse events.

From the acute studies, there were six studies which reported assessing AEs but had none (Canfora et al., 2017; Darzi et al., 2012; Mitrou et al., 2015a, 2015b; Van der Beek et al., 2016; T. Zhou et al., 2018). One case of nausea was reported for propionate (Byrne et al., 2016). For mixed SCFA administration, one study reported 6 incidences of increased belching across the control and intervention groups (Wolever et al., 1989).

From the chronic studies, three assessed AEs but had none to report (Ali et al., 2019; Chambers, Viardot, et al., 2015; Jasbi et al., 2019). Of the vinegar studies, there was one reported case of nausea, stomach-ache and headache (Derakhshandeh-Rishehri et al., 2014). One study reported 3 cases of flatulence after the consumption of bread containing IPE (Byrne et al., 2019). A separate study, also using propionate, reported cases of nausea, constipation and flatulence in both the intervention and control group and vomiting was reported in the intervention group but not the control (Venter et al., 1990).

2.5.6 **Compliance and withdrawals**

For acetate, a withdrawal rate of 40% was reported in one study (Van der Beek et al., 2016), as the researchers were not able to clip the catheter to the colonic mucosa. For acute vinegar studies, there was one study that reported 3 withdrawals due to time conflicts and a dislike for the intervention (W. Zhao et al., 2020). For chronic vinegar studies, one study had

an 18% withdrawal rate due to personal reasons, viral infections, seasonal allergies and one report of adverse events (Derakhshandeh-Rishehri et al., 2014). One study had an 8% withdrawal rate due to non-compliance with the intervention and consent withdrawal (Ali et al., 2019). One study had an 11% withdrawal rate due to vinegar intolerance, withdrawal and changes to medication (Gheflati et al., 2019). Jasbi and colleagues reported a 29% withdrawal rate due to dislike of the vinegar intervention (Jasbi et al., 2019). For chronic propionate, one participant was withdrawn due to side effects (Venter et al., 1990). Another study had an 18% withdrawal rate as the participants failed to complete the 24-week intervention period (Chambers, Viardot, et al., 2015). For Butyrate, there was a 2% withdrawal rate as the participant was lost to follow-up (Roshanravan et al., 2017, 2018).

2.6 Discussion

2.6.1 **Summarised discussion**

This systematic review and meta-analysis included 46 studies from 43 publications and a total of 913 participants. Acute vinegar intake was observed to lower postprandial glucose response compared to the control in both healthy volunteers and participants with T2D or pre-diabetes. In the meta-analyses for acute acetate and propionate administration, no significant differences were found for postprandial blood glucose and insulin. Similarly, meta-analyses for chronic propionate and vinegar intake found no significant effect on fasting and postprandial glucose or insulin. Chronic butyrate studies were insufficient and acute mixed SCFA studies were too variable to conduct a meta-analysis. No studies that explored the effect of acute butyrate, chronic acetate or chronic mixed SCFA administration on glycaemia were identified in the literature searches.

2.6.2 **Acute SCFAs and glycaemic response**

Acetate, vinegar, propionate and mixed SCFA studies could be reviewed for their acute effects on glycaemia. Doses of SCFAs varied from 12 to 200 mmol/L and the duration of the studies ranged from one to 18 hours. The acute postprandial hypoglycaemic effect of vinegar was consistent with the results of a recent meta-analysis (Shishehbor et al., 2017). However, in this meta-analysis participants were not categorised based on their health status, i.e. studies with participants with impaired glucose tolerance and healthy volunteers were pooled.

2.6.2.1 Acetate

No significant differences in postprandial glucose and insulin were found after oral administration and gastric, intravenous, or colonic infusions of acetate compared to a saline or sodium chloride control. This is considered to be consistent with prior investigations using acetate (Akanji & Hockaday, 1990; Scheppach, Wiggins, et al., 1988; Wolever et al., 1991). The administered doses and concentrations were similar to physiological volumes produced by the colonic microbiota and therefore it is likely that the observed responses reflect the normal metabolism of acetate (Freeland & Wolever, 2010; Scheppach, Cummings, et al., 1988; Van der Beek et al., 2016).

Acetate has been reported to enhance the clearance of plasma FFAs and inhibit lipolysis (Freeland & Wolever, 2010; González Hernández et al., 2019; Laurent et al., 1995; Scheppach, Cummings, et al., 1988), promoting improved insulin sensitivity (Defronzo, 2004). However, increased absorption of FFAs and the presence of acetate induces a

metabolic switch from glucose to lipid oxidation (Van der Beek et al., 2016), potentially lowering glucose uptake into tissues (Scheppach, Cummings, et al., 1988).

Acetate may also promote hepatic gluconeogenesis (Laurent et al., 1995; Scheppach, Cummings, et al., 1988), whilst reducing glycolysis (Laurent et al., 1995), resulting in the observed neutral effect on glucose production. Increased secretion of GLP-1 and PYY were reported in one study where acetate was administered intravenously (Freeland & Wolever, 2010), whereas elevations in PYY were seen in another, which administered acetate to the distal colon (Van der Beek et al., 2016). GLP-1 stimulates the postprandial release of insulin from pancreatic β-cells perhaps resulting in an improvement in insulin-mediated glucose uptake in peripheral tissues (Todd & Bloom, 2007). Increased PYY may elevate adiponectin concentrations and reduce TNF-α, an adipokine and cytokine associated with chronic low-grade inflammation and insulin resistance, as reported in two studies (Freeland & Wolever, 2010; Van der Beek et al., 2016).

Acetate may play a role in improving insulin sensitivity via multiple mechanisms and its effects should be investigated in future chronic acetate studies. However, the results lack directionality and it is unknown which site of administration is most beneficial. Scheppach and colleagues argue oral administration results in higher circulating acetate than rectal infusions (Scheppach, Cummings, et al., 1988), whereas Van der Beek and colleagues believe distal colonic infusions are superior (Van der Beek et al., 2016).

2.6.2.2 Vinegar

Vinegar, which contains acetic acid, has been reported to improve postprandial glycaemia, as reinforced by the results of this meta-analysis. However, the mechanisms are yet to be fully elucidated. In the included studies, acetic acid was administered in doses between 1 and 1.9g. Vinegar did not affect postprandial glucose in three studies (Hlebowicz et al., 2007; Mettler et al., 2009; Mitrou et al., 2015a), which was attributed to not controlling for confounders of glycaemic control or differences in food matrix used. Acetic acid is reportedly more readily absorbed in the gastrointestinal tract than acetate, which could contribute to the differential metabolic effects seen between acetate and vinegar (Brighenti et al., 1995; Feise & Johnston, 2020; González Hernández et al., 2019).

There are several mechanisms identified in the literature which may contribute to the improved postprandial glucose response. Vinegar may slow gastric emptying via duodenal receptor-mediated pathways either due to the fatty acids present or the lowered pH (Brighenti et al., 1995; Liatis et al., 2010; Liljeberg & Björck, 1998). However, slower gastric

emptying was not demonstrated in any of the included studies (Brighenti et al., 1995; Darzi et al., 2014; Hlebowicz et al., 2007; Leeman et al., 2005; Liatis et al., 2010; Östman et al., 2005; W. Zhao et al., 2020).

The organoleptic properties (e.g. vinegar's acidity), may delay the cephalic phase, lowering postprandial glycaemic response, slowing insulin and gut hormone secretion (Brighenti et al., 1995). There is some evidence that unpalatability or intolerability of a food ingredient like vinegar can affect metabolic response, for example, vinegar can be 'tasted' further down the gastrointestinal tract and these taste receptors may play a role in GLP-1 secretion (Darzi et al., 2014). Similarly, knockout of sweet taste receptor subunit T1r3 was associated with lower GLP-1 release in mice (Kokrashvili et al., 2009). However, this evidence is not conclusive (Darzi et al., 2014).

Even in cases where no change in postprandial blood glucose was reported, improvements in insulin sensitivity (C. S. Johnston et al., 2004) or insulin-mediated glucose disposal (Mitrou et al., 2015a, 2015b) were reported. This may be due to the upregulation of GLUT-4 receptors in muscle tissues or inhibition of lipolysis (González Hernández et al., 2019; Maruta et al., 2016; Mitrou et al., 2015a). Further, reductions in glycolysis and increases in glycogen repletion are also seen in rodent studies (Fushimi et al., 2001; C. S. Johnston et al., 2004; Mitrou et al., 2015a, 2015b; Ogawa et al., 2000). AMPK/cAMP-mediated increases in postprandial blood flow rate could contribute to glucose transport into peripheral tissues which tends to be lower in individuals with elevated insulin resistance (Mitrou et al., 2015a). Vinegar reportedly improved blood flow rates post-meal, compared to the control (Mitrou et al., 2015a).

There is some evidence to suggest that digestive amylases or disaccharidases are inhibited by the presence of acid from the vinegar (Hlebowicz et al., 2007; C. S. Johnston & Buller, 2005; Leeman et al., 2005; Ogawa et al., 2000).

2.6.2.3 Propionate

No significant differences were identified for postprandial glucose and insulin when acute propionate studies were meta-analysed. Propionate was administered orally in the form of sodium propionate (Chambers et al., 2018; Darwiche et al., 2001; Todesco et al., 1991), calcium propionate, propionate produced microbial fermentation in bread (Darzi et al., 2012) or IPE, to deliver propionate to the colon (Byrne et al., 2016). Two studies found that propionate had a significant effect on postprandial glucose (Darwiche et al., 2001; Todesco et al., 1991) and/or insulin (Darwiche et al., 2001).

Propionate enters the circulation via the portal vein and is processed by the liver, where 90% is oxidised or utilised by hepatocytes. This is reflected by the fact that propionate was often not present in serum samples, when acetate was, in mixed SCFA interventions (Alamowitch et al., 1996; Canfora et al., 2017; Wolever et al., 1989).

Some studies suggest propionate may upregulate endogenous glucose production. However, in a study using stable-isotopes, only 6% of propionate administered was found to be incorporated into glucose over 12 hours (Boets et al., 2017). Propionate may activate the sympathetic nervous system, indicated by significant elevations in adrenaline, which incurred amplified glucagon secretion via FFAR3 (G. K. Adler et al., 2021).

Some studies identified an increase in lipid oxidation via changes to the respiratory exchange ratio (Chambers et al., 2018), or the suppression of lipolysis in adipose tissues (Chambers et al., 2018), indicated by reductions in FFA post-intervention (Chambers et al., 2018; Darzi et al., 2012; Laurent et al., 1995). PYY elevated towards the end of the study visit in one study (Chambers et al., 2018), which could also induce a switch to lipid metabolism (Van der Beek et al., 2016).

Darwiche and colleagues used ultrasonography to identify differences in gastric emptying rate between sodium-propionate bread and normal bread. It was found that gastric emptying rates lowered after the intervention which has previously been associated with a more moderate glycaemic response (Darwiche et al., 2001; Liljeberg & Björck, 1998). Furthermore, propionate bread had a slower rate of digestibility *in vitro* (Todesco et al., 1991), which reinforces predictions that SCFA may inhibit digestive amylases (Darwiche et al., 2001; Todesco et al., 1991).

There appears to be differences in glycaemic effects seen with orally-administered propionate does compared with propionate produced through colonic microbial fermentation (Tirosh et al., 2019). Differences in the effect may partially be attributed to the signalling and absorption of SCFAs in different sites along the gastrointestinal tract (Tirosh et al., 2019).

2.6.2.4 Mixed SCFA

Infusions of mixed SCFAs were delivered to the stomach (Laurent et al., 1995), ileum (Alamowitch et al., 1996), colon (Canfora et al., 2017) and rectum (Wolever et al., 1989, 1991). No effects on glucose or insulin responses were found for the majority of the studies (Alamowitch et al., 1996; Canfora et al., 2017; Laurent et al., 1995; Wolever et al., 1989) but a significant increase in glucose was identified by Wolever and colleagues after the rectal infusion of acetate and propionate (Wolever et al., 1991). Some studies did not detect

propionate or butyrate in the circulation after the mixed infusions (Alamowitch et al., 1996; Wolever et al., 1989), as expected given that propionate is mostly metabolised in the liver (Alamowitch et al., 1996). Therefore, any changes in whole-body glucose tolerance should be attributed to acetate (Canfora et al., 2017).

Reduced insulin secretion was seen after acetate and propionate infusions (Wolever et al., 1991). Most studies found a reduction in FFAs after SCFA mixtures were administered (Alamowitch et al., 1996; Wolever et al., 1989). Interestingly, one group found propionate and acetate contributed to a larger reduction in FFA than when propionate or acetate was administered separately (Wolever et al., 1991). FFAs have an influence on insulin sensitivity and compete with glucose for substrate utilisation, thus decreases in FFAs may improve glucose disposal in peripheral tissues (Wolever et al., 1989). However, if FFAs are the preferred substrate, higher postprandial glycaemia may be maintained for longer (Wolever et al., 1989).

Furthermore, increased fat oxidation, likely related to elevated AMPK concentrations (den Besten et al., 2015; Yamashita et al., 2009), which was reported in one study could impinge upon glucose disposal in the liver and skeletal muscle (Canfora et al., 2017). Lastly, one group posited that propionate may inhibit acetate utilisation cells, therefore the ratio of SCFAs may contribute to differences in the metabolic effects seen (Wolever et al., 1991).

2.6.3 **Chronic SCFA and glycaemic response**

Vinegar, butyrate and propionate studies could be reviewed for their chronic effects on glycaemia. The duration of the studies ranged from two to 70 days. Chronic interventions with SCFA showed no pooled significant effect on fasting or postprandial glycaemia.

2.6.3.1 Vinegar

Although no significant differences were identified in the pooled effects of the meta-analysis, lowered fasting blood glucose was consistently reported after chronic vinegar supplementation (Ali et al., 2019; Gheflati et al., 2019; Hosseini et al., 2011; Jasbi et al., 2019; A. M. White & Johnston, 2007). Reduced glycated haemoglobin concentrations were seen in two studies (Ali et al., 2019; Hosseini et al., 2011) and lowered fasting insulin was also reported (Gheflati et al., 2019; Jasbi et al., 2019). These outcomes could be improved glucose disposal (Hosseini et al., 2011; A. M. White & Johnston, 2007) or lowered insulin resistance (Gheflati et al., 2019; Jasbi et al., 2019).

Researchers consistently inferred that acetic acid inhibited disaccharidase activity (Ali et al., 2019; Gheflati et al., 2019; A. M. White & Johnston, 2007) and slowed gastric emptying (Ali

et al., 2019; Gheflati et al., 2019; Hosseini et al., 2011; A. M. White & Johnston, 2007). However, this would be dependent upon whether starch-containing meals are consumed with or soon after vinegar ingestion. Some confounding variables did not appear to be accounted for, for example, a significant increase in fibre was seen in the intervention group which may have contributed to the improvements in glycaemia (Ali et al., 2019).

Vinegar contains both acetic acid and polyphenolic compounds (C. S. Johnston & Gaas, 2006), which could both affect glucose metabolism, making it difficult to disentangle which contributes to the metabolic effects reported, or whether they work in tandem. Polyphenols have been reported to reduce oxidative stress and improve scavenging of free radicals, as seen in one study which reported elevated antioxidant capacity via significant increases in 2,2-diphenyl-1-picrylhydrazyl and a trend towards lowered malondialdehyde, a marker of oxidative stress (Gheflati et al., 2019). Polyphenolic compounds may also improve insulin sensitivity via the PI3K/AKT/mTOR pathway (Martín & Ramos, 2021). Furthermore, one study compared red wine vinegar to an apple cider vinegar tablet control which did not contain polyphenols (Jasbi et al., 2019). In this study, fasting glucose, fasting insulin and insulin resistance were lower in the vinegar intervention compared with the control (Jasbi et al., 2019). Additionally, polyphenols inhibit α-amylase and α-glucosidase (Guo et al., 2018; Jasbi et al., 2019).

The types of vinegar used also varied substantially, red date, grape, apple, red wine and apple cider vinegar were used as interventions in the study and likely contain variable concentrations of acetic acid and polyphenols. One study combined grape vinegar and honey, which may not have been a comparable intervention to other studies included, as it appears to contribute 224g of sugar per day to the diet of the participants (Derakhshandeh-Rishehri et al., 2014). No significant difference in fasting blood glucose was reported for the control, however, HOMA-IR and fasting insulin were both elevated after the supplementation period (Derakhshandeh-Rishehri et al., 2014).

2.6.3.2 Butyrate

Chronic butyrate supplementation was explored in one study, separated into two publications (Roshanravan et al., 2017, 2018). When sodium-butyrate and inulin were supplemented together, fasting blood glucose and waist to hip ratio were lowered compared to the control (Roshanravan et al., 2017). However, supplementation of sodium-butyrate and sodium-butyrate and inulin resulted in significantly lower GLP-1 concentrations (Roshanravan et al., 2017). Effects from this study were attributed to increased insulin

sensitivity of adipocytes via FFAR2 (Kimura et al., 2013) and reduced whole body insulin resistance (Z. Gao et al., 2009). However, butyrate levels were not measured so there is no evidence it entered the circulation at an effective concentration. Furthermore, no significant differences were seen for QUICKI contradictory to a previous study in mice, which observed a 50% reduction in insulin resistance (Z. Gao et al., 2009; Roshanravan et al., 2018). Evidently, caution must be taken when drawing any conclusions from this study as it is the only one to investigate the effect of chronic butyrate supplementation on glycaemic responses in humans.

2.6.3.3 Propionate

Chronic propionate ingestion was explored using IPE (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Chambers, Byrne, Morrison, et al., 2019; Pingitore et al., 2017) by (10/20g dosage) and sodium propionate (Todesco et al., 1991; Venter et al., 1990). Two studies, both using sodium propionate, reported lowered postprandial (Todesco et al., 1991) and fasting glucose (Venter et al., 1990). As sodium propionate would be mostly absorbed in the upper gastrointestinal tract, whereas IPE was created to deliver propionate to the proximal colon, these results could demonstrate the effect of differing propionate absorption sites on glycaemia.

Todesco and colleagues attributed the improvements in glycaemic response to the inhibition of salivary amylase and subsequent increase in faecal bulk, post propionate bread consumption for seven days (Todesco et al., 1991). This was reinforced by reports of lowered starch digestibility *in vitro.* This research group also suggested that long-term supplementation could result in “adaptation of the absorptive capacity of the small intestine” alluding to altered rates of glucose absorption (Todesco et al., 1991).

The delivery of higher volumes of undigested starch to the colon due to inhibited amylase activity could alter the colonic microbiota. Certain species of bacteria, e.g. *Bifidobacterium* are associated with improvements in insulin sensitivity (Chambers, Byrne, Morrison, et al., 2019; Todesco et al., 1991). Researchers attributed this improvement in insulin resistance to the suppression of the proinflammatory cytokine, interleukin 8 increased populations of *Bifidobacterium* spp. which is inversely associated with insulin resistance and risk of T2D (Chambers, Byrne, Morrison, et al., 2019). Improvements in gut barrier integrity, reducing the risk of endotoxemia and chronic low-grade inflammation, may have also played a role (Chambers, Byrne, Morrison, et al., 2019).

The lack of significant response was attributed to unaltered GLP-1 or PYY (Byrne et al., 2019; Chambers, Viardot, et al., 2015; Pingitore et al., 2017), reduced glucose disposal due to a switch in substrate utilisation from glucose to lipids (Byrne et al., 2019; Chambers, Byrne, Morrison, et al., 2019) and propionate's potential contribution to hepatic gluconeogenesis (Byrne et al., 2019).

HOMA-IR was lowered in IPE and inulin intervention groups, after 20g of supplementation (Chambers, Byrne, Morrison, et al., 2019). Furthermore, β-cell function and insulin secretion were reportedly improved in another study using IPE, independent of GLP-1 secretion (Pingitore et al., 2017). Suggesting that propionate may directly stimulate FFAR2 on pancreatic β-cells to enhance glucose-mediated insulin secretion and prevent apoptosis as well as potentially influencing downstream insulin sensitivity (Pingitore et al., 2017).

2.6.4 **Limitations**

There are several limitations to this study. Firstly, uniform measures of glycaemia were not used to report glycaemic response, like area under the curve. Glycaemic outcome reporting should be standardised to facilitate comparison of results in future. Further, total area under the curve was analysed for acute outcomes, significant differences at specific time points or key postprandial phases (e.g., first-phase insulin response), may have been missed. Study heterogeneity was significant in some cases, but this was expected, due to the disparate methodologies and administration methods used for investigating SCFA. Most studies included had some concerns or a high risk of bias, mostly due to lack of information surrounding randomisation and reporting of results. These factors meant that the certainty of evidence for SCFA as a method of improving glycaemic control is low or very low, according to the GRADE criteria. Therefore, caution should be taken when drawing conclusions from the results.

2.7 Summary

This systematic review and meta-analysis found that acute vinegar (dietary form of acetic acid), significantly lowered postprandial glucose response in individuals with T2D, pre-diabetes and normal glucose control. No significant differences in postprandial glucose and insulin response were seen in acute acetate, propionate, or mixed SCFA administration. Further, no significant effects were seen on fasting and postprandial glucose or insulin after chronic administration of vinegar or propionate. No studies were identified for acute butyrate administration and chronic acetate administration. The results of this systematic review and meta-analysis do not align with current evidence from *in vitro* and animal studies. This is partially driven by the lack of well-designed human studies using physiological SCFA concentrations and homogenous sites of administration. Further studies delivering SCFA directly to sites of action in the colon to explore the effect upon glucose tolerance and insulin sensitivity are warranted. The effect of enhanced colonic propionate concentrations on fasting metabolic outcomes is investigated in **Chapter 3**. Furthermore, future work should attempt to understand mechanisms that may drive improvements in metabolic outcomes in humans, this is explored in **Chapter 4**.

Chapter 3: The effect of elevated colonic propionate delivery on weight gain prevention in adults aged 20 to 40

Research outputs from this chapter:

Pugh, J. E., Petropoulou, K., Vasconcelos, J. C., Anjum, A., Thom, G., McCombie, L., Tashkova, M., Alshehhi, S., Babalis, D., Holroyd, L., Sadiq, B. A., Prechtl, C., Preston, T., Chambers, E., Lean, M. J., Dhillo, W., Prevost, A. T., Morrison, D., & Frost, G. (2024). Increase in colonic PRopionate as a method of prEVENTing weight gain over 12 months in adults aged 20–40 years (iPREVENT): A multi-centre, double-blind, randomised, parallel-group trial. eClinicalMedicine, 76. https://doi.org/10.1016/j.eclinm.2024.102844

3.1 Summary of key findings

- Inulin-propionate ester had no significantly greater effect on weight gain prevention, compared with inulin over 12 months, in a cohort of 270 younger adults who were identified to be at greater risk of weight gain
- Fat-free mass, total body water and fasting glucose were significantly greater in the IPE group.
- Percentage of compliance (≥ 50% study product consumed) was 63% in the IPE group and 53% in the inulin group
- Higher rates of gastrointestinal effect-related adverse events were identified in the inulin study arm, which may have impacted compliance within this group

3.2 Introduction

More than 60% of the UK population is overweight or obese (NHS, 2020). Definitions, prevalence and economic burden of obesity are reported in **Chapter 1, Section 1**. Yet once an individual becomes obese, only one in 210 men and one in 124 women can return to a normal weight (Fildes et al., 2015). Further, a study investigating surgical and non-surgical weight loss programmes reported significant regains in body weight over one year (mean ± SD) 1.8 ± 7.5 and 1.7 ± 7.0 kg, respectively (Bond et al., 2009). There is a pressing need for research focussing on weight gain prevention.

Weight gain often accelerates upon reaching adulthood (Katsoulis et al., 2021). The Coronary Artery Risk Development in Young Adults (CARDIA) study found that weight gain ranged from 0.96 kg to 0.55 kg per year, dependent upon demographic, between the ages of 25 to 35 years (Lewis et al., 2000). This cumulative weight gain is associated with a deterioration in biochemical markers of cardiovascular disease and type 2 diabetes. Substantial weight gain over the 10 years resulted in elevations in LDL cholesterol, triglycerides, fasting insulin and blood pressure (Norman et al., 2003). This period can set the trajectory for weight gain and chronic disease risk in later life. Therefore, averting weight gain at this life stage could preserve good metabolic health as the population ages.

As mentioned in **Chapter 1,** there is strong evidence to suggest that higher daily fibre intake helps manage weight or can contribute to weight loss. The mechanisms by which dietary fibres influence energy balance are unclear but may be associated products of their microbial fermentation in the colon, SCFAs. The effects of SCFAs in energy balance is not yet elucidated. Current evidence for the impacts of SCFA on appetite regulation, energy expenditure and substrate metabolism are explored in **Chapter 1, Section 4**.

The data from chronic SCFA administration in humans have yet to reach a consensus owing to difficulties in selectively increasing SCFA production. In one study, chronic increases in colonic propionate via IPE), inhibited body weight gain and hepatic lipid accumulation (Chambers, Viardot, et al., 2015). Further, Malkova and colleagues reported that IPE intake in combination with a moderate energy deficit reduced body weight and fat mass over a four-week intervention (Malkova et al., 2020). Whereas body weight change was not significantly different after six weeks of propionate supplementation compared to cellulose and inulin controls (Chambers, Byrne, Rugyendo, et al., 2019), nor after one week of propionate administration (Todesco et al., 1991). Notably, one study body weight was reported to be higher in the IPE arm, compared to the inulin control after a six-day intervention (Byrne et

al., 2019). Few studies have explored the role of chronic acetate or butyrate administration in humans. Further research is required before conclusions can be drawn on the effect of chronic SCFA administration on body weight gain and energy balance.

Although there is evidence to suggest that increasing fibre intake has a positive effect on body weight, encouraging human subjects to increase fibre intake to the levels used in rodent studies (5-20% total energy intake), is not feasible. Furthermore, general fibre supplementation makes it difficult to regulate gut microbiota composition and the type and extent of SCFA production in the colon. Furthermore, elevating fibre intakes to this extent would induce unpleasant side effects such as bloating, cramping, gas and changes in stool frequency and consistency. Moreover, SCFAs absorbed in the upper gut may affect metabolism differently from those absorbed in the lower areas of the colon (Van der Beek et al., 2016). IPE was developed as a delivery vehicle for propionate to the proximal colon, releasing 36.2 mmol propionate, equivalent to the fermentation of 60g of fibre in 10g IPE (Chambers, Viardot, et al., 2015). Per 10g of IPE, there is 2.4g of esterified propionate, >80% (1.9g) of which is delivered to the distal colon (Chambers, Viardot, et al., 2015; Polyviou et al., 2016). Studies using a ^{13}C-propionate variant of the IPE, demonstrated that bound propionate was stable until it reached the colon (Chambers, Viardot, et al., 2015; Polyviou et al., 2016).

Previous clinical studies in overweight, middle-aged adults demonstrated that IPE prevented weight gain and lowered abdominal adiposity over six months of intake. The next logical step was to investigate the impact of IPE on preventing weight gain in young adults aged 20 to 40 years old, a period of rapid weight accumulation that sets a weight trajectory for later life. The aim of this Chapter is to determine if using IPE to selectively raise propionate concentrations, could prevent weight gain and improve body composition and fasting biochemistry in young adults, whilst using inulin, a fermentable fibre, as a positive control. An exploratory mechanistic sub-study was also conducted, which aimed to investigate the physiological mechanisms by which IPE may influence appetite, substrate utilisation and energy expenditure in humans. This forms **Chapter 4.**

3.3 Purpose of the Study

3.3.1 **Hypothesis**

I hypothesised that inulin-propionate ester would prevent weight gain in adults between the age of 20 to 40 years over 12 months, compared to the inulin control.

3.3.2 **Aims**

The primary aim of this study was to investigate whether inulin-propionate ester could prevent weight gain in a cohort at greatest risk of weight gain (i.e., overweight, young adults), over a 12-month intervention period.

3.3.3 **Outcomes**

The primary outcome of this study was body weight (kg).

The secondary outcomes of this study were:

- BMI (kg/m^2)
- Fat mass (kg)
- Water mass (kg)
- Fat-free mass (kg)
- Waist and hip circumference
- Blood pressure
- Fasting blood glucose, insulin, high density lipoprotein, LDL and total cholesterol, triglycerides
- Physical activity level measured using the international physical activity questionnaire (IPAQ)
- Energy intake at baseline, 2-, 6- and 12-months
- Alcohol, smoking, e-cigarette, and recreational drug use
- Compliance
- Adverse events

3.4 Methods

3.4.1 **Study design**

This study was a multi-centre, double-blind, randomised, parallel-group trial, registered on 1st March 2018 (ISRCTN16299902). Participants were randomly allocated to consume inulin or inulin-propionate ester. The study was conducted at two sites, the NIHR Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital and the Glasgow Clinical Research Facility.

3.4.2 **Ethical approval**

This study received ethical approval from the London Hampstead Research Ethics Committee (REC) on 24th January 2019, REC reference: 19/LO/0095 and was conducted in accordance with the Declaration of Helsinki.

3.4.3 **Participants**

Inclusion criteria:

- Males and females aged 20 to 40 years (inclusive)
- BMI of 24.00-27.00 kg/m^2 if of South Asian ethnicity or 25.00-30.00 kg/m^2 if non-South Asian
- Stable medication for 3 months at screening visit
- Written informed consent

One of the following criteria, selected because they have been independently associated with weight gain in adults (Bes-Rastrollo et al., 2006; Pietiläinen et al., 2008; Vergnaud et al., 2012) :

- Self-reported weight gain of 2 kg or more over 12 months
- Low self-reported physical activity (per international physical activity questionnaire [IPAQ] score)
- Low self-reported fruit and vegetable intake of <2 servings per day
- High self-reported intake of sugar-sweetened beverages of >1 serving per day

Exclusion criteria:

- Any diagnosed chronic disease: type 1 or 2 diabetes, cancer, kidney disease, heart disease or organic acidaemia (propionic acidaemia, methyl malonic acidaemia)

- Any diagnosed gastrointestinal condition: coeliac disease, inflammatory bowel disease and irritable bowel syndrome
- Bowel reconstruction surgery
- Pregnancy or lactation
- Antibiotic use in the last 3 months
- Vitamin B12 deficiency and anaemia
- Taking part in a weight loss program or consuming a weight loss product
- Weight loss of 3 kg or more in the last 3 months
- Any other gastrointestinal upset (e.g. diarrhoea, constipation) in the past 2 weeks
- Any other reason in the opinion of the investigator or the study doctor

3.4.4 **Study visits**

The iPREVENT main study consisted of five study visits in total, including the screening visit. Participants attended the ICRF on the day of the visit and were requested to have refrained from intense physical activity and drinking alcohol for the day before the visit. Participants were also asked to fast for 12 hours prior to the visit so that fasting blood samples could be taken, at all visits excluding screening and 2-months visit. The mechanistic sub-study visits took place at baseline and 12-month points, this study will be covered in **Chapter 4.** Further illustration of the study visits can be seen in **Figure 3.1**.

3.4.4.1 Screening visit

Participants who were eligible for the study based on their pre-screening questionnaire were invited to attend the CRF for a screening visit. At this visit, the study was described to the participants, and they were asked to provide their informed consent. After consenting to participation, a blood sample was taken to assess full-blood count (FBC). Body composition (body weight, body fat, body water, fat free mass) was measured using electronic scales which could conduct bioelectric impedance (Tanita BC-418MA). Waist and hip circumference were measured in centimetres. Questionnaires were conducted to determine participants' physical activity levels (IPAQ) and lifestyle factors (alcohol, smoking, recreational drug use). Blood pressure and pulse rate were also recorded.

3.4.4.2 Baseline or randomisation visit

Once the participant was determined to be eligible for the study, they were invited for their randomisation visit within one month of their screening visit. Fasted blood samples were taken to assess blood glucose, serum insulin and HDL, LDL and total cholesterol and

triglyceride concentrations. Body composition, waist and hip circumference, blood pressure and changes in physical activity or lifestyle factors were all measured using the same methodology as the screening visit. Randomisation was done using minimisation with a random element to balance the arms by research centre, sex, and BMI within ethnicity (South Asians: 24.00–25.49 kg/m^2 and 25.50–27.00 kg/m^2 or non-South Asians: 25.00–27.49 kg/m^2 and 27.50–30.00 kg/m^2) and whether they take part in the mechanistic sub-study. Minimisation will be conducted by researchers using sealed envelope software (Open-source software, www.sealedenvelope.com).

3.4.4.3 2-month, 6-month and 12-month visits

At the 2-month, 6-month and 12-month intervals, participants were asked to attend the CRF. For blood sampling at the 6- and 12-months visits, participants were asked to fast for 12 hours before the study visit and abstain from alcohol consumption and intense physical activity the day before the visit. Body weight, waist and hip circumference and body composition was measured per procedures above. Participants were asked to return used and unused sachets to determine their compliance. IPAQ and lifestyle factors questionnaires were repeated. Any adverse events that had occurred since the previous visit were recorded. The participants were allocated sufficient sachets to last until their next study visit.

3.4.5 **Data collection methods**

3.4.5.1 Body weight and body composition

Body weight and body composition was measured using electric and bioelectrical impedance scales (Tanita BC-418MA, Tanita, Japan). Participants were asked to void their bladder and remove excess layers of clothing, jewellery and shoes and socks beforehand. Participants were asked to wear the same clothes to each study visit to ensure measurements were kept as consistent as possible.

3.4.5.2 Blood sample collection

Fasting blood samples were collected in vacutainers via a needle inserted into the antecubital vein. Samples for plasma glucose, cholesterol and lipid profile were immediately delivered to the pathology laboratory at Hammersmith hospital. Samples for serum insulin analysis were processed in the CRF laboratory. These samples were centrifuged at 2500 RCF at a temperature of 4ºc and stored in the -80 freezer until assays could be conducted at the end of the study. Insulin was measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) assay (DY8056-05, Biotechne, USA).

3.4.5.3 Waist and hip circumference

Waist and hip circumference were measured in centimetres using standard disposable measuring tapes. Measurements were conducted by finding the widest part of the hips to measure the maximum hip circumference. Waist circumference was measured at the mid-point between the lowest rib and the highest point of the iliac crest.

3.4.5.4 Blood pressure and pulse rate

Participants were first asked to rest for 5 minutes or more in sedentary position before blood pressure and pulse rate could be measured. Participants were discouraged from talking or moving whilst the blood pressure measurement was taken. Blood pressure and pulse rate were measured three times and an average reading was recorded.

3.4.5.5 Physical activity

Physical activity levels were assessed using the IPAQ short questionnaire to detect changes during the study.

3.4.5.6 Lifestyle factors

At each visit, participants were interviewed to determine any changes in their smoking, e-cigarette or recreational drug use and their alcohol consumption throughout the trial period.

3.4.5.7 Compliance

Participants were asked to complete a food supplement diary and were asked to keep all used and unused sachets. The sachets were counted, and supplement diary was reviewed during the study visit.

3.4.5.8 Changes to dietary habits

For seven days before each visit, participants were instructed to make detailed notes of all food and drink that was consumed in as greater detail as possible, so that changes in the diet or alterations in energy intake could be identified.

3.4.5.9 Changes in medication

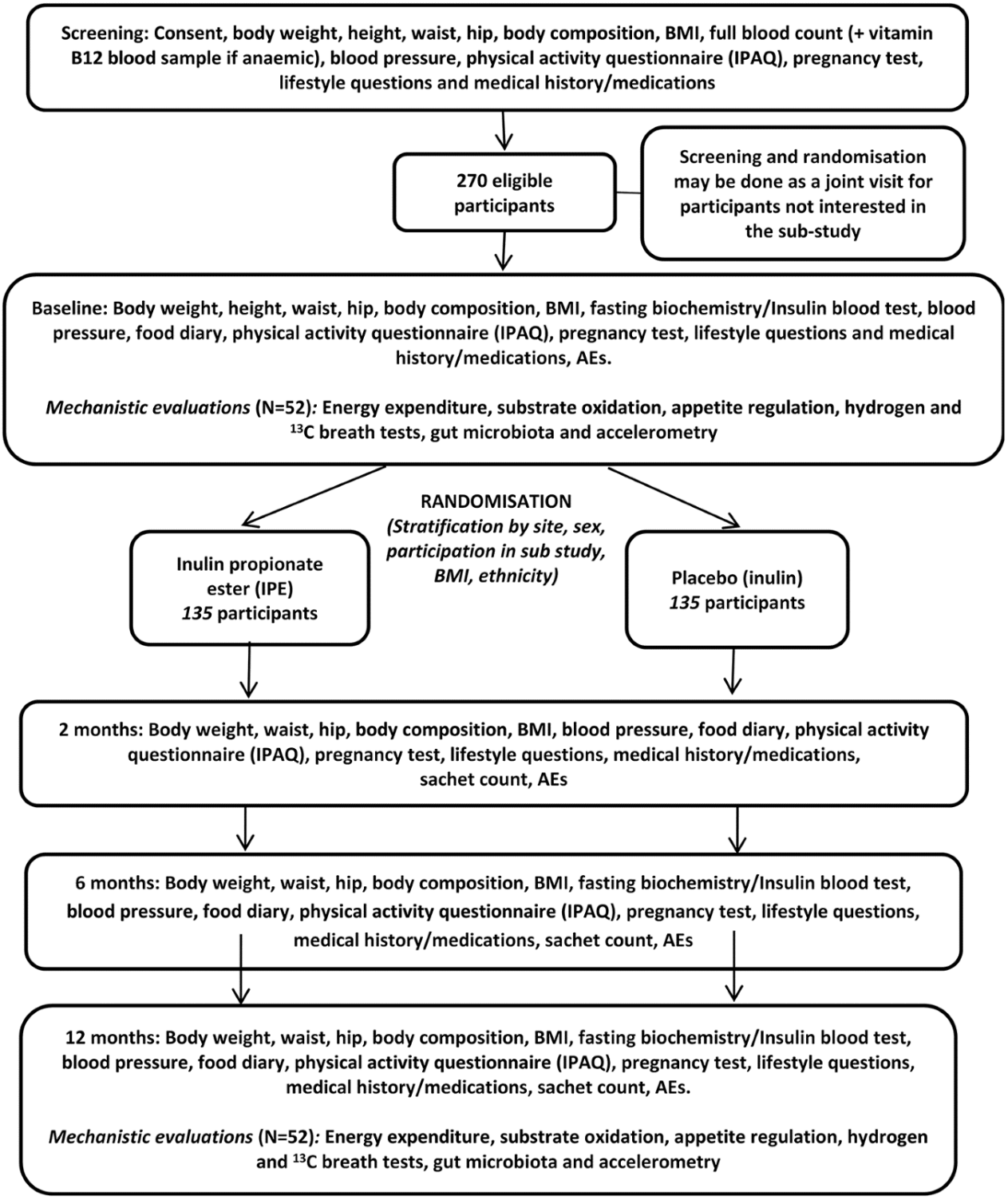
Participants were asked to recall any changes to their medication or if they had been prescribed any new medication since the previous visit.

3.4.6 **Interventions**

Inulin-propionate ester was created for the targeted delivery of propionate to the colon. Propionate is chemically bound to the polysaccharide inulin. The 10 g per day dose of IPE provided 7.3 g of inulin and 2.7 g of esterified propionate to the diet (Chambers, Viardot, et

al., 2015). The production methods for IPE are previously established and described (Chambers, Viardot, et al., 2015). Inulin is a highly fermentable polysaccharide and looks identical to IPE. Participants received boxes of unlabelled, identical-looking trial interventions of 10g of IPE and 10g of inulin in pre-packed, foil-backed sachets. Instructions were to consume one sachet per day, mixed in a cold drink/water, at any time with their normal diet. Inulin served as a positive control to differentiate between the effects on gut microbiota and metabolism arising from the inulin component of IPE and those specifically attributable to propionate.

Figure 3.1: **Study flow chart for iPREVENT**



3.4.7 **Safety reporting**

3.4.7.1 Adverse events (AEs) and severe adverse events (SAEs)

Definition

Adverse events: An AE is any untoward medical occurrence in a clinical trial subject. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding) or symptom, whether it is considered related to the trial protocol or intervention.

Severe adverse events: Any untoward medical occurrence that requires hospitalisation, results in a disability or incapacity, or is life-threatening, a congenital abnormality or medically important or an adverse event that results in death.

3.4.7.2 Side effects

Expected side effects of inulin and IPE are increased gastrointestinal events such as bloating, cramping and gas due to elevated fibre intake. Any unexpected side effects were documented as adverse events.

3.4.8 **Statistical analysis**

The statistical analysis for this section was conducted by Joana Vasconcelos (JCV) and Toby Prevost (ATP).

3.4.8.1 Sample Size Calculation

In the randomised proof of concept trial, the difference between arms in the change in body weight over 24 weeks was 1.4kg (95% CI: -0.3 to 3.1), p=0.099. Using a Bayesian method recommended for preliminary trials in which evidence in the 95% CI is translated into probabilities,(Burton et al., 1998) there was a 95% posterior probability of an underlying positive between-arm difference favouring the intervention. The posterior probability of intervention-favouring differences greater than 1kg, 1.5kg, and 2kg were respectively 69%, 47% and 25% based on 24-week intervention. The difference increased in magnitude through successive 8-week, 16-week, and 24-week time points. By 24 weeks there were significant reductions in the proportion of intervention participants gaining 3%, and 5% of body weight from a mean baseline of 90kg. A 2kg between-arm 12-month effect size was therefore chosen. This agreed with a weight gain prevention trial over 9 months in young adults,(Allman-Farinelli et al., 2016) which aimed to detect a 2kg effect and achieved 4.3kg, with a pooled SD for body weight change of 4.35kg, and 81% retention.

On this basis a sample size of 270 randomised participants (135 per arm) was chosen to provide 90% power to detect a 2kg difference between arms in mean body weight change over 12 months using a two-sided 5% level significance test, assuming a 4.35kg SD and with 25% dropout allowance (68 participants).

3.4.8.2 Primary Outcome Analysis

This trial aimed to understand whether intake of IPE reduced further weight gain, compared with control. An explanatory element of the trial aimed to understand the mechanisms of the causal pathway of such body weight change and any limitations from compliance. Therefore, analyses were primarily conducted in the 'Intention to Treat' population. The analysis of the primary endpoint incorporates the earlier correlated interim measurements of body weight in a linear mixed effects model and is adjusted for baseline continuous body weight and other categorical randomisation stratifiers. Further specification of the role of timepoint, and correlation structure is detailed in the statistical analysis plan (SAP) (Vasconcelos et al., 2022).

3.4.8.3 Secondary endpoints analysis

Where possible, continuous secondary endpoints have been adjusted for their baseline to improve the precision of estimated intervention effects. Repeated measures have been analysed using linear mixed effects models adjusting also for randomisation stratifiers. Comparisons between arms for binary outcomes are summarised as differences in proportions. 95% confidence intervals have been used to make inferences from estimated effect sizes. For SAEs, the Wilson score-based method with no continuity correction was used when there were fewer than five events in either of the arms (Newcombe, 1998). Where possible, continuous secondary endpoints were adjusted for their baseline to improve the precision of estimated intervention effects. Repeated measures were analysed using linear mixed-effects models adjusting also for randomisation stratifiers. Comparisons between arms for binary outcomes were summarised as differences in proportions and estimated 95% confidence intervals were used to make inferences about effect sizes. Confidence intervals excluding zero, for the analysis of a difference in means or proportions, were labelled as tentatively statistically significant due to multiple secondary outcome tests. p-values were reserved for the powered analysis of the primary outcome.

IBM SPSS Statistics (RRID: SCR_019096), version 28.0.1, was used for all statistical analyses. The final approved SAP, following the protocol, took precedence for undertaking the analysis of the main trial.

3.5 Results

3.5.1 **Recruitment and baseline characteristics**

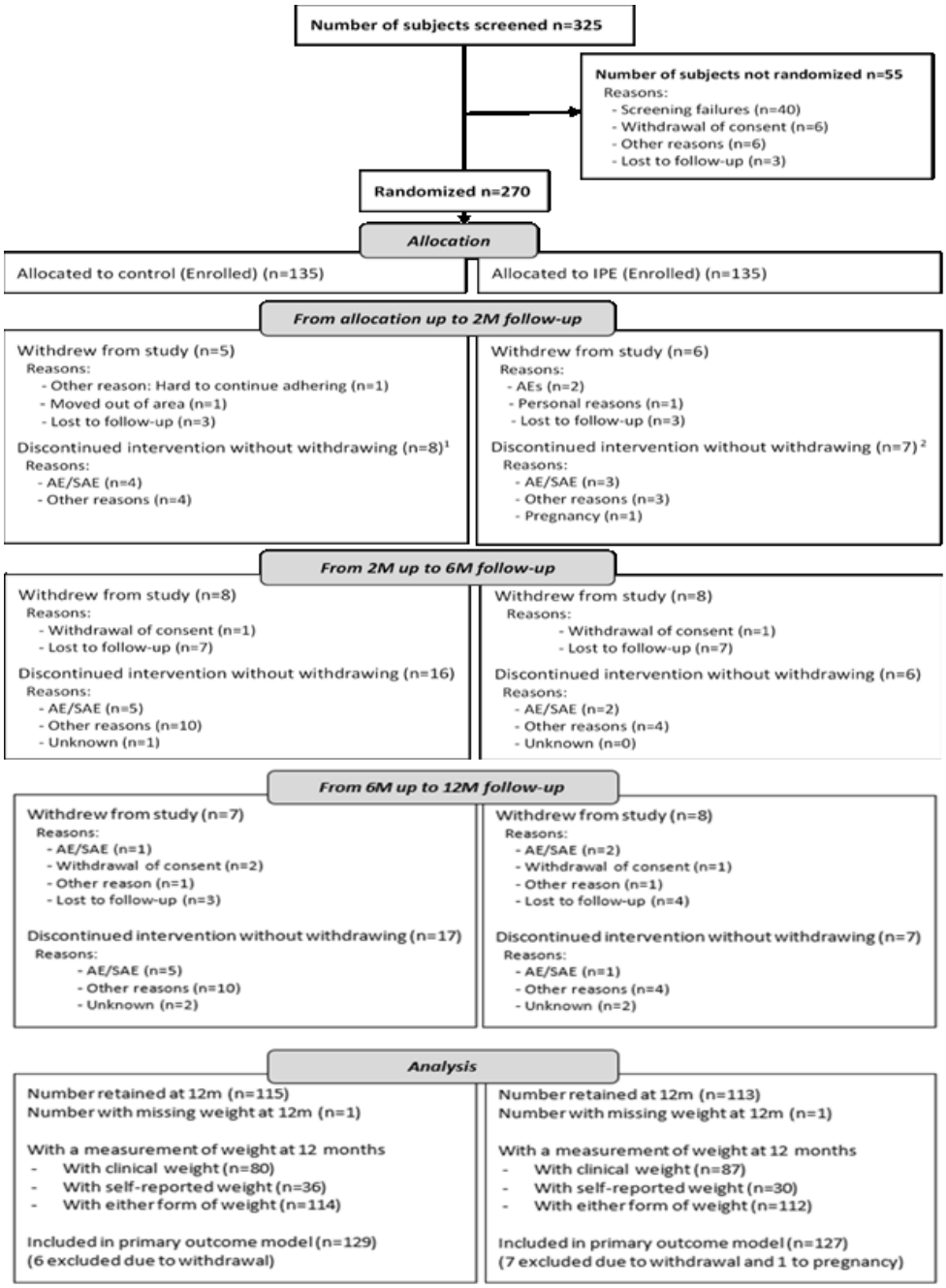
Between July 2019 and October 2021, 325 individuals were screened for this study and 270 participants were randomly allocated to the inulin (n=135) or IPE (n=135) group. Recruitment was paused from March to September 2020 due to the COVID lockdowns. The mean (Standard deviation [SD]) age was 30.2 (5.4) years and BMI was 27.5 (1.5) kg/m^2. Baseline characteristics were well balanced between the two study arms (**Table 3.1**). The trial CONSORT diagram (**Figure 3.2**) illustrates the participants' flow through the study stages.

Table 3.1: Baseline socio-demographic and clinical characteristics by arm

	Control N=135 N (% total)	IPE N=135 N (% total)	Total N=270 N (% total)
Age Mean (SD), (Min, max)	30.1 (5.7) (21, 40)	30.3 (5.2) (20, 40)	30.2 (5.4) (20, 40)
Ethnicity			
White	95 (70%)	90 (67%)	185 (68%)
Asian	20 (15%)	14 (10%)	34 (13%)
Mixed	6 (4%)	7 (5%)	13 (5%)
Black	4 (3%)	8 (6%)	12 (4%)
Any other ethnic group	10 (7%)	16 (12%)	26 (10%)
Smoking status:			
Current	17 (13%)	25 (19%)	42 (16%)
Ex-smoker	28 (21%)	25 (19%)	53 (20%)
Never	90 (67%)	85 (63%)	175 (65%)
Vaping status:			
Current	11 (8%)	8 (6%)	19 (7%)
Ex-vaper	3 (2%)	4 (3%)	7 (3%)
Never	121 (90%)	123 (91%)	244 (90%)
Drinking status[ǂ]:			
Current	110 (81%)	106 (79%)	216 (80%)
Ex-drinker	9 (7%)	12 (9%)	21 (8%)
Never	16 (12%)	16 (12%)	32 (12%)
Recreational drugs taken in the past year	6 (4%)	7 (5%)	13 (5%)

Weight (kg)	**79.1 (10.6)**	**79.6 (10.9)**	**79.3 (10.7)**
Height (cm)	169.5 (9.3)	169.6 (9.5)	169.6 (9.3)
Waist (cm)	93.2 (8.2)	94.0 (8.7)	93.6 (8.5)
BMI (Kg/m2)	27.4 (1.5)	27.5 (1.6)	27.5 (1.5)
‡ one participant in the IPE arm failed to report drinking status.			

Figure 3.2: CONSORT Study Flow Diagram



[1] One of these participants withdrew later in the 2M to 6M period.

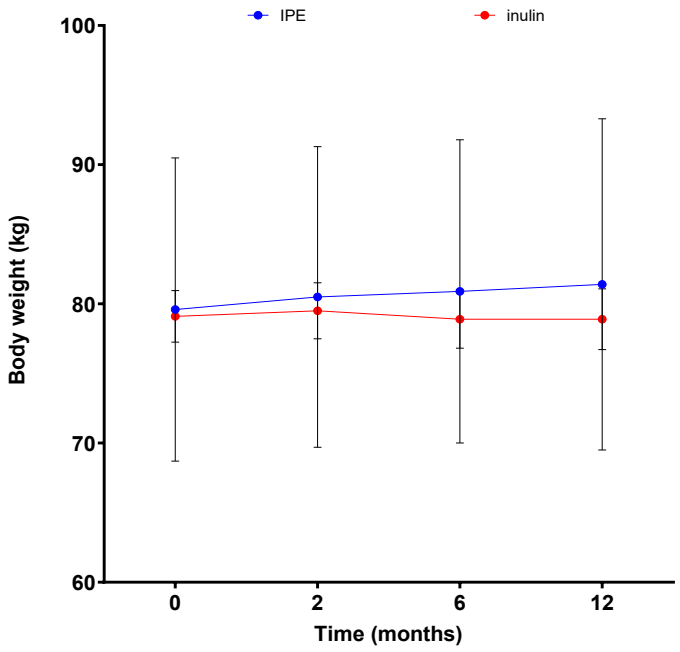
[2] Two of these participants withdrew later in the 6M to 12M period.

3.5.2 **Primary Outcome Results**

Participants could not attend the research facility due to COVID-19 lockdowns, so researchers switched to collecting self-reported weight and conducted the visits remotely. The SAP was amended accordingly to ensure these weights could be used in the primary endpoint analysis. The clinical weight was used as the 'principal weight' if it was provided. If the clinical weight was not provided, self-reported weights were included in the primary endpoint analysis. Clinical weight was provided by 62% (167/270) of participants whereas principal weights were recorded for 84% (226/270) of participants at 12-months, meeting the required 80% for the power calculation.

At baseline, the mean (standard deviation) unadjusted principal weight was 79.6 (10.9) for IPE and 79.1 (10.6) for inulin. At 12 months, the mean unadjusted principal weights were 81.4 (11.9) and 78.9 (11.8), for IPE and inulin respectively. The adjusted weight difference of 1.02 (95% confidence intervals [CI]: -0.37 to 2.41) was calculated from the clinical and principal weights (**Table 3.2, Figure 3.3**).

Figure 3.3: **Principal weight of IPE and inulin participants at each visit timepoint**



Mean (standard error [SE]) body weight at each time point.

Abbreviations: IPE, inulin-propionate ester

Table 3.2: Clinical and Principal weight at baseline and the 12-month visit

	Baseline				12-month visit				Adjusted difference in means (IPE – Control) (95% CI)
	IPE	**N**	**Inulin**	**N**	**IPE**	**N**	**inulin**	**N**	
Clinical Weight (kg)	79.6 (10.9)	135	79.1 (10.6)	135	80.8 (11.1)	87	79.8 (10.5)	80	1.02 (-0.37 to 2.41)
Principal Weight (kg)	79.6 (10.9)	135	79.1 (10.6)	135	81.4 (11.9)	112	78.9 (11.8)	114	

3.5.3 Secondary outcome results

Fat-free and body water mass significantly increased in the IPE group compared to inulin with adjusted mean differences (95% CIs) of 1.07 (0.21 to 1.93) and 0.72 (0.10 to 1.33), respectively. There were no significant differences in BMI, waist or hip circumference, waist-hip ratio or body fat mass between IPE and inulin at 12-months. There was a significant rise in fasting blood glucose in the IPE group compared to the inulin with an adjusted mean difference of 0.11 (0.01 to 0.21). There were no significant differences in other fasting biochemistry measures between IPE and inulin (**Table 3.3**).

Table 3.3: Body composition and fasting biochemistry at baseline and the 12-month visit

	Baseline				12-month visit				Adjusted difference in means (IPE – Control) (95% CI)
	IPE	**N**	**Inulin**	**N**	**IPE**	**N**	**inulin**	**N**	
BMI (Kg/m^2)	27.5 (1.6)	135	27.4 (1.5)	135	27.9 (2.4)	87	27.6 (2.2)	80	0.31 (-0.23 to 0.85)
Waist/hip ratio	0.87 (0.08)	135	0.86 (0.07)	135	0.90 (0.10)	85	0.86 (0.07)	78	-0.01 (-0.02 to 0.01)
Body fat mass (kg)	24.8 (5.7)	135	24.2 (6.2)	135	25.0 (6.4)	79	24.5 (6.9)	74	0.36 (-0.64 to 1.35)
Fat-free mass (kg)	54.8 (11.1)	134	55.0 (10.9)	135	56.1 (10.7)	86	55.2 (10.5)	77	1.07 (0.21 to 1.93)
Body water mass (kg)	39.5 (7.9)	135	39.7 (7.8)	134	40.5 (7.6)	86	40.0 (7.5)	78	0.72 (0.10 to 1.33)
Diastolic blood pressure (mm/Hg)	72.1 (9.8)	135	70.3 (9.7)	135	71.2 (9.7)	86	71.4 (8.9)	79	-0.64 (-2.71 to 1.44)
Systolic blood pressure (mm/Hg)	116.2 (11.3)	135	113.2 (10.5)	135	115.7 (11.6)	86	115.8 (10.6)	79	-1.30 (-3.84 to 1.24)
Glucose (mmol/L)	4.59 (0.04)	127	4.65 (0.04)	125	4.74 (0.04)	85	4.66 (0.04)	80	0.11 (0.01 to 0.21)
Ln Insulin (pmol/L)	4.50 (0.97)	130	4.52 (0.88)	131	4.47 (0.86)	84	4.60 (0.80)	75	0.03 (-0.13 to 0.20)
Total cholesterol (mmol/L)	4.70 (0.08)	134	4.58 (0.06)	133	4.66 (0.09)	86	4.69 (0.10)	80	-0.10 (-0.31 to 0.10)
LDL cholesterol (mmol/L)	2.90 (0.07)	131	2.72 (0.06)	132	2.82 (0.08)	86	2.78 (0.09)	79	-0.12 (-0.30 to 0.05)
HDL cholesterol (mmol/L)	1.35 (0.03)	133	1.37 (0.03)	133	1.37 (0.03)	86	1.40 (0.04)	80	0.00 (-0.07 to 0.07)

3.5.4 **Changes in lifestyle factors**

No significant changes were detected in physical activity (**Table 3.4**) or smoking and drinking status (**Table 3.5**) over the 12-months.

Table 3.4: Changes in physical activity levels from Baseline to the 12-month visit

	Baseline		12m	
Physical activity level	Control % (n)	IPE % (n)	Control % (n)	IPE % (n)
High	17% (23)	18% (24)	24% (23)	24% (24)
Moderate	40% (54)	38% (51)	38% (36)	41% (41)
Low	43% (58)	44% (60)	39% (37)	34% (34)
Total complete	135	135	96	99
No. missing	0	0	29% (39)	27% (36)

Table 3.5: Changes in lifestyle factors from baseline to the 12-month visit

	Baseline		12m	
	Control	**IPE**	**Control**	**IPE**
	N=135	**N=135**	**N=135**	**N=135**
	% (n)	**% (n)**	**% (n)**	**% (n)**
Smoking				
Current	14% (19)	19% (25)	11% (13)	14% (16)
Ex-smoker	19% (26)	19% (25)	22% (25)	22% (25)
Never	67% (90)	63% (85)	67% (77)	63% (71)
No. missing	0	0	15% (20)	17% (23)
Vaping				
Current	9% (12)	6% (8)	9% (10)	7% (8)
Ex-vaper	2% (3)	3% (4)	5% (6)	4% (4)
Never	89% (120)	91% (123)	86% (99)	89% (100)
No. missing	0	0	15% (20)	17% (23)
Drinking				
Current	81% (110)	79% (106)	82% (94)	79% (88)
Ex-drinker	7% (9)	9% (12)	6% (7)	10% (11)
Never	12% (16)	12% (16)	12% (14)	11% (12)
No. missing	0	1% (1)	15% (20)	18% (24)
Recreational drugs				
No	95% (128)	96% (129)	92% (107)	94% (107)
Yes	5% (7)	4% (6)	8% (9)	6% (7)
No. missing	0	0	14% (19)	16% (21)

3.5.5 **Compliance and withdrawals**

Compliance with IPE and inulin was similar at 2 months. By 6 months, compliance is 11% higher and at 12 months compliance is 16% higher in the IPE group. The percentage of participants who reached the compliance threshold of greater than 80% of sachets consumed, dropped throughout the study period. The percentages of participants meeting the threshold at the 2-months, 6-months and 12-months visit were 62%, 50% and 40%,

respectively (**Table 3.6**). Participants who withdrew from the study or discontinued the supplement between study visits were included in the compliance analysis.

Table 3.6: Changes in Compliance at each study visit

Percentage compliance levels	Control N=135	IPE N=135	Total N=270
2 months			
>80%	61% (82)	64% (86)	62% (168)
50% to <80%	10% (13)	16% (22)	13% (35)
<50%*	30% (40)	20% (27)	25% (67)
≥50%	70% (95)	80% (108)	75% (203)
6 months			
>80%	45% (61)	56% (75)	50% (136)
50% to <80%	24% (32)	14% (19)	19% (51)
<50%⁺	31% (42)	30% (41)	31% (83)
≥50%	69% (93)	70% (94)	69% (187)
12 months			
>80%	32% (43)	48% (65)	40% (108)
50% to <80%[1]	22% (29)	15% (20)	18% (49)
<50%[2]	47% (63)	37% (50)	42% (113)
≥50%	53% (72)	63% (85)	58% (157)

* This included eleven (5 in control and 6 in IPE) participants who dropped out from the study at or between baseline and 2M follow-up.

⁺ This included eleven (5 in control and 6 in IPE) participants who dropped out from the study at or between baseline and 2M follow-up plus 16 (8 in each arm) who dropped out from 2M and 6M follow-up.

[1]This included 2 participants (one in each arm) who dropped out from the study between the 6M and the 12M follow-up.

[2]This included the 40 (19 in control and 21 in IPE) participants who dropped out from the study.

Eleven (5 in control and 6 in IPE) at or between baseline and 2M follow-up, 16 (8 in control and 8 in IPE) between 2M and 6M follow-up, and 13 (6 in control and 7 in IPE) between 6M and 12M follow-up).

Complete withdrawal rates were consistent at all visits between the inulin and IPE groups (**Table 3.7**). Whereas supplement discontinuation diverged after the 2 month visit. Rates of discontinuation were 10% and 15% for IPE and 18% and 30% for inulin at 6 month and 12-month visits.

Table 3.7: Participant withdrawal rates at each study visit

	IPE			Control			Total
	Up to 2M	Up to 6M	Up to 12M	Up to 2M	Up to 6M	Up to 12M	Up to 12M
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
No. withdrawing	6 (4%)	14 (10%)	22 (16%)	5 (4%)	13 (10%)	20 (15%)	42 (16%)
No. discontinued without withdrawing	7 (5%)	13 (10%)	20 (15%)	8 (6%)	24 (18%)	41 (30%)	61 (23%)
Total (sum of two above)	13 (10%)	27 (20%)	42 (31%)	13 (10%)	37 (27%)	61 (45%)	103 (38%)

3.5.6 **AEs and SAEs**

There were 339 AEs in 151 (56%) participants with 160 AEs in 75 (56%) participants in the IPE arm, and 180 in 76 (56%) participants in the control arm. There were four SAEs, two in the control arm and two in the IPE arm. **Tables 3.8** and **3.9** summarise the adverse and severe adverse events.

Table 3.8: Summary of adverse events by arm

	IPE (n=135)	**Control (n=135)**	**Difference in proportions (95% CI) (IPE – Control)**
Participants reporting ≥1 AE	75 (56%)	76 (56%)	-0.7% (-12.6 to 11.1%)
Participants reporting a SAE	2 (1.5%)	2 (1.5%)	0.0% (-3.9% to 3.9%)
Death	0 (0%)	0 (0%)	0.0% (-2.8% to 2.8%)
Adverse events of special interest:			
Participants reporting ≥1 AE in Gastrointestinal disorders	30 (22%)	42 (31%)	-8.9% (-19.4% to 1.6%)
Participants reporting at least one AE in infection	22 (16%)	25 (19%)	-2.0% (-11.3% to 6.8%)
Participants reporting at least one AE in Food sup taste/texture[+]	9 (6%)	6 (4%)	2.2% (-3.2% to 7.7%)

No. participants discontinuation from study due to AE[+]	4 (3%) [*]	1 (1%)	2.2% (-1.6% to 6.7%)

[+]3 participants had an AE related to stopping the supplement because they "did not like taking supplement". AE, adverse event; SAE, serious adverse event

Table 3.9: Serious adverse events

	Arm	Days duration	Severity	When (months after randomisation)	Relationship to study treatment	Outcome
Stab wound	IPE	30	Severe	10	Not related	Resolved
Hospitalisation	IPE	61	Severe	8	Not related	Resolved
Renal stone removal	Control	1	Moderate	4	Not related	Resolved
Meningitis bacterial	Control	17	Severe	6	Not related	Resolved

3.6 Discussion

3.6.1 **Summarised Discussion**

In the present study, IPE and inulin had no differential effect on weight gain prevention. Neither group experienced the predicted weight gain of 2 kg over the year-long study period. This result was encouraging as the study was conducted during the COVID-19 lockdowns, a period in which several observation studies suggest accelerated weight gain throughout the population. Furthermore, the study participants were specifically selected for their phenotypic susceptibility to further weight gain. It is also worth noting that there was no additional intervention incorporated into the study design, such as exercise or dietary advice, to promote a negative energy balance. The lack of significant weight gain seen in this cohort could reinforce the importance of fibre intake on satiety and energy homeostasis.

3.6.2 **Withdrawals, compliance and safety**

Total withdrawals from the study did not vary substantially between the IPE and inulin intervention. Thus, any effect seen was not influenced by differences in withdrawal rates. The study characteristics of the participants who continued the study versus those who withdrew were not significantly different. However, there was a significant difference in compliance between the two groups, with withdrawals from the study treatment almost doubling in the inulin control group versus the IPE group at the 12-month visit point. Higher numbers of gastrointestinal-related adverse events in the inulin group may have been part of the reason for the higher withdrawal rates. Other reasons are unknown and may require further exploration. Neither group reported any unexpected, related AEs indicating that IPE and inulin have good safety profiles.

3.6.3 **Primary Outcome**

There was no significant difference in the primary outcome between IPE and inulin groups, suggesting that propionate does not have a superior effect on weight gain prevention than inulin alone in this cohort of younger adults. In previous studies in a middle-aged cohort, significant weight gain was prevented in 96% of IPE-consuming participants and 75% of inulin-consumers over 6 months (Chambers, Viardot, et al., 2015). IPE-consumers had significantly lower body weight, BMI and body fat mass in comparison to the pre-intervention values, whereas there were no significant differences in the group consuming cellulose (Malkova et al., 2020). Other chronic IPE studies reported no significant change in body weight over a 6-week study period, however body weight was not the primary outcome (Chambers, Byrne, Rugyendo, et al., 2019). It is worth noting that neither group reached

the predicted 2 kg weight gain threshold, perhaps reinforcing the beneficial effect of increased fibre intake on energy balance, regardless of the presence of propionate. Given that this cohort were specifically selected for their phenotypically higher susceptibility to weight gain, this result is even more encouraging. Moreover, no further intervention, such as physical activity or dietary advice was given to the participants, to achieve a negative energy balance. Participants were asked to maintain current physical activity levels and dietary habits throughout the trial period. Furthermore, this trial was conducted between July 2019 and October 2022 and many participants were enrolled during the peak of the COVID-19 pandemic. Studies indicate that COVID-19 had a significantly impacted habitual diet and physical activity (Ammar et al., 2020; Curtis et al., 2021; Tison et al., 2020). Further, a meta-analysis of six studies indicated that the average body weight after the first lockdown (March-May 2020) was 1.57 kg heavier than at baseline (Bakaloudi et al., 2022). Given the circumstances of the trial and the absence of intervention other than IPE or inulin, within this free-living population, the lack of weight gain is encouraging.

3.6.4 **Secondary Outcomes**

IPE has consistently improved body composition. In the present study, a significant rise in fat-free and body water mass was detected but no significant alterations in fat mass or body weight were observed. Enhanced lean mass has been observed in rodents supplemented with SCFAs. However, the mechanism by which SCFA or propionate would enhance lean mass is to be elucidated. There is evidence to suggest that propionate has a lactate-sparing effect as it is the preferred substrate for gluconeogenesis. Enhanced concentrations of lactate within the body produce an anabolic effect which may promote myocyte proliferation. Arguably, any effects on lean tissue are likely to occur via the liver where the majority of propionate is metabolised, for example, enhanced fat oxidation, resulting in increased energy expenditure. However, this is more likely to lead to a reduction in fat mass rather than lean mass. This finding warrants further investigation.

Previous studies in middle-aged individuals have observed lowered visceral adipose tissue mass (Chambers, Byrne, Rugyendo, et al., 2019) and total body fat mass after a modest energy deficit (Malkova et al., 2020). This is consistent with evidence from rodent studies which suggest that SCFAs may enhance fat oxidation, preventing further adipose tissue deposition (W. Zhang et al., 2023). There was a slight, non-clinically significant increase in fasting glucose in the IPE group and no other changes in the fasting biochemistry. Given that there were no significant changes to fat mass or body weight in either group, this was

expected. However, in previous studies, there have been improvements to biomarkers associated with the risk of chronic disease. Elevated insulin sensitivity (Chambers, Byrne, Rugyendo, et al., 2019; Chambers, Viardot, et al., 2015) and insulin secretion (Pingitore et al., 2017) along with reductions in cholesterol (Chambers, Viardot, et al., 2015) and inflammatory markers (Chambers, Byrne, Rugyendo, et al., 2019), have been reported.

3.6.5 **Comparison with Prior Evidence**

There appear to be substantial differences in the IPE and inulin responses of middle-aged individuals compared with this younger cohort. The physiological and psychosocial differences between the two groups have not been thoroughly explored. There is a possibility that differences within the colonic environment could play a role. Younger adults may be less sensitive to SCFA flux as SCFA concentrations within younger people tend to be naturally higher (Salazar et al., 2019). In the present study, the IPE group had an upward trend in body weight whereas the inulin group were weight stable, whereas results of the two study arms diverged in the opposite direction in the previous study (Chambers, Viardot, et al., 2015). The microbial populations within the colon of younger individuals may be more sensitive to inulin, enhancing fermentation and SCFA production which could have had an independent effect on appetite and energy balance.

Dietary habits and meal timing may also have contributed to the differences in the effect seen. Young adults tend to eat outside of the home, follow inconsistent meal patterns and snack throughout the day (Almoraie et al., 2021; Kant & Graubard, 2015; Lhuissier et al., 2013; Malam et al., 2014). These eating habits are associated higher energy intake (Lachat et al., 2012; Myhre et al., 2015). Hyperpalatable foods and other external cues driving appetite may have overpowered any satiety signal by propionate in the colon. Previous studies using 13C-labelling indicate that IPE reaches the colon and releases propionate three to four hours post-ingestion (Polyviou et al., 2016). As propionate is a short-term signalling molecule, if the timing of the main meal is inconsistent or outside of this window, there may be no discernible effect on fullness and energy intake. Further, there is no evidence of a difference in gut response, for example, gut transit or fermentation time between these population groups (Bitar et al., 2011). Hence, the release and sensing of propionate should be similar in all healthy adults.

3.6.6 **Limitations**

The merits of this study were the duration, large sample size, acceptable compliance rates (63% participants consuming ≥ 50% sachets) and blinded, randomised-controlled design.

However, this study is not without limitations. The dosage of IPE was based on studies in an older population who may have a lower basal SCFA production. Therefore, a 10g dose may have been insufficient to exert a positive effect on this younger cohort.

Dietary intake was monitored throughout the intervention but due to substantial energy intake misreporting (~40% below estimated energy expenditure); it was not deemed to reliable enough for interpretation. This does not allow us to account for the effect of dietary changes on body weight, differences in dietary habits between individuals or the effect of meal timing on satiety signalling from IPE. However, there is evidence on a national scale to suggest that both dietary quality and volume of fermentable fibres consumed lowered in 2020, during the study period (Public Health England, 2020).

Furthermore, the control arm was treated with inulin, a fermentable fibre that may affect body weight. The present study did not include a negative control, like cellulose as in previous investigations. This is because the aim was to isolate the effect of propionate alone on weight gain prevention to remove the effect of the inulin backbone. Therefore, the most appropriate control was inulin.

In this study, fat-free mass increased in the IPE group, it is worth noting that the use of bioelectrical impedance to measure body composition is less accurate than other 'gold-standard' measures such as DEXA and is also dependent upon hydration status. Therefore, it is difficult to determine whether lean mass increased through IPE intake.

3.7 Summary

This clinical trial found no significant differences in body weight gain between the inulin and IPE cohort after 12-months of supplementation. The results of this trial do not align with previous studies using IPE in middle-aged adults who were overweight or obese. The disparity in the results could be in part attributed to physiological and social differences between younger and older adults such as stronger hunger signalling lower concentrations of satiety hormones and more sporadic eating patterns. Future work should investigate the effect of IPE on the preservation or growth of lean mass and associated mechanisms such as, enhanced fat oxidation. These will be explored in **Chapter 4**.

Chapter 4: The effect of enhanced colonic propionate flux on weight gain prevention in adults aged 20 to 40 years: An exploratory mechanistic sub-study.

4.1 Summary of key findings

- Increasing concentrations of propionate in the colon by using the vehicle, IPE, had no effect on appetite hormones, energy expenditure or energy intake, compared with the inulin control
- High rates of withdrawal associated with changes in lifestyle post-COVID meant that the study was underpowered for the primary outcome
- Low sample size at follow up may partly explain why the results from this study do not reflect the increased gut hormone release observed in previous studies using IPE

4.2 Introduction

Emerging evidence indicates that SCFAs influence energy balance and subsequent control of body weight (Byrne et al., 2015; Canfora et al., 2015; Morrison & Preston, 2016). Faecal transplants from mouse twins with differing body weight phenotypes to germ-free mice resulted in increased circulating propionate and butyrate and an enhanced ability to break down fermentable carbohydrates when the microbiota from the lean mouse is transplanted (Ridaura et al., 2013). Additionally, germ-free mice colonised with bacteria from mice who had undergone Roux-en-Y bariatric surgery were detected to have significantly greater caecal SCFA concentrations compared to those colonised with bacteria from the sham surgery controls (Liou et al., 2013). These findings suggest changes in the gut microbiome after weight loss can have positive metabolic effects and highlight the bidirectional relationship between weight control and colonic microbiota.

Weight loss and improved insulin sensitivity were associated with circulating levels of propionate, butyrate, and isobutyrate in patients with severe obesity following bariatric surgery (Martínez-Sánchez et al., 2023). Salazar and colleagues reported a non-significant increase in faecal propionate post-Roux-en-Y bariatric surgery whilst two other research groups found significant reductions in faecal SCFA post-surgery (Farup & Valeur, 2020; Meijer et al., 2022; Salazar et al., 2022). However, individuals with obesity generally have higher faecal SCFA concentrations (K. N. Kim et al., 2019) and stool SCFA concentrations do not necessarily reflect colonic concentrations (Beek et al., 2016).

The effect of propionate on weight gain prevention in humans is yet to be fully understood and is explored in greater detail in **Chapter 3**. In summary, propionate delivery to the colon has prevented weight gain in a cohort of middle-aged adults and when combined with an exercise intervention in women who were overweight (Chambers, Viardot, et al., 2015; Malkova et al., 2020). However, in similar studies using propionate or sodium propionate, the same effect was not observed (Chambers, Byrne, Morrison, et al., 2019; Todesco et al., 1991). Further research is required to understand the mechanisms by which SCFAs can influence energy balance in humans. However, potential pathways are described in **Chapter 1, Section 4** and summarised below.

In vitro, SCFA stimulate the secretion of satiety hormones GLP-1 and PYY from enteroendocrine L-cells by activating FFAR2 and 3 (Christiansen et al., 2018; Larraufie et al., 2018; Nishida et al., 2021). Enhanced PYY and GLP-1 concentrations have been

reported in acute (Canfora et al., 2017; Chambers, Viardot, et al., 2015; Freeland & Wolever, 2010) and chronic SCFA interventions in humans (Roshanravan et al., 2017).

In rodents, there is evidence that all three SCFA can directly affect the central nervous system (CNS) and appetite centres in the brain to enhance satiety or promote energy expenditure (Cook et al., 2021; G. Frost et al., 2014; Z. Gao et al., 2009; Z. Li et al., 2018). In humans, acute IPE intake reduced BOLD signalling in brain reward response centres, slowing reaction time to energy-dense meal images and lowering their appeal ratings (Byrne et al., 2016). These effects were independent of any changes to anorectic hormones.

SCFA modulation in central or peripheral satiety signalling also appears to influence energy intake. In rodents, administration of acetate and butyrate significantly reduced energy intake (G. Frost et al., 2014; Z. Gao et al., 2009; Z. Li et al., 2018). Similarly, individuals consuming IPE also exhibited lower energy intakes during an *ad-libitum* meal compared with an inulin control (Byrne et al., 2016, 2019; Chambers, Viardot, et al., 2015; Polyviou et al., 2016), However, these reductions in food intake have not been associated with significant alterations in subjective appetite, assessed by visual analogue scales (VAS). However, evidence suggests that circulating gut hormone concentrations and VAS ratings of subjective appetite are significantly correlated (Lim et al., 2023). While not yet demonstrated, the reduction in energy intake demonstrated in SCFA interventions may be associated with faster satiation, rather than changes in satiety response. This could be explored in future by assessing both *ad-libitum* meal energy intake and duration.

SCFAs have been shown to enhance expression of genes related to thermogenesis and lipid metabolism and activate the sympathetic nervous system through activation FFAR3 on the vagus nerve (den Besten et al., 2015; Kimura et al., 2011; Van der Beek et al., 2014; W. Zhang et al., 2023). In rodents, SCFA administration enhances fat oxidation both alongside (den Besten et al., 2015; Z. Gao et al., 2009; Hattori et al., 2010), and independently of increases in total energy expenditure (Z. Li et al., 2018). Enhanced fat oxidation and thermogenesis may contribute to an increase in total energy expenditure aiding overall energy balance.

Research in human studies is limited by challenges in targeting delivery of SCFA and accurately measuring both energy intake and expenditure in larger study cohorts. Colonic SCFA infusions in overweight and obese men elevated resting energy expenditure and fat oxidation, particularly when using high-acetate or high-propionate infusions (Canfora et al., 2017). Our research group demonstrated an increase in resting energy expenditure and fat

oxidation after oral intake of sodium propionate (Chambers et al., 2018), an observation which was repeated in follow-up studies (A. Sukkar, 2021). Although these effects were not observed by our group when IPE for colonic propionate delivery. Interestingly, acetate increased fat oxidation when administered to the distal, not proximal colon (Van der Beek et al., 2014). However, in the same study, acetate had no observed effect on fasting energy expenditure as measured by indirect calorimetry (Van der Beek et al., 2014).

As outlined in **Chapter 2** of this thesis, SCFA may modulate hepatic and whole-body glucose homeostasis via various proposed mechanisms. However, data in human subjects are insubstantial and inconclusive and these outcomes warrant further investigation.

The current body of evidence regarding the impact of SCFAs on physiological mechanisms related to appetite regulation and energy balance is limited, particularly in humans. *In vitro* and animal models have inherent limitations and may not accurately reflect normal physiological responses in humans. The aim of this Chapter is to identify the physiological mechanisms through which the SCFA propionate influences appetite and energy balance in humans.

4.3 Purpose of the Study

4.3.1 **Hypothesis**

I hypothesised that inulin-propionate ester would affect appetite and energy expenditure in adults between the ages of 20 to 40 years over 12 months, compared to the inulin control.

4.3.2 **Aims**

The primary aim of this study was to determine whether inulin-propionate ester would alter incremental AUC (iAUC) of the anorectic gut hormones GLP-1 and PYY, from baseline to 12 months, compared to the inulin control.

4.3.3 **Outcomes**

The primary outcome of this study was GLP-1 and PYY iAUC change from baseline to follow-up (at 12 months)

The secondary outcomes of this study were changes in:

- Appetite regulation measured by energy intake measured by an *ad-libitum* meal and subjective appetite responses (measured by VAS) from baseline to follow-up
- Energy homeostasis indicated by energy expenditure and whole-body substrate oxidation measured by indirect calorimetry
- Glucose and insulin concentrations from baseline to follow-up
- Physical activity from baseline to follow-up
- Breath hydrogen from baseline to follow-up

Secondary outcomes explored in the study but not included in this thesis:

- Gut microbiota: 16S rRNA profiles from stool sample
- Impact on neuroendocrine cell number: Proliferation in intestinal organoids using the level of SCFA and other metabolites identified from nuclear magnetic resonance spectroscopic analyses of stool
- Hepatic lipid metabolism: Stable isotope tracers of fat oxidation (^{13}C palmitate) and De Novo Lipogenesis
- Total body water through dilution analysis of deuterated water

4.4 Methods

4.4.1 **Study design**

This study was a randomised, double blind, placebo-controlled parallel group trial.

4.4.2 **Ethical approval**

Ethical approval from the London Hampstead Research Ethics Committee (REC) for this study was received on 24th January 2019, REC reference: 19/LO/0095 and was conducted in accordance with the Declaration of Helsinki.

4.4.3 **Participants**

Inclusion and exclusion criteria was per **Chapter 3**, **Section 3.4.3**.

4.4.3.1 Mechanistic study-specific exclusion criteria

Exclusion criteria for the mechanistic study only were:

1. Known anaemia or per screening FBC results (Haemoglobin levels of <130g/L for males and <110g/L for females)

2. Allergies or intolerances to any of the ingredients in the set sub-study meals

4.4.4 **Study visits**

The iPREVENT mechanistic study consisted of a screening visit and two eight-hour study visits.

4.4.4.1 Screening visit

Participants who were eligible for the study based on their pre-screening questionnaire were invited to attend the Imperial Clinical Research Facility (CRF) for a screening visit. At this visit, the study was described to the participants, and they were asked to give informed consent. Screening visit measurements included: a blood sample to assess full blood count (FBC), body composition (body weight, body fat, body water, fat-free mass) measured using electronic scales which could conduct bioelectric impedance (Tanita MC-780MA), blood pressure and waist and hip circumference. Participants were asked about their physical activity using the International Physical Activity Questionnaire (IPAQ) and their smoking, drinking and electronic cigarette use.

4.4.4.2 Baseline or randomisation visit

Once the participants were determined to be eligible for the study, they were invited for their randomisation visit within one month of their screening visit. Randomisation was done using minimisation with a random element to balance the arms by research centre, sex, and BMI within ethnicity (South Asians: 24.00–25.49 kg/m^2 and 25.50–27.00 kg/m^2 or non-South Asians: 25.00–27.49 kg/m^2 and 27.50–30.00 kg/m^2) and whether they are taking part in the mechanistic sub-study. Minimisation was conducted by researchers using sealed envelope software (Open-source software, www.sealedenvelope.com).

Before the baseline visit, participants wore a physical activity monitor and completed a food diary for seven days. The day before the study visit, participants abstained from alcohol consumption and intense physical activity. The evening prior to the study visit, participants consumed a standard meal to keep evening nutrient intake consistent the evening before the baseline and follow-up (e.g., a ready meal). This meal was then recorded to be repeated before the 12-month visit. Before bed, participants drank deuterated water (2H2O) (approximately 2-3 g/kg body water). Participants were also asked to collect a stool sample the day before or morning of the study visit day. This sample was kept on ice until it was transported to the facility. Participants fasted for 12 hours before attending the CRF for their visit at 8 am the following morning. During this period, they were allowed to drink water.

Upon arrival at the CRF, participants were asked to void their bladder so urine collection could commence thereafter. Then a cannula was placed in the antecubital forearm vein so blood sample collection could begin.

4.4.4.3 12-month follow-up visit

Before the 12-month follow-up visit, participants wore a physical activity monitor and completed a food diary for seven days. The day before the study visit, participants abstained from alcohol consumption and intense physical activity. The evening prior to the study visit, participants consumed the same standard meal as consumed at baseline and deuterated water (2H2O) (approximately 2-3 g/kg body water). Participants were asked to collect a stool sample in the evening before or morning of the study visit day. This sample was stored and transported to the facility in an insulated cool bag. Participants fasted for 12 hours before attending the CRF for their visit at 8 am the following morning. During this period, they were allowed to drink water. Upon arrival at the CRF, participants were asked to void their bladder so urine collection could commence thereafter. Then a cannula was placed in the antecubital forearm vein so blood sample collection could begin.

4.4.5 **Study interventions and measurements**

4.4.5.1 Interventions

Inulin-propionate ester was created for the targeted delivery of propionate to the colon. Propionate is chemically bound to the polysaccharide inulin. The 10 g per day dose of IPE provided 7.3 g of inulin and 2.7 g of esterified propionate to the diet (Chambers, Viardot, et al., 2015). The production methods for IPE are established and have been previously described (Polyviou et al., 2016). Inulin is a highly fermentable polysaccharide and looks identical to IPE. Inulin served as a positive control to differentiate between the effects on gut microbiota and metabolism arising from the inulin backbone of IPE and those specifically attributable to propionate. IPE for this study was produced at a pilot-plant by Moorepark Technology Limited (Fermoy, Ireland) using the previously referenced protocol. Safety of the products was assessed by microbiological and heavy metal testing by an external accredited laboratory.

4.4.5.2 Standard meals

At timepoint 0 minutes, a standardised, liquid breakfast meal (Ensure Plus, Abbott, UK: 500 kcal, 67.3g carbohydrates, 13.4g fat and 20.8g protein) was provided as a mixed-meal tolerance test. The 10g dose of IPE or inulin along with 200 mg of [U-^{13}C] palmitic acid was dissolved into the standard breakfast. A second dose of deuterated water was given with

the breakfast, to achieve and maintain a plasma water enrichment of 0.3% for the measurement of fasting and postprandial hepatic *De Novo* Lipogenesis. The nutrient composition of the breakfast was consistent for all participants. Lunch was served at 180-minute time point and consisted of a cheese sandwich on white bread and salted potato crisps (Tesco, UK: 10g carbohydrates, 5.1g fat, 3.1g protein and <0.5g fibre per 100 kcal). Meal size was adapted depending on the basal metabolic rate (BMR) of the participant; if BMR <1600 kcal per day 550 kcal lunch, 1600 – 1900 kcal per day = 650 kcal and >1900 kcal per day = 750 kcal lunch. Basal metabolic rate was calculated using the Schofield equation (W. N. Schofield, 1985). This meal was selected as it has a low dietary fibre content and is a commonly consumed food in the UK ensuring it would be widely acceptable to participants.

4.4.5.3 Blood sample collection

A cannula was placed into the forearm antecubital vein and 10 mL blood was taken at each time point for assessment of plasma gut hormones (GLP-1, PYY), glucose, β-hydroxybutyrate, palmitic acid within triacylglycerol lipid fraction and serum insulin. A total of 12 blood samples were taken at timepoints -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360, and 420 minutes. Samples were processed in the CRF laboratory by centrifuging at 2500 RCF at a temperature of 4ºC. Samples were stored in -80ºC freezers until analyses could be conducted at the end of the study.

4.4.5.4 Visual Analogue Scales for Appetite

Subjective appetite measurements were taken using a standardised 100 mm visual VAS to assess subjective hunger, fullness, desire to eat, appetite for a meal, snack, something sweet or something savoury, thirst, anxiety, nausea, sleepiness and stress. These 12 measurements were taken at time points -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360, and 420 minutes. Participants were asked to draw a vertical line on the scale at the place corresponding to their current state. The scale ranged from 0 = not at all to 100 = extremely. The placement of the lines was measured post-study visit so composite appetite scores could be calculated.

4.4.5.5 Ad-libitum meal

At 420 minutes, participants were served a meal of dried, durum wheat penne pasta, olive oil and tomato sauce (Tesco, UK: 126.5 kcal, 21.4g carbohydrate, 2.9g fat and 3.3g protein per 100g). The meal was served in excess; so that participants' intake was not influenced them finishing the amount provided. They were asked to eat until they felt comfortably full.

The standardised meal was weighed before and after meal intake to determine the weight (g) of the meal consumed. This was converted into kilocalories to provide an objective energy intake measurement.

4.4.5.6 Indirect Calorimetry

Whole-body energy expenditure and substrate oxidation were measured using a gas exchange monitor (GEM Nutrition, Cheshire, UK). Indirect calorimetry measurements were taken four times throughout the study visit day, fasting measurements were taken from time points -30 to 0 minutes, and then fed measurements were taken at 30 to 60, 210 to 240 and 390 to 420 minutes.

4.4.5.7 Breath sampling

Breath samples were taken every 15 minutes (from -30 minutes to 420 minutes, except at 0, 45, 225 and 405 minutes) by asking participants to blow lightly into glass tubes using a drinking straw for 5 seconds at a time before tightly securing the lid. This was to measure the appearance of ^{13}C in breath CO_2 in breath tests to measure whole-body fatty acid oxidation (Pramfalk et al., 2015).

Breath hydrogen measurements using a Gastrolyser (Bedfont Scientific Ltd., Maidstone, UK) were also taken at -30, 60, 120, 180, 240, 300, 360 and 420 minutes.

4.4.5.8 Physical Activity

Accelerometry was recorded for seven days leading up to the two study visit days. The GENEActiv (Activinsights Ltd., Kimbolton, UK) device measured physical activity duration and intensity, step count, time spent sedentary and sleep duration. Participants were encouraged to wear the monitor as much as possible over this period for optimal data collection.

Figure 4.1: **Study flow chart for iPREVENT**

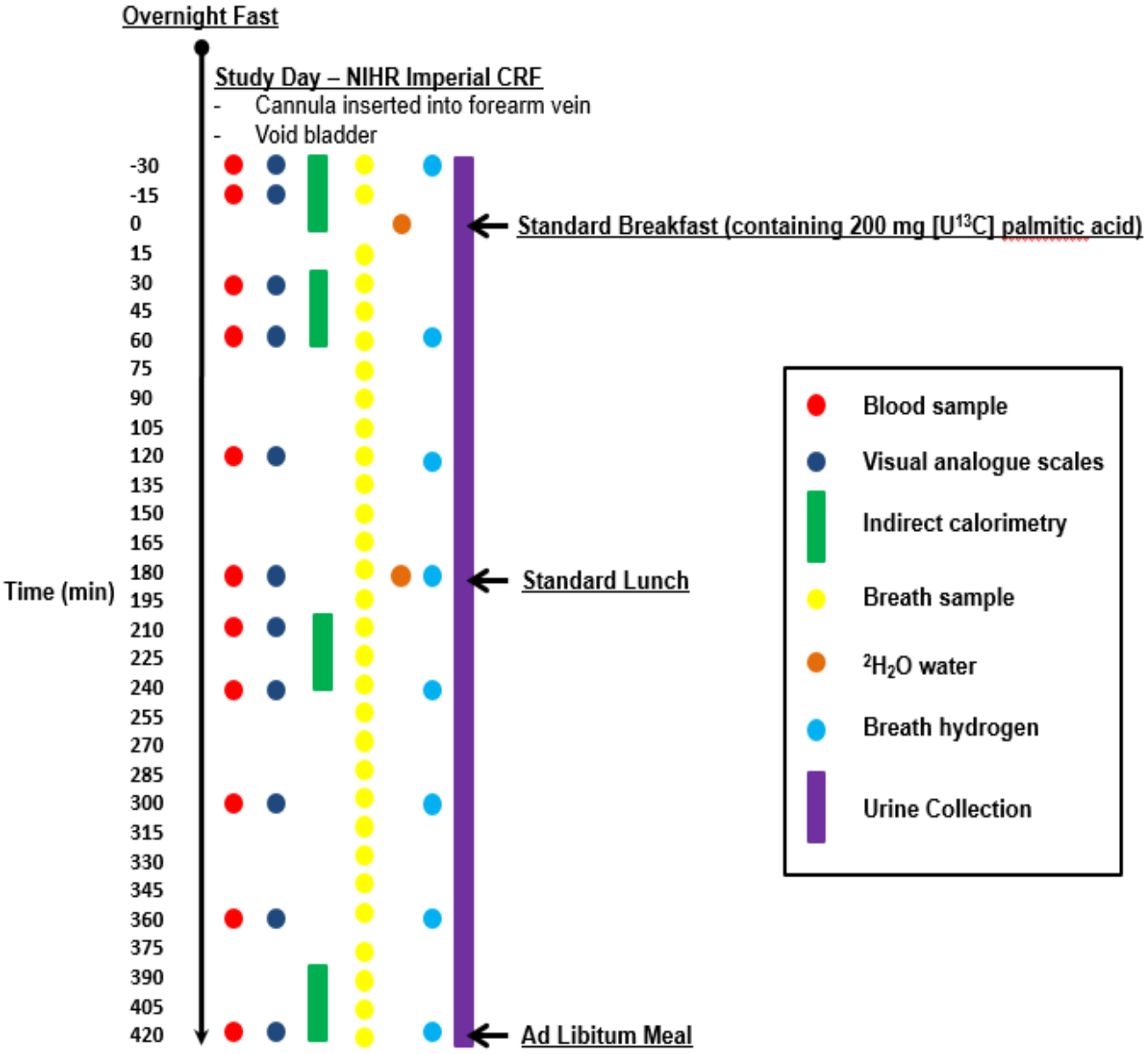


Figure 4.2: **Study meals for iPREVENT**

A **B**



(A) Standard Lunch served to iPREVENT participants: cheese sandwich on white bread and ready-salted potato crisps. (B) Ad-libitum pasta meal served to all iPREVENT participants: Tesco brand tomato and basil pasta sauce with penne pasta, cooked using a standard protocol.

4.4.6 **Sample Processing and Analysis**

4.4.6.1 Gut hormone analysis

Gut hormone concentrations (PYY and GLP-1) were measured using established in-house radioimmunoassays (RIA). The methodology has been previously described elsewhere (Adrian et al., 1985; Kreymann et al., 1987). Mean CV % of assay was 2.8% of assay for GLP-1 and 2.7% for PYY.

4.4.6.2 Subjective appetite

Subjective appetite for hunger, fullness, desire to eat and feeling sick, were measured from the left end of the line using a 100 mm ruler. This value was used as their score for that timepoint.

4.4.6.3 Ad-libitum Energy intake

To calculate energy intake from the ad-libitum meal, the following formula was used:

Energy intake = (meal weight before – meal weight after eating) * (energy content of *ad-libitum* meal per 100g [kcal]/100)

4.4.6.4 Indirect calorimetry

Gas measurements for oxygen consumption and carbon dioxide production were used to calculate substrate oxidation and energy expenditure (Frayn, 1983; J. B. de V. Weir, 1949). To calculate protein oxidation a nitrogen excretion constant was taken from a previous study using IPE (Byrne et al., 2019).

4.4.6.5 Glucose and Insulin Analysis

Glucose was measured using a colourimetric enzymatic activity assay (GL364, Randox, UK). Mean CV % of assay was 3.0%. Insulin was measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) (DY8056-05, Biotechne, USA). Mean CV % of assay was 5.1%. Both assays were conducted in line with the manufacturers' instructions.

4.4.6.6 Physical Activity

Data was read and processed using GeneActiv R Markdown Analysis Package (available from https://activinsights.com/support/geneactiv-support/), to produce reports on physical activity and sleep.

4.4.7 **Safety reporting**

Adverse events and side effects were reported per methodology stated in **Chapter 3, Section 3.4.7**.

4.4.8 **Statistical analysis**

The exploratory statistical analysis for this Chapter was conducted by JCV and ATP, unless otherwise stated. Statistical analysis was conducted in IBM SPSS Statistics (RRID: SCR_019096), version 28.0.1. Methodology for statistical analysis conducted by JCV and ATP was as follows:

As the sub-study sample size was smaller, within-participant imputation was used when up to four post-baseline measurements were missing in measures where 10 or 11 time points, were expected to be present. For measurements missing at the end of the series of measurements, the last-observation-carried-forward approach was used. For all other missing measurements, a straight-line imputation approach was taken between observed measurements, using the values and timings of adjacent observed measurements.

For the 12m follow-up visit, pre-baseline/baseline measurements were also imputed using the baseline measurement at the baseline visit from the same person on two occasions.

Due to non-negligible differences between groups in the baseline visit iAUC in small groups, the change from 12-month visit to baseline visit in the iAUC was analysed, to account for baseline differences. The number of participants present at the 12-month follow-up was lower than anticipated, so no attempt was made to further adjust for baseline using multiple regression as had been originally planned. Similarly, sensitivity analysis to imputations was not undertaken due to the small numbers with data at both time points, together with a lack of plausible pre-determined alternative assumptions.

Due to skewness in the data or small numbers present at the 12-month visit, the analysis to compare the two arms was expressed in terms of the more robust difference in medians, rather than means, and reporting the interquartile range (IQR) defined here as the first to the third quartile. The 95% confidence intervals for the difference in medians between two independent samples was obtained using the Hodges-Lehmann estimator. An exception for this was the accelerometer outcome data, where daily data allowed more appropriately distributed summary averages to be calculated per outcome, and the nonparametric Bootstrap method was used to calculate 95% confidence intervals. Confidence intervals excluding zero, for the analysis of a difference in means or proportions, were labelled as tentatively statistically significant due to multiple secondary outcome tests. Further detail is provided in the statistical analysis plan for the iPrevent Clinical Trial (Vasconcelos et al., 2022).

4.4.8.1 Area under the curve Analysis

For this study, iAUCs and time course data were used to summarise responses at each of the baseline and 12-month follow-up visits. The iAUC was used for all mechanistic outcome unless indicated otherwise in the following sections, for clinical relevance and compatibility with previous research. To calculate the incremental AUCs, imputation was used when up to four post-baseline measurements were missing in measures where 10 or 11 were expected to be present. For measurements missing at the end of the series of measurements, the last observation carried forward approach was used. For all other missing measurements, a straight-line imputation approach was taken between observed measurements, using the values and timings of adjacent observed measurements.

For plasma gut hormones, glucose, and serum insulin, the iAUC is presented in two distinct time segments: from -30 to 180 minutes and from 180 to 420 minutes. This separation was based on the theory that IPE or insulin would reach target sites within the colon and exert a physiological effect by 180 minutes. This is based on previous evidence using ^{13}C-labeled IPE to investigate the rate of IPE metabolism in the colon (Polyviou et al., 2016). Therefore, this division allows for the detection of any differential effects occurring after the 180-minute threshold.

4.4.8.2 Post-hoc Analyses

Post-hoc analysis was conducted by the author using JASP Statistical analyses (JASP Team (2024). JASP (Version 0.19.0) [Computer software]). Given the number of withdrawals at follow-up an intention to treat “last observation carried forward” exploratory analysis was conducted on the primary outcome of fasting and postprandial iAUC gut hormone data, using linear mixed models. To account for any changes from fat-free mass energy expenditure iAUC at BL and 12-months was corrected for fat-free mass by dividing energy expenditure iAUC by fat-free mass. Linear mixed models were used to detect any differences in effect.

4.4.8.3 Graph Preparation

The Graphs were prepared by the author using GraphPad Prism (version 10.3, for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). The Data on the graph represents all participants at baseline (IPE; n=25, inulin; n=25), rather than only participants who completed both baseline and follow-up visits. Data on graph represents Mean ± SEM.

4.4.9 **Sample Size Calculation**

For the mechanistic study, 34 volunteers (17 per group) would provide sufficient statistical power to detect a 15 pmol/L effect size in PYY and GLP-1 concentrations between groups, with 90% power, 5% significance level, SD 13 pmol/L. These differences were based on previously published findings that report enhanced gut hormone release following IPE supplementation (Chambers, Viardot, et al., 2015; Polyviou et al., 2016). A subsample of 52 volunteers (26 per group), was chosen, to allow for a 70% retention rate.

4.5 Results

4.5.1 **Recruitment and Baseline Characteristics**

A subgroup of 52 participants from the iPREVENT main study were enrolled in this mechanistic study. Two participants were withdrawn from the sub-study at baseline due to multiple cannula failures. One participant was willing to continue with the sub-study after cannulation failure, this meant that no blood samples were taken from this participant during their baseline visit. One participant withdrew from the sub-study visit at T180 as they refused to eat the standard lunch meal. The mean age of these participants was greater 31.8 (5.5) years versus 27.5 years for the whole study cohort (**Table 1.1** and **1.2**). A total of 24 participants (IPE n=16; inulin n=8) underwent sub-study visit at 12 months. There were no significant differences in participant characteristics within both study arms between baseline and 12 months. The exception to this was cholesterol which increased by 2.36 mmol/L (p=0.027).

Table 4.1: Demographic characteristics by arm and timepoint

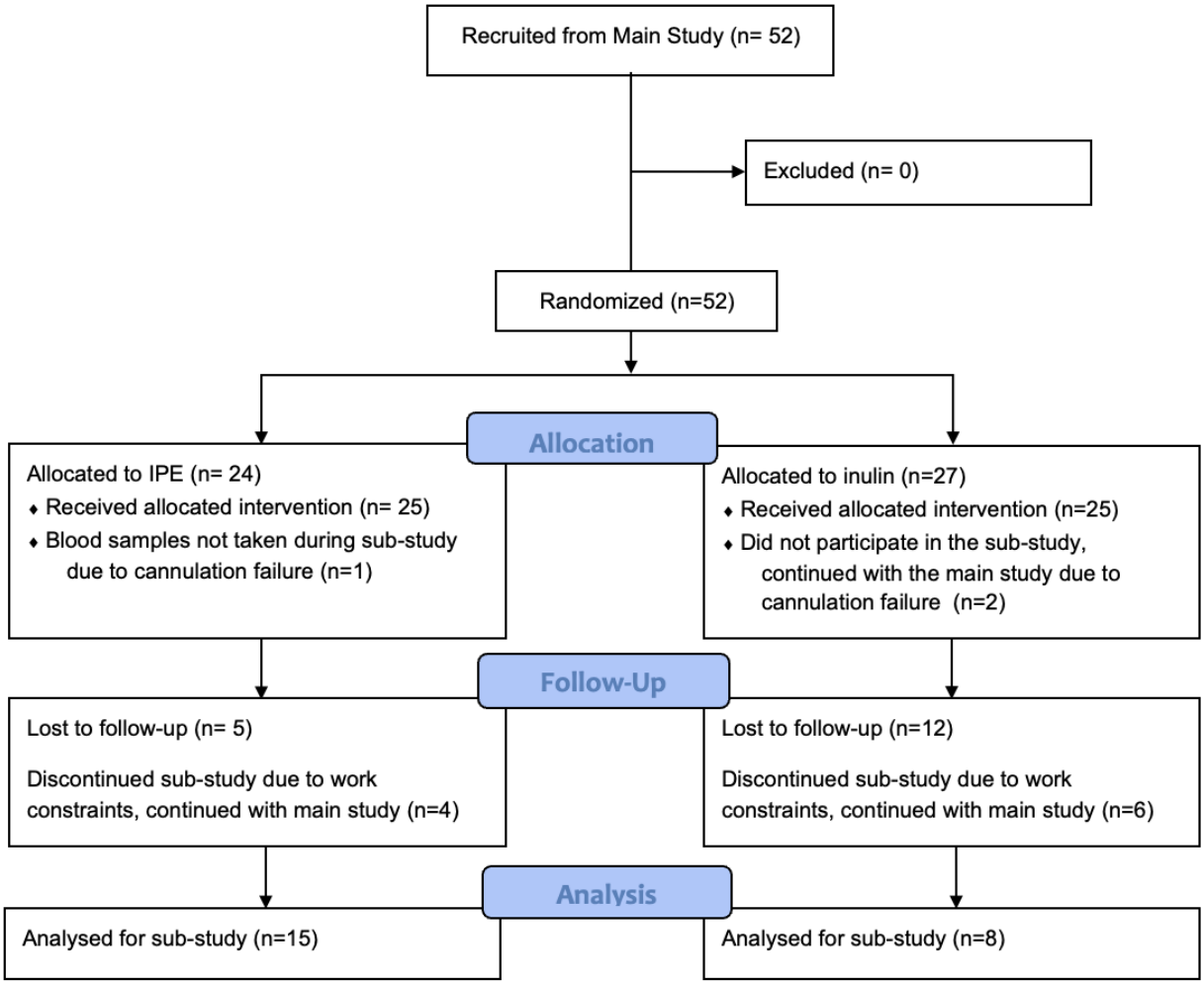
	IPE BL n (%)	IPE FU n (%)	Inulin BL n (%)	Inulin FU n (%)
Sex				
Male	7 (28)	7 (44)	5 (19)	2 (25)
Female	18 (72)	9 (56)	22 (81)	6 (75)
Ethnicity				
White	16 (64)	10 (62)	18 (67)	5 (63)
Black	2 (8)	0 (0)	2 (7)	2 (25)
Asian	4 (16)	3 (19)	3 (19)	1 (12)
Any other ethnic group	3 (12)	3 (19)	2 (7)	0 (0)

Table 4.2: Baseline characteristics by arm and timepoint

		Baseline			Follow-up			Follow-up – baseline* (p value)
	Group	N	Mean	SD	N	Mean	(SD)	
Age (years)	IPE	25	31.8	(5.8)				
	Inulin	27	31.8	(5.1)				
Weight (kg)	IPE	25	78.1	(9.6)	16	78.2	(12.1)	-0.87 (0.39)
	Inulin	27	75.0	(9.4)	8	76.0	(7.4)	
Fat Mass (kg)	IPE	25	24.7	(4.9)	16	22.2	(4.9)	-1.47 (0.15)
	Inulin	27	24.3	(4.5)	8	23.4	(4.7)	
Water Mass (kg)	IPE	25	38.0	(6.3)	16	39.8	(7.1)	0.53 (0.60)
	Inulin	27	36.3	(6.5)	8	37.8	(6.2)	
Fat Free Mass (kg)	IPE	25	53.3	(9.1)	16	56.7	(11.2)	-0.76 (0.46)
	Inulin	27	50.7	(9.3)	8	52.7	(8.7)	
BMI (kg/m^2)	IPE	25	27.4	(1.5)	16	26.4	(1.7)	-0.99 (0.33)
	Inulin	27	27.2	(1.4)	8	27.1	(1.1)	
Fasting glucose (mmol/L)	IPE	25	4.6	(0.5)	14	4.5	(0.5)	0.47 (0.65)
	Inulin	26	4.7	(0.4)	8	4.6	(0.3)	
Fasting Insulin (pmol/L)	IPE	25	86.2	(63.6)	16	62.7	(54.1)	-0.88 (0.39)
	Inulin	26	69.6	(57.1)	8	70.3	(56.3)	
Fasting Cholesterol (mmol/L)	IPE	25	4.4	(1.0)	16	4.7	(1.0)	2.36 (0.027)
	Inulin	26	4.5	(0.9)	8	4.6	(0.9)	

*Difference between baseline and 12 month visit values assessed by paired samples t-tests or Wilcoxon signed rank tests.

Figure 4.3: CONSORT Study Flow Diagram



4.5.1 **Plasma GLP-1 and PYY**

Table 4.3 demonstrates that there were no significant differences between arms at baseline and follow-up within or between the IPE and control study arms for GLP-1 and PYY iAUC. **Figure 4.4** shows concentrations of GLP-1 and PYY over the study visit day (-30 mins to 420 mins) (**A & B**). There was no difference in fasted concentrations of PYY and GLP-1 at T -30 and -15. There were no significant differences between $iAUC_{0\text{-}180\ mins}$ or $iAUC_{180\text{-}420\ mins}$ for GLP-1 and PYY concentrations for IPE and inulin at baseline and follow-up (**C-F**). Conclusions did not change after conducting an intention to treat analysis where fasting and iAUC GLP-1 and PYY concentrations were carried across from baseline in instances where participants did not attend their follow-up visit.

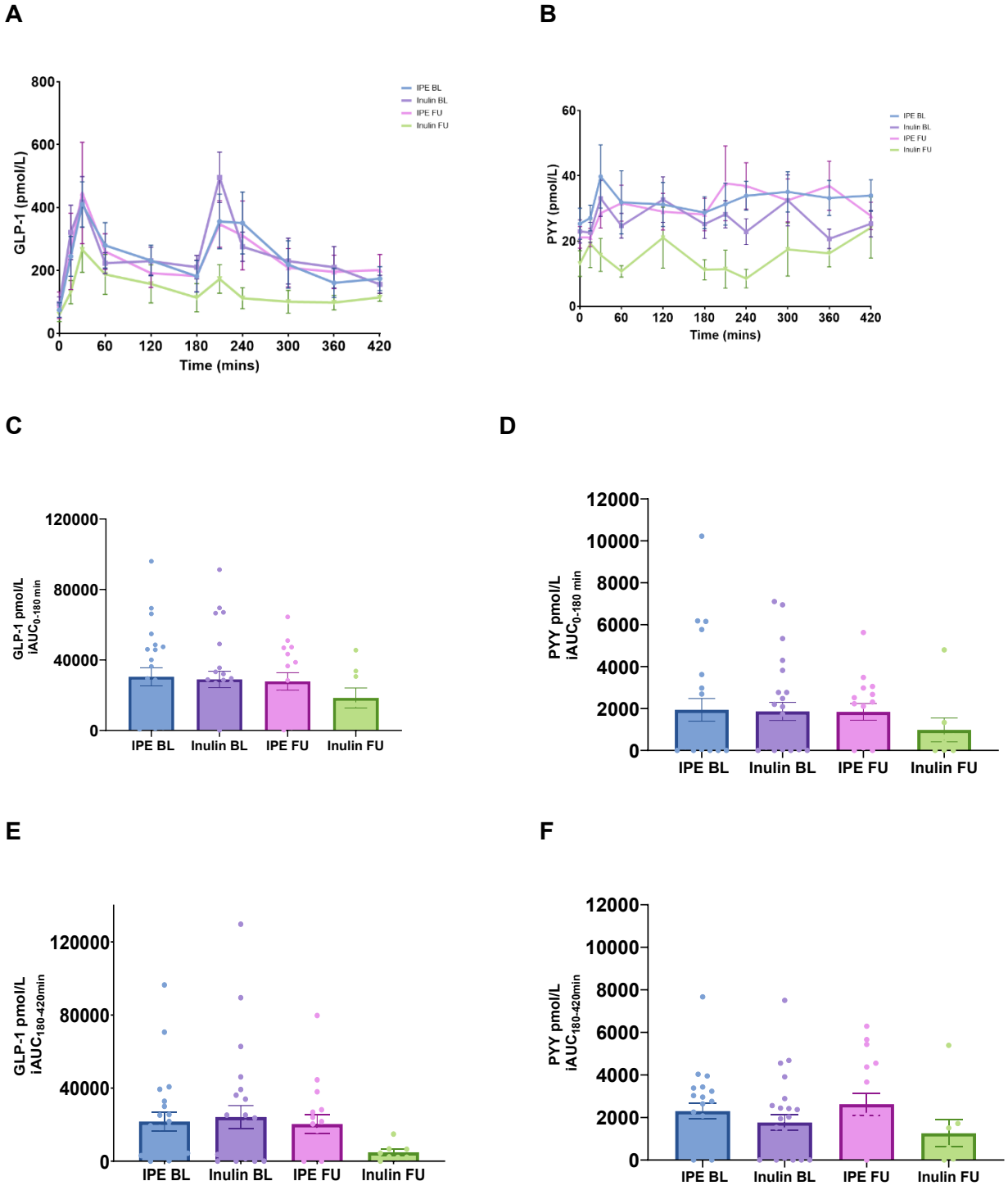
Table 4.3: Medians (IQR) of the incremental iAUC of GLP-1 and PYY

	Baseline*		**12m follow-up**		**Change from baseline**		**Difference between arms**
	IPE	inulin	IPE	Inulin	IPE	Inulin	
	N=15**	N=8	N=16	N=8	N=15	N=8	**(95% CI)**
GLP-1 (pmol/L)	67019 (15985 - 116443)	47547 (20464 - 56880)	58295 (20278 - 94274)	31665 (8653 - 49388)	-7214 (-36780 - 25174)	-8486 (-34067 - 15281)	1272 (-33734 to 38013)
PYY (pmol/L)	3980 (822 - 8494)	1893 (916 - 5651)	6124 (1008 - 9842)	1304 (108 - 2734)	437 (-5068 to 5078)	-150 (-4251 to 769)	586 (-5174 to 6629)

*For those with measurements at both baseline and 12m follow up visits.

**One participant with a follow-up measurement was excluded from this baseline summary due to having only two measurements at baseline.

Figure 4.4: **Gut hormone concentrations following intake of inulin or IPE at baseline and follow-up visit**



(A & B) Fasting and postprandial GLP-1 and PYY concentrations. (C & D) $iAUC_{0\text{-}180}$ for GLP-1 and PYY. (E & F) $iAUC_{180\text{-}420}$ for GLP-1 and PYY. Data represent mean ± SEM. Blue: IPE BL (n=25), purple: inulin BL (n=24), pink: IPE FU (n=16), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; GLP-1, glucagon-like peptide-1; iAUC, incremental area under the curve; IPE, inulin-propionate ester; PYY, peptide YY

4.5.2 **Subjective appetite scores**

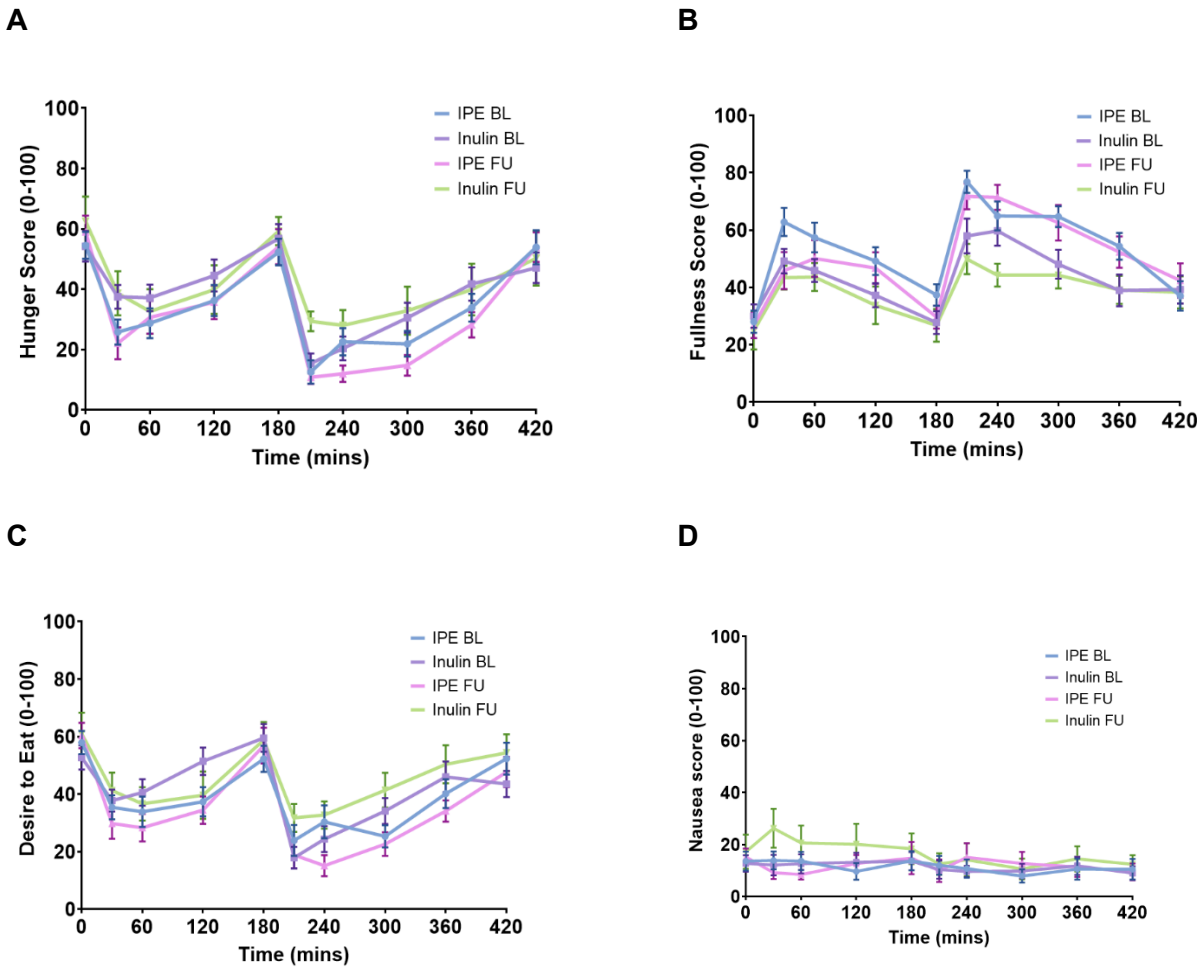
Table 4.4 demonstrates that there were no significant differences between arms at baseline to follow-up within or between the IPE and inulin study arms hunger, fullness, desire to eat or feeling sick. **Figure 4.5** shows changes in subjective appetite: hunger, fullness, desire to eat and sickness over the study visit day (-30 mins to 420 mins) (**A-D)**. Breakfast and lunch were consumed at 0 minutes and 180 mins, respectively. There were no differences in the $iAUC_{0\text{-}480\ mins}$ the curve for hunger, fullness, desire to eat or feeling sick at baseline and follow-up, within groups or between IPE and inulin.

Table 4.4: Medians (IQR) of the iAUC for Subjective appetite scores

	Baseline*		**12m follow-up**		**Difference (95% CI) between arms at f-up**	**Change from baseline**		**Difference (95% CI) between arms of change**
	IPE N=16	Inulin N=8	IPE N=16	Inulin N=8		IPE N=16	Inulin N=8	
Desire to eat (mm)	4 (0 - 382)	194 (27 - 4094)	128 (0 - 773)	76 (0 - 3890)	52 (-2804 to +264)	11 (-193 – 467)	-118 (-648 - 113)	129 (-240 to +1074)
Fullness (mm)	13693 (7815 - 20252)	7382 (2290 - 8186)	10174 (5482 - 17559)	6247 (1878 - 12821)	3927 (-1928 to +9978)	-998 (-7770 - 3166)	-268 (-5200 - 4277)	-730 (-7062 to +3717)
Hunger (mm)	106 (1 - 1135)	253 (0 - 4276)	174 (0 - 425)	0 (0 - 1527)	174 (-1079 to +206)	-23 (-489 - 411)	0 (-301 - 596)	-23 (-681 to +1044)
Nausea (mm)	879 (13 - 1744)	279 (73 - 2444)	26 (0 - 1118)	55 (0 - 4284)	-28 (-1428 to +533)	-297 (-1448 - 1106)	-41 (-144 - 2214)	-256 (-2648 to +886)

*for those with measurements at both baseline and 12m follow up visits.

Figure 4.5: Subjective appetite scores following intake of inulin or IPE at baseline and follow-up visit



Change in feelings of hunger (A), fullness (B), desire to eat (C) and sickness (D) from T0-420 min. Data are represented as mean ± SEM. Blue: IPE BL (n=24), purple: inulin BL (n=23), pink: IPE FU (n=14), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; iAUC, incremental area under the curve; IPE, inulin-propionate ester.

4.5.3 **Ad-libitum Energy intake**

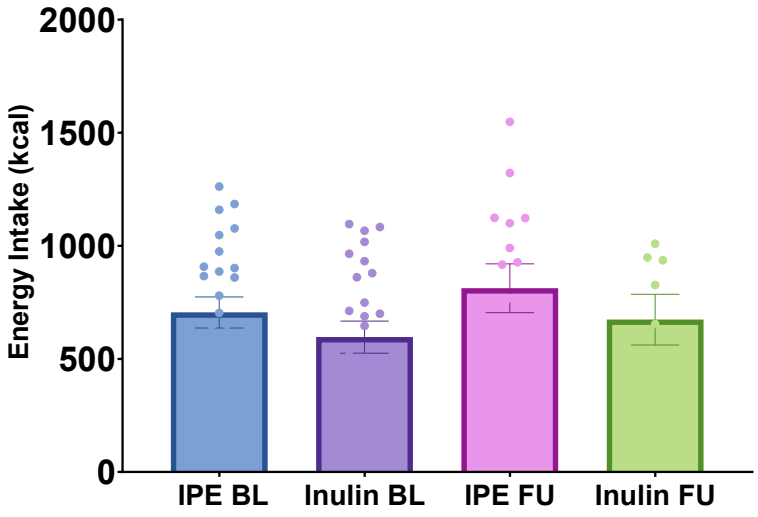
Table 4.5 shows a statistically significant difference, indicated by the 95% confidence intervals, in mean change in *ad-libitum* meal intake between the study arms of 169 (311 to 26) grams at follow-up. **Figure 4.6** shows mean energy intake (kilocalories) per study arm at baseline and follow-up.

Table 4.5: Intake (g) of ad-libitum meal (meal weight before – meal weight after)

	Baseline		**12m follow-up**		**Changes from baseline**		**Difference in means**
	IPE Mean (SD) N=15	Inulin Mean (SD) N=8	IPE Mean (SD) N=15	Inulin Mean (SD) N=8	IPE Mean (SD) N=15	Inulin Mean (SD) N=8	**(95% Cis)**
Ad-libitum meal intake (g)	619 (257)	679 (143)	640 (329)	531 (250)	20 (161)	-148 (146)	169 (311 to 26)

CI, confidence intervals; IPE, inulin-propionate ester; SD, standard deviation

Figure 4.6: *Ad-libitum* energy intake (kilocalories) following intake of inulin or IPE at baseline and follow-up



Data represent mean ± SEM. Blue: IPE BL (n=24), purple: inulin BL (n=24), pink: IPE FU (n=15), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; iAUC, incremental area under the curve; IPE, inulin-propionate ester.

4.5.4 **Energy expenditure and substrate oxidation**

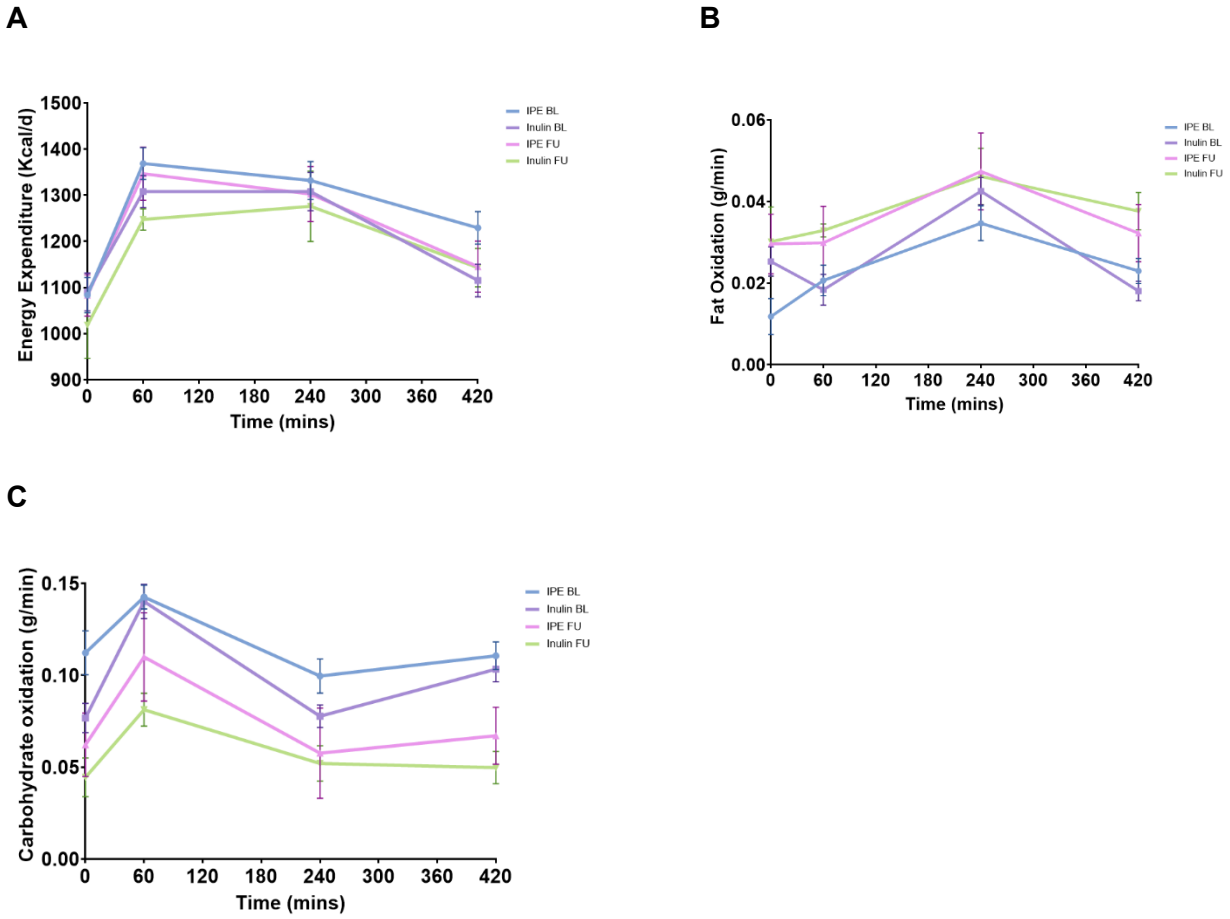
Table 4.6 shows that there were no differences in the iAUC for energy expenditure, fat and carbohydrate oxidation, within, or between inulin and IPE study arms. **Figure 4.7** shows changes in energy expenditure, fat and carbohydrate oxidation over the study visit day (-30 mins to 420 mins) (**A-C)**. Breakfast and lunch were consumed at 0 minutes and 180 mins. Conclusions did not change when energy expenditure was corrected for fat-free mass.

Table 4.6: Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for energy expenditure and substrate oxidation

	Baseline*		**12m follow-up**		**Difference (95% CI)**	**Change from baseline**		**Difference (95% CI)**
	IPE N=13	Inulin N=6	IPE N=14	Inulin N=7	**between arms at follow up**	IPE N=13	Inulin N=6	**between arms of change**
Energy expenditure (kcal)	102219 (60333 - 161471)	34461 (24195 - 70383)	76246 (28429 - 129345)	99198 (9816 - 137038)	-22952 (-65063 to 69397)	-49237 (-97538 to 43923)	32035 (-31915 to 109039)	-81272 (-165218 to +34569)
Respiratory exchange ratio (RER)	1 (0 - 14)	9 (2 - 27)	2 (1 - 10)	1 (0 - 16)	0 (-4 to 13)	2 (-2 to 6)	-4 (-18 to 9)	6 (-9 to +19)
Carbohydrate oxidation (g/min)	6 (1 - 18)	10 (6 - 22)	4 (2 - 14)	4 (1 - 12)	1 (-9 to 8)	0 (-4 to 6)	-6 (-16 to 5)	5 (-4 to +17)
Fat oxidation (g/min)	6 (2 - 11)	0 (0 - 4)	4 (2 - 6)	8 (0 - 9)	-4 (-4 to 7)	-3 (-7 to 2)	3 (-2 to 10)	-6 (-13 to +2)

*for those with measurements at both baseline and 12m follow up visits.

Figure 4.7: Energy expenditure, fat and carbohydrate oxidation following intake of inulin or IPE at baseline and 12 months visit



Fasting and postprandial energy expenditure (A), fat oxidation (B), carbohydrate oxidation (C). $IAUC_{0\text{-}420}$ for energy expenditure (D), fat oxidation (E) and carbohydrate oxidation (F). Data represent mean ± SEM. Blue: IPE BL (n=24), purple: inulin BL (n=25), pink: IPE FU (n=16), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; iAUC, incremental area under the curve; IPE, inulin-propionate ester.

4.5.5 **Plasma glucose and serum insulin**

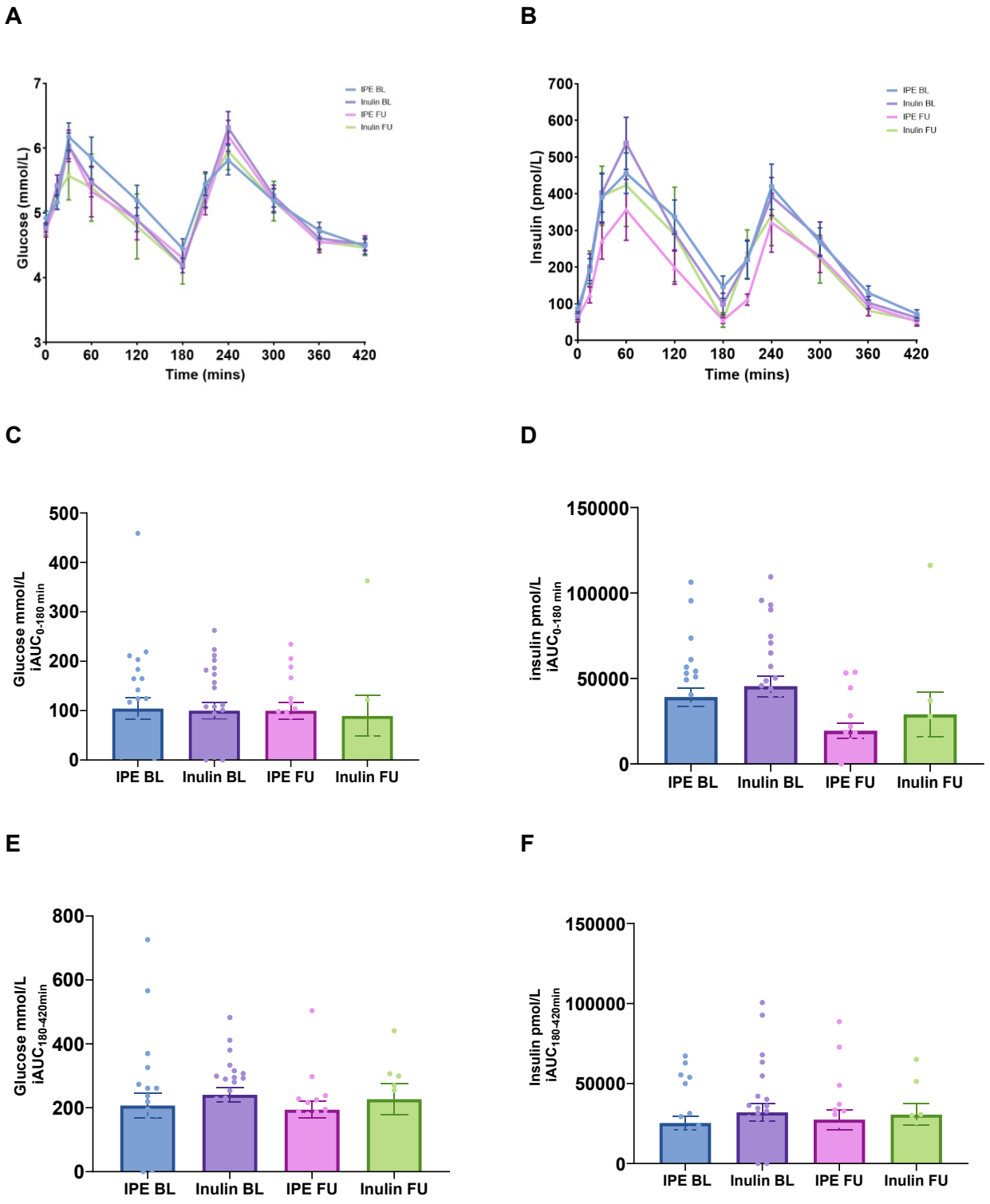
Table 4.7 demonstrates that there were no significant differences between arms at baseline to follow-up within or between the IPE and control study arms for glucose and insulin iAUC. **Figure 4.8** shows changes in glucose and insulin over the study visit day (-30 mins to 420 mins) (**A & B)**. Breakfast and lunch were consumed at 0 minutes and 180 mins, respectively. There were no significant differences between $iAUC_{0\text{-}180\ mins}$ or $iAUC_{180\text{-}420\ mins}$ for glucose and insulin concentrations for IPE and inulin at baseline and follow-up (**C-F**).

Table 4.7: Medians (IQR) of the iAUC of plasma glucose and serum insulin

	Baseline*		**12m follow-up**		**Difference (95% CI)**	**Change from baseline**		**Difference (95% CI)**
	IPE N=15	Inulin N=8	IPE N=16	Inulin N=8	**between arms at follow up**	IPE N=15	Inulin N=8	**between arms of change**
Glucose iAUC (mmol/L)	189 (71 - 293)	204 (122 - 281)	231 (136 - 291)	142 (75 -263)	89 (-56 to +162)	2 (-45 - 66)	-28 (-112 - 67)	30 (-81 to +122)
Insulin iAUC (pmol/L)	43,839 (29,268 - 84,992)	66,735 (38,990 - 10,6919)	42,961 (18,056 - 79,609)	42,591 (28,537 - 101,735)	+370 (-40,628 to +22879)	-4,885 (-31,520 - 19,557)	-3,914 (-37,425 - 16,011)	-971 (-29868 to +24549)

*For those with measurements at both baseline and 12m follow up visits.

Figure 4.8: Glucose and insulin concentrations following intake of inulin or IPE at baseline and 12 months visit



(A & B) Fasting and postprandial glucose and insulin concentrations. (C & D) $iAUC_{0\text{-}180}$ for glucose and insulin. (E & F) $iAUC_{180\text{-}420}$ for glucose and insulin. Data represent mean ± SEM. Blue: IPE BL (n=24), purple: inulin BL (n=25), pink: IPE FU (n=16), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; iAUC, incremental area under the curve; IPE, inulin-propionate ester.

4.5.6 **Physical activity**

At the baseline visit, there were 21 participants with all 7 days of data for all outcomes and three had fewer than three days of data. At follow-up, 14 participants had 7 days of data and 9 had three or fewer days of data. Sleep duration was statistically significant between arms with a change from baseline -74 (-124 to -29) minutes, no other measurements of physical activity were different between the two study arms (**Table 4.8**).

Table 4.8: Means (SD) of the different physical activity measurements

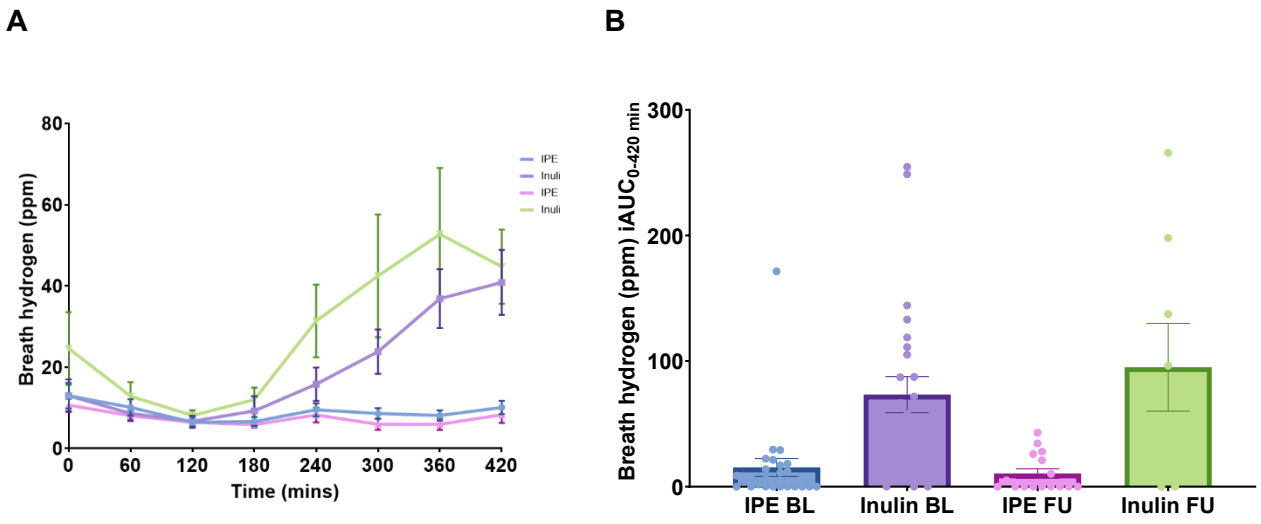
	Baseline		**Follow-up**		**Change from baseline**		**Difference (95% CI) between arms of the change from baseline**
	IPE Mean (SD) N=16	Inulin Mean (SD) N=7	IPE Mean (SD) N=16	Inulin Mean (SD) N=7	IPE Mean (SD) N=16	Inulin Mean (SD) N=7	
Sleep duration (mins)	432 (66)	379 (50)	374 (60)	394 (60)	-58 (76)	15 (42)	-74 (-124 to -29)†
Step count	9406 (5130)	8473 (2988)	10655 (5227)	9992 (2188)	1250 (3371)	1519 (1992)	-269 (-2485 to 1913)
Moderate + Vigorous (mins)	134 (66)	121 (48)	151 (84)	132 (51)	17 (60)	11 (22)	5.8 (-26.5 to 39.4)
METs	1278 (367)	1277 (395)	1461 (391)	1423 (457)	183 (306)	146 (253)	36 (-198 to 269)
PA duration⁺ (mins)	352 (99)	358 (116)	405 (104)	401 (136)	53 (86)	43 (75)	10 (-59 to 77)

MET, metabolic equivalent of tasks; PA, physical activity; SD, standard deviation; Con, inulin control; IPE, inulin-propionate ester.
⁺defined as the sum of light+ moderate + vigorous
†Statistically significant

4.5.7 **Breath Hydrogen**

Figure 4.9 shows changes in breath hydrogen concentrations between IPE and inulin study arms at baseline and 12 months (A) and breath hydrogen iAUC 0-420 (B). There were no significant differences in breath hydrogen within or between groups at baseline or follow-up.

Figure 4.9: Breath hydrogen concentrations following intake of inulin or IPE at baseline and 12 months visit



(A) Concentrations of fasting and postprandial breath hydrogen for IPE and inulin at baseline and follow-up, (B) $iAUC_{0\text{-}420}$ for breath hydrogen. Data represent mean ± SEM. Blue: IPE BL (n=24), purple: inulin BL (n=23), pink: IPE FU (n=14), green: Inulin FU (n=8). Abbreviations: BL, baseline; FU, follow-up; iAUC, incremental area under the curve; IPE, inulin-propionate ester.

4.6 Discussion

4.6.1 **Summarised Discussion**

It was hypothesised that SCFA may improve metabolic health by stimulating satiety and enhancing energy expenditure (Byrne et al., 2015; Canfora et al., 2015; Morrison & Preston, 2016). I hypothesised that enhancing propionate in the colon would stimulate increased secretion of GLP-1 and PYY in overweight, younger adults at risk of further weight gain. However, after 12-months, no significant differences in GLP-1 and PYY iAUC were detected between the IPE and inulin study arms. There were also no significant differences in secondary outcomes, except for energy intake at the *ad libitum* meal, which was higher in the IPE intervention group.

4.6.2 **Withdrawals**

This mechanistic study was worse impacted by lifestyle changes during the COVID-19 pandemic than the main clinical trial. There were much higher withdrawal rates, with less than 50% of participants returning for the follow-up visit. This meant that the primary outcome was substantially underpowered, and that caution should be taken when drawing conclusions drawn from this study.

4.6.3 **Primary Outcome**

In this study, no differences were seen in GLP-1 and PYY secretion after acute and chronic inulin and IPE intake. In a preliminary study, an acute 10g intake of IPE increased secretion of GLP-1 by seven-fold and PYY by three-fold (Chambers, Viardot, et al., 2015). The design of the present study differed from the previous in two ways. Firstly, inulin or IPE were given in the baseline study breakfast mixed-meal tolerance test in the current study. The addition of the intervention to the study meals at baseline prevented the comparison of the acute effects of IPE or inulin with the gut hormone response to a mixed meal without any intervention. Notably, the previous study had a crossover design meaning that participants acted as their own controls, lowering the risk of individual differences in metabolism or the gastrointestinal microbiota impacting study outcomes.

Gut hormone secretion was not significantly different after long-term IPE intake, consistent with previous studies with a chronic IPE intervention (Chambers, Byrne, Morrison, et al., 2019; Chambers, Viardot, et al., 2015; Malkova et al., 2020). More surprisingly, GLP-1 and PYY iAUC trended lower at follow-up in IPE and inulin conditions. This may have been driven by alterations in the colonic environment, for example, desensitisation or downregulation of

FFAR2 and 3 or reduced proliferation of enteroendocrine cells responsible for GLP-1 and PYY release, returning gut hormone concentrations to baseline levels (Chambers, Viardot, et al., 2015). However, studies in rodents reported enhanced L-cell proliferation even after 10 weeks of inulin supplementation (Brooks et al., 2017). The results at 12 months could also be attributed to an upregulation of proteins involved in gut hormone degradation, like DPP4 (Tolhurst et al., 2012).

It is also worth noting that inulin *per se* is a fermentable carbohydrate which promotes SCFA production by colonic microbiota (Rahat-Rozenbloom et al., 2017). Therefore, inulin alone could influence gut hormone secretion and appetite. However, inulin had no effect on anorectic gut hormone secretion or satiety responses in men who were overweight or obese (Beek et al., 2018).

The lack of acute or chronic response to enhanced flux of colonic propionate poses the question of whether the 10g dose was suitable for a cohort of younger adults with naturally higher SCFA concentrations (Salazar et al., 2019). Further, younger adults also appear to have lower fasting and postprandial PYY secretion and rate subjective hunger higher than older adults (Johnson et al., 2020). Suggesting that even if there were any effects on GLP-1 or PYY secretion, they may not have been powerful enough to dampen hunger signals in this cohort.

4.6.4 **Secondary Outcomes**

Contrary to previous findings where energy intake was suppressed (Byrne et al., 2016; Chambers, Viardot, et al., 2015; Polyviou et al., 2016), the IPE group had a significantly greater energy intake during the follow-up *ad-libitum* meal. This result was linked with trends towards an increased desire to eat and lower fullness that was detected in the VAS for subjective appetite. However, like in previous studies, IPE did not significantly affect subjective appetite responses. There is evidence to suggest that the stress of the clinical environment or study participation has an effect on appetite and energy intake (Chandarana et al., 2009). Hence, some of the previous interventions were designed to include an acclimatisation visit so participants were familiarised with the *ad libitum* meal (Byrne et al., 2019; Chambers, Viardot, et al., 2015). This was not incorporated into the current study design and may have contributed to a lower energy intake at the baseline visit.

Although SCFAs may increase resting energy expenditure and lipid oxidation, as seen in both humans (Canfora et al., 2017; Chambers et al., 2018) and animals (Z. Gao et al., 2009; Hattori et al., 2010), no changes in fasting or postprandial energy expenditure and fat

oxidation were detected. As mentioned, IPE and inulin were given at breakfast at the baseline visit, therefore it is not possible to determine whether the absence of inulin or propionate would have resulted in lower fat oxidation and energy expenditure at baseline. Furthermore, any acute effect of enhanced colonic propionate on energy expenditure was maintained at 12 months, indicated by the lack of significant differences between the baseline and follow-up measurements. One previous investigation identified a 7% increase in resting energy expenditure after seven days of IPE supplementation, however the effects of this were lost after controlling for changes in lean mass (Byrne et al., 2019). Further, no difference was seen after controlling for lean body mass in the present study. Unaltered resting energy expenditure and fat oxidation aligns with the observation of no weight change in the main cohort for this study (**Chapter 3**). However, these results fail to provide an explanation for the significant increase in fat-free mass post-IPE intake. Furthermore, in the sub-study cohort, there was a non-significant trend towards lowered fat mass and body weight. Since no changes in physical activity levels were detected, it is unlikely that increased physical activity contributed toward the effect on lean mass. Therefore, the factors behind this change in body composition are yet to be elucidated.

No differences in postprandial glucose and insulin were detected at baseline or follow-up in the present study, aligning with data from some previous studies (Byrne et al., 2016, 2019). However, other studies have demonstrated acute reductions in glucose AUC (Chambers, Viardot, et al., 2015), enhanced insulin sensitivity and improved glucose homeostasis after chronic IPE consumption (Byrne et al., 2019; Pingitore et al., 2017). There is reasonable understanding of the potential mechanisms by which SCFAs modulate glucose homeostasis and insulin sensitivity (Canfora et al., 2015; Frampton et al., 2020; Morrison & Preston, 2016). However, these theoretical findings are difficult translate into concrete evidence in human subjects (Cherta-Murillo et al., 2022). One key reason could be that, except for acetate, circulating SCFA concentrations may be too low to have a direct effect on sites involved in glucose homeostasis, such as the pancreas (Cummings et al., 1987). Alternatively, it could simply be due to a lack of adequately powered and well-designed human intervention studies exploring the role of SCFA on glycaemic response.

Propionate acts has a gluconeogenic substrate in the liver and intestine, where it has the potential to indirectly influence energy balance and glucose homeostasis (Boillot et al., 1995; Mithieux et al., 2009). Therefore, the role of the liver and gastrointestinal tract in propionate utilisation and metabolism requires further exploration.

There were no significant differences in breath hydrogen between the two study arms. However, breath hydrogen trended markedly lower in the IPE arm compared with the control. In past ^{13}C-labelling studies, propionate was delivered to the colon within three hours (Polyviou et al., 2016). In the present investigation, breath hydrogen was the sole measure of colonic IPE delivery. It is also unknown if delayed satiety signalling due to slower transit and catabolism of IPE contributed to the increase in energy intake observed at the *ad libitum* meal. A key question from this trial is whether propionate was effectively released in colon during the study visit. However, a standardised production methodology (Polyviou et al., 2016), was used for IPE synthesis and optimal colonic IPE absorption. There is no reason why this iteration of the compound would have been metabolised differently. Moreover, individual variations in gut transit time and the fact that there is 25% less fermentable fibre in IPE might have had some impact on breath hydrogen. However, it is unlikely to fully account for the magnitude of the effect seen in this trial. Further investigation is required to adequately interpret these results.

No significant differences in METs scores or duration of moderate and intense physical activity were identified between baseline and follow-up. There was significantly less sleep detected in the IPE than inulin arms at follow-up. A potential mechanism for the effect of IPE on sleep is activation of the nucleus accumbens, which plays a role in sleep-wake behaviour and arousal (Qiu et al., 2012). In previous work, IPE has been shown to lower BOLD signalling in the nucleus accumbens (Byrne et al., 2016).

4.6.5 **Allocation of study product**

As the results of this study do not reflect the data from previous research using IPE, the correct allocation of the study product was investigated. Serendipitously, the question of correct allocation of study intervention and control products was raised during the study. The two compounds were tested by an independent third party and the presence of propionate within the intervention product was confirmed. Despite correct blinding and allocation, the direction of effect opposed prior evidence.

4.6.6 **Limitations**

Few studies have examined the effects of increased colonic propionate concentrations using standardised methodologies to measure appetite response, energy expenditure and fat oxidation. This study is the first to investigate the mechanisms by which SCFA affect appetite and energy balance in younger, overweight adults. However, it is not without limitations.

The present study data indicates that elevated colonic propionate production did not acutely increase plasma concentrations of PYY and GLP-1 or lower energy intake of the *ad-libitum* meal compared with inulin. As inulin and IPE were given in the baseline visit breakfast, it was not possible to detect any acute differences between IPE or inulin intake with a *null* comparison. Notably, any effects of IPE or inulin on anorectic gut hormone release and energy expenditure were preserved after 12-months supplementation.

As previously mentioned, participants in both groups withdrew from this study before the 12 month follow up. This resulted in non-significant findings where previously positive results were observed. Underpowered outcomes also increase the risk of a type-one error, making it difficult to determine whether the lack of effect seen was because IPE does not affect appetite or is due to the small sample size.

Breath hydrogen was used as the sole indicator of IPE and inulin delivery to the colon in this study, whereas previous studies have used ^{13}C-labelling or measured concentrations of plasma propionate to confirm IPE reached the colon. Although there were no significant differences in breath hydrogen detected at baseline and follow-up, breath hydrogen within the IPE study arm did not trend towards an increase at any point during the two visits. Therefore, we cannot confirm with certainty whether IPE was metabolised at the target site during the study observation period, which could have dampened any predicted effects on satiety and energy expenditure.

Lastly, an older cohort with a higher BMI was recruited for the study to determine effective IPE dose (Chambers, Byrne, Morrison, et al., 2019). It is possible that 10g IPE was inadequate to elicit an effect on gut hormones or other outcomes in the present study in younger adults.

4.7 Summary

This clinical trial found no significant differences in GLP-1 and PYY between the inulin and IPE groups after acute or chronic supplementation. It is difficult to determine whether this was due to the underpowered sub-study primary outcome, the provision of IPE and inulin at the baseline visit or because the dose was inadequate to elicit an effect on satiety or energy expenditure in younger adults. Future work should investigate the physiological differences between younger and older populations and the effect of these differences on appetite and energy balance.

Chapter 5: General Discussion

5.1 Summary of findings

- Findings from the systematic review indicate oral, gastric, intravenous, colonic or rectal administration of acetate, propionate or butyrate did not affect glycaemic response. However, acetic acid, consumed in the form of vinegar, lowered acute postprandial glucose response in metabolically healthy individuals and those with T2D. However, it is difficult to draw conclusions from this evidence because there are few studies which investigate the effects of SCFA on glycaemia in humans and certainty of the available evidence was low or very low according to the GRADE criteria.

- In the RCT, the delivery of propionate to the colon, using IPE, in a year-long intervention did not prevent weight gain to a greater extent than the fermentable fibre, inulin. Fat-free mass was elevated in the IPE group, suggesting propionate may preserve lean mass. Encouragingly, substantial weight gain was not detected in either group, reinforcing the potential role of fermentable fibres in energy homeostasis.

- In the RCT, the delivery of propionate to the colon, via intake of IPE, did not affect satiety hormone secretion, energy expenditure or subjective appetite in younger adults after 12 months of intake. This may, in part be a result of the small sample size, due to high rates of withdrawal, that led to an underpowered primary outcome. Arguably, the acute effects of inulin and IPE on satiety hormones and energy expenditure were preserved from baseline to follow-up as there were no significant differences in the response at the two study visits.

5.2 Introduction

Fibre plays an important role in energy balance, weight gain prevention, appetite and blood glucose regulation (Reynolds et al., 2019). Current fibre intakes are low, representing less than a quarter of intake from palaeolithic diets (Eaton, 2006). Low fibre intakes may partly contribute to the increased prevalence of obesity and other related diseases, like T2D (O'Keefe, 2019). The benefits of dietary fibre are partially attributed to SCFA, a product of anaerobic fermentation of dietary fibre in the colon (Cummings & Macfarlane, 1991). Receptors for SCFAs are present at metabolically active sites of the body like the liver, vagus nerve or pancreas and have been shown to influence appetite, energy expenditure and substrate metabolism in animal studies and a limited number of human interventions (Beek et al., 2016; Canfora et al., 2015; Morrison & Preston, 2016; A. H. Sukkar et al., 2019). The number of studies investigating the role of SCFA on metabolic responses in humans is limited and the quality of evidence is low (Cherta-Murillo et al., 2022).

SCFAs are unpalatable and rapidly digested in the upper gastrointestinal tract, meaning that oral administration in interventions is unrealistic. Further, measuring SCFA concentrations and localised responses to SCFA in the vastly inaccessible colon is challenging. Past studies using dietary fibre have increased SCFA production but controlling the ratios of SCFAs produced is difficult. A potential solution is to deliver SCFAs directly to the colon by esterifying them to a fermentable carbohydrate 'carrier', ensuring they remain bound until they reach their natural site of production in the distal gut.

Findings from this research group suggest propionate is an appropriate SCFA to investigate the effects of enhancing colonic SCFA on appetite regulation, energy expenditure and body composition (Byrne et al., 2019; Chambers, Byrne, Rugyendo, et al., 2019; Chambers, Viardot, et al., 2015; Malkova et al., 2020; Pingitore et al., 2017; Polyviou et al., 2016).

The work presented in part of the thesis explored the current evidence for the role of SCFA in metabolic responses in humans and the effect of increased colonic propionate on weight maintenance in young adults susceptible to weight gain. Furthermore, relevant mechanisms driving satiety and increased energy expenditure were explored in the mechanistic sub-study.

5.3 Detailed Discussion

In **Chapter 2**, acute and chronic administration of SCFAs, acetate, propionate and butyrate, were found to have no significant effect on glucose or insulin. Vinegar, a dietary source of acetic acid, reduced acute glucose responses in healthy individuals and those with impaired glucose regulation. These results align with findings from a previous meta-analysis on vinegar (Shishehbor et al., 2017), though in this study, participants were not separated by health status. In the present meta-analysis, no differences in effects were detected between healthy individuals and those with pre-diabetes or T2D. The existing studies exploring the roles of acetate, propionate and butyrate on glycaemic regulation are limited in number and quality. Additionally, variability in administration methods and compositions of intervention substances contributed to significant heterogeneity. This variability made it difficult to identify which routes of administration, sites of action, SCFA type or composition affect glucose homeostasis. The variability also meant that some outcomes such as those from acute, mixed-SCFA interventions could not be analysed by meta-analysis.

The glucose-lowering effect of orally administered vinegar could suggest that SCFA action on glycaemic regulation may occur in the upper GI tract. The bitterness of vinegar and the rapid rate it is absorbed into the small intestine is associated with slower gastric emptying, enhanced GLP-1 release and inhibition of digestive amylases or disaccharidases (Brighenti et al., 1995; Darzi et al., 2014; Feise & Johnston, 2020; González Hernández et al., 2019; Hlebowicz et al., 2007; C. S. Johnston & Buller, 2005; Leeman et al., 2005; Liatis et al., 2010; Liljeberg & Björck, 1998; Ogawa et al., 2000). However, the included studies provided no explicit evidence for the physiological mechanisms described (Brighenti et al., 1995; Darzi et al., 2014; Hlebowicz et al., 2007; Leeman et al., 2005; Liatis et al., 2010; Östman et al., 2005; W. Zhao et al., 2020). Notably, studies using sodium propionate, which is also more readily absorbed in the upper gastrointestinal tract reported lower fasting and postprandial glucose responses (Todesco et al., 1991; Venter et al., 1990).

The limited research investigating the metabolic effects of colonic SCFA delivery prompted the design and implementation of the study, described in **Chapters 3** and **4**. The compound, inulin-propionate ester was used to investigate the effects of SCFA delivery to the colon. Propionate was selected for its ability to bind to FFAR2 and 3 and because it does not undergo interconversion to other SCFA. This study found that IPE did not affect postprandial glucose or insulin response, compared with inulin alone. Interestingly, chronic IPE intake

was associated with an increase in fasting glucose concentrations relative to the inulin control, though it remained within the healthy range (World Health Organization, 2024a). This increase in fasting glucose may reflect the role of propionate as a gluconeogenic precursor. However, as mentioned in earlier chapters, the contribution of colonic propionate to glucose production is approximately 6% (Boets et al., 2017). However, limited evidence in humans from another group suggests that acute propionate may stimulate glucagon and noradrenaline secretion, contributing to enhanced endogenous glucose production (Tirosh et al., 2019). These observations could partially explain the increase in fasting glucose. The role of SCFA in modulating glucose and insulin responses warrants further investigation to understand these conflicting findings.

In **Chapter 3**, IPE did not prevent weight gain more effectively than inulin. Similar results were seen in some previous studies that were not powered to detect a difference in body weight (Byrne et al., 2019; Chambers, Byrne, Rugyendo, et al., 2019; Todesco et al., 1991). However, one study found that IPE did effectively prevent weight gain (Chambers, Viardot, et al., 2015). This study selected participants with a susceptibility to further weight gain and the study was conducted during the COVID-19 pandemic when accelerated weight gain has been reported (Bakaloudi et al., 2022; Dicken et al., 2021). Given this, the results are encouraging and potentially reinforce the role of fermentable fibres in weight management.

Results from **Chapter 4** demonstrated no differences in subjective appetite response, gut hormones or energy expenditure from baseline to the 12-month follow-up between inulin and IPE. Interestingly, the IPE group exhibited a significantly higher *ad libitum* meal intake at 12 months (**Chapter 4**), which did not translate into weight gain overall. In previous studies, one of the most consistent findings was a reduction in *ad libitum* energy intake after acute and chronic IPE supplementation (Byrne et al., 2016, 2019; Chambers, Viardot, et al., 2015; Polyviou et al., 2016). This could suggest that any protective effect of SCFA on energy balance is not driven by satiety but instead by modulating energy expenditure or fat oxidation. However, this was not shown in this study and the small sample size of the sub-study (**Chapter 4**) makes it difficult to extrapolate any findings to the larger, main study population (**Chapter 3**).

The lack of changes in weight and body fat mass suggests that dietary intake remained consistent during the study period. However, the dietary intake data were of poor quality, with recorded intakes 40% below the expected energy intake, indicating substantial energy intake underreporting. This lack of quality dietary data (**Chapter 3**) prevents detection of a potentially confounding effect from dietary intake and limits assessment of the effects of IPE

or inulin on energy intake in a free-living context. A better understanding of dietary intake, especially given the observed increase in *ad libitum* energy intake seen at 12 months, could offer insights into how SCFA may influence energy balance mechanisms beyond appetite regulation. Although **Chapter 4** explored these mechanisms, no significant effects were identified. This does not preclude the possibility of such effects, as propionate enhanced energy expenditure and fat oxidation in previous human studies (Byrne et al., 2019; Chambers et al., 2018; Malkova et al., 2020). Additionally, without food diaries, it is difficult to control dietary fibre intake, which affects SCFA production and energy balance. However, National Diet and Nutrition Survey data showed a 2 g decrease in dietary fibre intake over the study period likely associated with change in diet linked with the COVID-19 lockkdowns (Office for Health Improvement and Disparities, 2021).

In **Chapter 4**, breath hydrogen was used as a proxy to confirm IPE hydrolysis and subsequent release in the colon. This may have been insufficient to establish whether IPE was released. It may have been more beneficial to use ^{13}C-labelled IPE as in prior studies (Polyviou et al., 2016). Although no significant differences in breath hydrogen were detected, levels within the IPE group appear lower, potentially indicating insufficient release of IPE in the colon. Slightly lower breath hydrogen in the IPE is expected as less fermentable fibre present. However, there did not appear to be a rise at the expected time of colonic delivery ie. 180 to 240 minutes (Polyviou et al., 2016). If propionate was not released from the vehicle molecule, this could have driven the lack of effect on appetite and energy expenditure seen in **Chapter 4** and the lack of significant effect on weight gain seen between inulin and IPE in **Chapter 3**. Any extrapolation from the exploratory sub-study to the main study population should be viewed with caution. Lower breath hydrogen within the IPE group could be due to a higher population of non-hydrogen producers within this group as is seen in 20% of the population (Hammer et al., 1996, 2022). However, this is unlikely as participants were randomly allocated to study arms.

IPE supplementation was associated with an increase in fat-free mass. Although this did not coincide with a reduction in fat mass (**Chapter 3**) or increases in energy expenditure or fat oxidation (**Chapter 4**). Body composition was assessed using bioelectrical impedance analysis (BIA), a convenient but less accurate method than alternative methods like DEXA (Kistorp et al., 2000). In a previous study using MRI, lower intra-abdominal adipose mass was detected after IPE supplementation (Chambers, Viardot, et al., 2015). Both fat-free and water mass were significantly increased in the IPE group, this may be indicative of the sensitivity to BIA in hydration status. These observations may reflect changes in hydration

rather than gains in lean muscle mass, making it difficult to interpret the intervention’s effects on body composition.

5.4 Future work

Proposed future work should:

1. Investigate whether certain individuals responded better to IPE, considering factors like adherence to the intervention, lean mass, age and gender.
2. Understand if regular eating patterns and physical activity influenced weight outcomes in response to IPE.
3. Assess the rate of IPE fermentation to understand whether it was released at target sites.
4. Use multiple measures, such as isotopic tracers, to assess colonic delivery and SCFA metabolism.
5. Compare the effects of IPE and inulin with a non-fermentable fibre as a negative control.
6. Refine age-specific SCFA dose
7. Investigate other methods of dietary intake assessment to reduce the risk of energy intake underreporting in future studies

5.5 Conclusions

There are several novel aspects of this part of the thesis. The systematic review and meta-analysis in **Chapter 2** is the first to investigate the effects of all SCFAs on glycaemic control in individuals with both healthy and impaired glucose control. The review highlights the lack of high-quality studies investigating the impacts of SCFA on human metabolism. **Chapters 3** and **4** describe one of the few studies to deliver propionate to the colon using IPE, to assess its effect on weight gain prevention, appetite and energy expenditure. Furthermore, the present study has the longest IPE intervention duration of one year, with the aim of better understanding the long-term effects of SCFA on metabolism. **Chapter 3** and **4** describe the first study to assess the effects of IPE in younger adults, the population most susceptible to weight gain, whilst providing mechanistic insight into the role of SCFA in regulating of appetite and energy expenditure in this group. Overall, further investigation is warranted to fully understand the role of SCFAs on appetite, energy balance and substrate metabolism in humans, particularly in younger populations most at risk of weight gain.

Part 2: The Effect of Resistant Starch on Glycaemic Control in Individuals with Type 2 Diabetes

Chapter 6: Introduction

6.1 Type 2 Diabetes

Obesity is strongly associated with the onset of chronic disease, particularly T2D. The risk of developing T2D increases markedly with rising BMI, escalating from approximately 18% in adults with a healthy BMI to 72% among those with Class 2 obesity (Narayan et al., 2007). Additionally, the risk in T2D is partially driven by elevated glucose concentrations and compensatory hyperinsulinemia induced by intake of readily digestible carbohydrates, sugar and saturated fat prevalent within the hyper-palatable, processed foods characteristic of the modern Western diet (Levy et al., 2021; Srour et al., 2020). This section examines the development of T2D and evaluates current treatments, with an emphasis on present dietary interventions for improved blood glucose control.

6.1.1 **Defining T2D**

T2D is characterised by chronic elevations in blood glucose and is a complex, heterogenous metabolic disorder. In essence, the development of T2D is driven by insulin resistance, which impairs glucose uptake into peripheral tissues and pancreatic β-cell dysfunction which leads to inadequate insulin secretion (Taylor, 2013). The WHO diagnose T2D using the following criteria (World Health Organization & International Diabetes Federation, 2006):

Having symptoms typical of T2D such as polydipsia, polyuria, fatigue and unintentional weight loss as well as meeting one of the following criteria:

- Fasting plasma glucose concentrations ≥ 7 mmol/L
- Postprandial plasma glucose concentrations ≥ 11mmol/L two hours after a 75 g glucose dose provided as part of an oral glucose tolerance test.
- A random plasma glucose sample of ≥ 11mmol/L
- HbA1c ≥ 6.5% (48 mmol/mol)

6.1.2 **The Prevalence and burden of T2D**

T2D is the ninth leading cause of mortality worldwide (World Health Organization, 2023a). In the UK, approximately 4.4 million people live with diabetes, of whom 95% have T2D (Diabetes UK, 2024). Care costs associated with T2D are three times higher than costs for non-diabetic individuals (Stedman et al., 2020). Like obesity, T2D was historically more prevalent in high-income countries whereas now 75% of new diagnoses are in low- and middle-income countries (International Diabetes Foundation, 2021). A trend associated with the shift towards an inexpensive, highly processed and energy-dense diet.

Poorly managed T2D can lead to chronic complications including organ damage or failure, nephropathy and CVD (Nowotny et al., 2015). T2D is associated with a two-fold increased risk of heart disease and strokes and is implicated in 11% of vascular disease deaths (The Emerging Risk Factors Collaboration, 2010). In the UK, the management of diabetes and diabetes-related conditions costs the NHS £10.7 billion annually, with an additional £3.7 billion per year in indirect societal costs (Hex et al., 2024).

6.1.3 Evaluation of T2D management strategies

The primary aim of T2D management is to control blood glucose concentrations as monitored by HbA1c level. The threshold for good control in an individual with T2D is between 48 to 53 mmol/mol (NICE, 2015). Conventional treatment of T2D combines lifestyle modifications like increased physical activity or dietary changes, with pharmacological interventions including metformin, sulphonylureas, sodium-glucose co-transporter-2 (SGLT2) inhibitors and GLP-1 receptor agonists. However, lifestyle interventions such as calorie restriction and increased physical activity can be ineffective in the long-term as they rely on individual motivation and efficacy (Schellenberg et al., 2013).

For individuals with both T2D and obesity, bariatric surgery and VLCDs can be effective. Bariatric surgery achieves remission rates of 58% after one year, whilst weight loss of ≥10 kg yields remission rates of 75% in the first year (Aminian et al., 2020; Lean et al., 2024). However, these approaches may not be appropriate or achievable for all. Most individuals with T2D are struggling to maintain good glucose control and many see deterioration in HbA1c as the disease progresses. Data from NHANES (2009-2020) indicates that only approximately 25% of individuals with T2D maintained an HbA1c ≤ 53 mmol/mol over an 11-year period (Hankosky et al., 2023). There remains a gap in available methods of maintaining normoglycaemia in individuals with T2D, underscoring the need for novel population-level interventions to improve blood glucose control.

6.1.4 Dietary interventions for the management of T2D

Effective dietary interventions can have a positive effect on mitigating T2D risk and slowing T2D progression.

The Mediterranean diet consists of minimally processed foods, healthy fats, and lean protein sources. A systematic review has reported that this dietary pattern is associated with lowered HbA1c in subjects with T2D from several studies (Martín-Peláez et al., 2020). However, barriers to adoption and maintenance of this diet are the inaccessibility and cost

of fresh produce alongside the absence of guidance on intakes of high-fat, energy-dense foods such as nuts, which may increase total energy intake.

Low carbohydrate diets (LCDs), defined by a daily carbohydrate intake of less than 130g, may also improve glycaemic control. This is equivalent to less than half of the reference daily carbohydrate intake for UK adults. A systematic review of eight studies (n=264) indicated that individuals following a low carbohydrate diet had higher rates of diabetes remission (HbA1c <48 mmol/mol), compared with control diets at 57% versus 31%, respectively (Goldenberg et al., 2021). However, LCDs have been associated with a non-significant increase in LDL-cholesterol, potentially compromising diet quality (Kirkpatrick et al., 2019). Further, adherence to this particular dietary pattern is challenging and many studies reported diminished efficacy beyond six months of intervention (Goldenberg et al., 2021).

The Dietary Approaches to Stop Hypertension (DASH) Diet was originally designed to lower blood pressure but has proven beneficial for individuals with T2D. It emphasises intake of wholegrain cereals, legumes, fruits, vegetables, olive oil, nuts, seeds, fish, and lean meats and whilst it is a recommended dietary intervention for individuals with T2D, there is little evidence regarding its efficacy in improving glycaemic control or T2D remission. However, DASH diet adherence is associated with a lowered risk of T2D development (Jannasch et al., 2017).

High dietary fibre intake is a common characteristic of the Mediterranean, DASH and low glycaemic index diets (discussed in **Section 6.1.5**). A systematic review of 42 trials (n=1789) including subjects with prediabetes, type 1, or type 2 diabetes demonstrated a dose-response relationship where a daily dietary fibre intake of 35 g compared with the average intake of 19 g, significantly improved HbA1c, fasting plasma glucose, fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR) (Reynolds, Akerman, et al., 2020). This suggests that fibre or non-digestible carbohydrate intake may play an important role in glycaemic control. As mentioned in **Chapter 1,** current average UK intakes of fibre fall at 18g per day, which may be insufficient to reap any metabolic benefits (Public Health England & Food Standards Agency, 2021).

6.1.5 **Glycaemic index and T2D management**

The glycaemic index (GI) was developed to quantify the rate at which a given food elevates blood glucose concentrations relative to an equivalent carbohydrate amount from a standard reference, such as white bread or pure glucose (D. J. Jenkins et al., 1981). The GI is

measured on a scale from 0 to 100, with values ≤ 55 categorised as low, 56–70 as medium, and > 70 as high. Glycaemic load (GL), a complementary measure, predicts blood glucose responses by accounting for both GI and the available carbohydrate content (total carbohydrate – dietary fibre) of the food (Das et al., 2007). GL is calculated by the following formula:

GL = GI x available carbohydrate content (g) / 100

Foods with a high GI are rapidly digested, causing sharp increases in blood glucose after food intake. Whereas, low GI foods tend to take longer to digest and absorb in the gastrointestinal tract, leading to a gradual rise in postprandial glucose (D. J. Jenkins et al., 1981). Dietary GI was first proposed as a predictor of T2D and chronic disease risk in the 1980s (D. J. A. Jenkins et al., 1983). More recently, two large-scale cohort studies involving over 100,000 participants reported that both dietary GI and GL were significant predictors of T2D risk, independent of fibre and whole grain intake (Miller et al., 2024). Additionally, high-GI diets have been associated with increased T2D and cardiovascular disease risk (D. J. A. Jenkins et al., 2024). Further, evidence pooled from eleven RCTs involving individuals with diabetes suggests that low GI diets improve glucose control, indicated by a significant decrease in HbA1c and fewer incidences of hyperglycaemia (Thomas & Elliott, 2009).

The GI of a food can be modified through factors that reduce starch digestibility or slow absorption of glucose from the gastrointestinal tract. These include macronutrient composition (D. J. Jenkins et al., 1981), ‘preloading’ with protein or fat (Gentilcore et al., 2006; Jakubowicz et al., 2014) and food structure or processing methods (Cai et al., 2021). **Section 6.3** explores how the properties and structure of carbohydrate-containing foods, as well as processing methods, influence glycaemic response.

6.2 Physiological Mechanisms of Glucose Homeostasis

As a vital energy substrate, glucose concentrations must be precisely regulated. In healthy individuals, fasting plasma glucose concentrations range between 3.9 and 5.6 mmol/L (World Health Organization, 2024a). Individuals with T2D regularly surpass this range having fasting glucose concentrations of ≥ 7 mmol/L. Glucose homeostasis is achieved by maintaining a balance between glucose intake and endogenous glucose production via gluconeogenesis and glycolysis (Röder et al., 2016).

In the fed state, glucose and other monosaccharides are derived from dietary carbohydrates and absorbed by enterocytes in the intestine (Thorsen et al., 2014). Glucose travels via the liver, where roughly 34% is absorbed into the peripheral circulation (Moore et al., 2012). From there, insulin action on muscle and adipose tissue increases glucose uptake to stabilise glucose concentrations in the circulation (Stöckli et al., 2011). During fasting, endogenous glucose production, stimulated by glucagon, maintains normal plasma glucose concentrations (Surmely et al., 1999).

The sensing and regulation of glucose concentrations involves interaction between an array of organs, hormones and neural factors to preserve glucose homeostasis. This section provides a brief overview of the mechanisms involved to better understand the therapeutic targets for glucose regulation.

6.2.1 **Glucose Regulating Hormones**

The body regulates glucose concentrations through various hormones including insulin, glucagon, amylin, GIP, GLP-1, adrenaline, cortisol and growth hormone (Aronoff et al., 2004).

Insulin, a 51-amino acid peptide hormone, is synthesised by β-cells of the pancreatic islets of Langerhans in response to elevated plasma glucose and amino acid concentrations. GLUT2, facilitates glucose uptake into the pancreatic cells (L. Wu et al., 1998), where concentrations must reach the ‘set point’, which in humans is approximately 5 mmol/L, to trigger insulin secretion (Alcazar & Buchwald, 2019). Insulin secretion occurs in two phases: an initial phase, triggered by a postprandial increase in plasma glucose and a second secretion phase, which is sustained until glucose concentrations return to fasting levels (Alcazar & Buchwald, 2019).

Insulin promotes glucose uptake into splanchnic and peripheral tissues, facilitating its utilisation and storage by the muscles and white adipose tissues (Moore et al., 2012). As insulin is an anabolic hormone, it promotes the storage of glucose as glycogen

(glycogenesis) and fat (lipogenesis) in peripheral tissues (Aronoff et al., 2004). Insulin also concurrently downregulates catabolic processes, such as glycogenolysis and gluconeogenesis, inhibiting endogenous glucose production in the liver (Saltiel & Kahn, 2001). The effects of insulin are short-lived, it is rapidly cleared from the circulation, with a half-life of only 3 to 10 minutes (Arnolds et al., 2010).

GLP-1 is secreted by enteroendocrine L-cells in the gastrointestinal tract in response to the presence of lipids, protein and glucose in the lumen (Campbell & Drucker, 2013; van der Klaauw et al., 2013). Dietary fibre and SCFAs have also been shown to stimulate GLP-1 secretion from EECs potentially explaining why these cells are highly concentrated in the distal gut (Christiansen et al., 2018; Psichas, Sleeth, et al., 2015). GLP1R are found in the hypothalamus, NTS, pancreatic β-cells, Stomach, Myenteric plexus neurons, small intestine, colon, lung, kidney and heart GLP1R are found in the hypothalamus, NTS, pancreatic β-cells, stomach, Myenteric plexus neurons, small intestine, colon, lung, kidney and heart (P. J. Larsen et al., 1997; Pyke et al., 2014; Turton et al., 1996). Dietary fibre and SCFA, present in the colon, can stimulate GLP-1 secretion from EECs potentially explaining why these cells are highly concentrated in the distal gut (Christiansen et al., 2018; Psichas, Sleeth, et al., 2015). The characteristics of GLP-1 and its role in appetite regulation is explored in **Chapter 1, Section 1.1.2**.

GIP, secreted by enteroendocrine K-cells, is also released in response to the presence of dietary carbohydrates or fat in the gastrointestinal tract (Campbell & Drucker, 2013). The GIP receptor (GIPR) is located on pancreas, small intestine and adipose tissues (Hammoud & Drucker, 2023).

Both GIP and GLP-1 are incretins as they are hormones that bind to receptors on pancreatic β-cells and stimulate insulin secretion (Dupre et al., 1973; Kjems et al., 2003). Incretins are defined as “gut-derived hormones, members of the glucagon superfamily, released in response to nutrient ingestion, mainly glucose and fat. They stimulate pancreatic insulin secretion in a glucose-dependent manner and play an important role in the local gastrointestinal and whole-body physiology” (Saini & Badole, 2015). Incretins stimulate approximately 50-70% of insulin secretion in response to food intake (T. Wu et al., 2010). Furthermore, GIP and GLP-1 have been shown to contribute to reduced β-cell apoptosis, enhanced insulin synthesis and GLP-1 alone has been associated with improved β-cell function (Cornu et al., 2009; S.-J. Kim et al., 2005).

Glucagon is produced from proglucagon by pancreatic α-cells and intestinal L-cells in response to low blood glucose concentrations (Aronoff et al., 2004). Glucagon receptors are found in the liver, brain, pancreas, kidney, intestine, skeletal muscle and adipocytes (Kemp & Habener, 2004). The primary function of glucagon is to increase hepatic glycogenolysis and gluconeogenesis, maintaining glycaemia to facilitate normal physiological functions. Glucagon administration increases hepatic glucose output in both animals and humans (Jiang & Zhang, 2003).

6.2.2 **Glucose transport**

Glucose transporters play a role beyond facilitating glucose entry into cells and the expression of transporters is specific to the role of the organ in glucose regulation. GLUT1 is predominantly located in the brain, placenta and red blood cells, while GLUT3 is found in neurons and the placenta (Alexander et al., 2023). Both GLUT proteins facilitate a steady flow of glucose into cells, aiding the maintenance of euglycemia. However, GLUT3 tends to be located on tissues with a particularly high energy demand (Custódio et al., 2021).

GLUT2 is located on key organs involved in glucose homeostasis, namely, pancreatic β-cells, intestines, kidneys, liver and the CNS (Thorens et al., 1988). GLUT2 plays a key role in maintaining intracellular glucose at similar levels to concentrations in the circulation (Thorens, 2015). This allows for accurate glucose sensing and the rapid stimulation of glucose-stimulated insulin secretion (Thorens, 2015).

GLUT4 is expressed in skeletal muscle, heart and adipose tissue (Alexander et al., 2023). These receptors are translocated to the surface of the cell membrane in response to elevated circulating insulin, thereby facilitating the clearance of glucose into the peripheral tissues to lower circulating glucose concentrations (Stöckli et al., 2011). Skeletal muscle provides a substantial glucose sink, absorbing more than 80% of glucose in the postprandial state (DeFronzo et al., 1981), with GLUT4 responsible for facilitating this uptake (S. Huang & Czech, 2007). Interventions that increase GLUT4 expression, translocation or activity have the potential to improve postprandial glucose tolerance.

6.2.3 **Organs involved in glucose homeostasis**

The pancreas plays a central role in glucose regulation as it is responsible for secreting insulin during elevated circulating glucose concentrations from pancreatic β-cells (Röder et al., 2016). The pancreas also releases other glucoregulatory hormones, like GLP-1, GIP and glucagon, which are secreted by α-cells (Röder et al., 2016). The pancreas has an

exocrine function and supports digestion by releasing amylase, lipase and trypsin, along with bicarbonate to neutralise stomach acid (Henderson et al., 1981).

The liver is a key regulator of glucose homeostasis. The presence of GLUT2 transporters allows for the rapid and accurate detection of changes in glucose concentrations (Thorens, 2015). Elevations in glucose concentrations in the hepatic portal vein enhanced glucose uptake into the splanchnic organs by 400% (DeFronzo et al., 1978). Insulin promotes the conversion of glucose to glycogen for storage and inhibits gluconeogenesis and glycolysis in the liver (Moore et al., 2012). During the fasted state, signalling from glucagon, cortisol or elevated FFA concentrations promote hepatic, endogenous glucose production. Glycogen stores are broken down and glucose is synthesised using pyruvate, glycerol and amino acids, like alanine and glutamine (Moore et al., 2012).

The kidneys also play a role in glucose homeostasis and are estimated to contribute 20% of all endogenous glucose produced (Mather & Pollock, 2011). In the fed state, renal gluconeogenesis is suppressed by increased circulating insulin, to a greater degree than that observed in the liver (Mather & Pollock, 2011).

In peripheral tissues, insulin increases the rate of glucose utilisation by promoting glycolysis in muscle and white adipose tissues and glycogenesis in skeletal muscles (Frayn, 2013; Shulman et al., 1990). These peripheral tissues rapidly remove glucose from the circulation during periods of hyperglycaemia (DeFronzo et al., 1981; Mårin et al., 1987). In adipose tissue, lipolysis is suppressed in the presence of insulin, lowering release of FFAs (Hayashi, 2021).

6.2.4 **Glucose dysregulation**

Insulin resistance is defined as “a decreased tissue effect of insulin per unit delivered” (C. J. Schofield & Sutherland, 2012). It occurs when insulin fails to enhance glucose uptake into tissues or elicit other metabolic effects, like reducing lipolysis or inhibiting gluconeogenesis, when glucose and insulin concentrations are elevated which further exacerbates glycaemic dysregulation (C. J. Schofield & Sutherland, 2012). Insulin resistance is driven by obesity, central adiposity, endocrine disturbances, inflammation, elevated FFAs and age (Frayn, 2013; C. J. Schofield & Sutherland, 2012).

Inadequate insulin secretion arises when there is a heightened demand for insulin in response to a state of hyperglycaemia, eventually pancreatic β-cells can no longer meet this demand and begin to fail (Frayn, 2013). Prolonged hyperglycaemia, often coupled with elevated FFA concentrations to the point of lipotoxicity, damages β-cell function and causes

a decline in β-cell mass (Sharma & Alonso, 2014). Elevated concentrations of leptin and proinflammatory cytokines, alongside increased oxidative stress also contribute to this decline in β-cell function and mass (Poitout et al., 2010; Wajchenberg, 2007). At the time of T2D diagnosis, β-cell mass and function may be reduced by 40-60% compared with normal levels (G. C. Weir et al., 2020; Wysham & Shubrook, 2020).The combination of insulin resistance and inadequate insulin secretion typically leads to the development of impaired glucose control and eventually, T2D.

6.3 Dietary carbohydrates, resistant starch and glycaemic control

Over the past century, there has been a dramatic change in the type of carbohydrates consumed. Populations have transitioned from traditional plant-based diets with a high proportion of indigestible fibre to diets high in processed, easily digestible carbohydrates. Whilst the development of T2D and other chronic disease is attributed to a multitude of factors, chronic disease diagnoses have increased during this transition. An umbrella review investigating the role of diet in T2D development reported that intake of whole grains and cereal fibre was linked to lowered T2D incidence, whilst red and processed meat, alongside sugar-sweetened beverages were positively associated with T2D risk (Neuenschwander et al., 2019). Therefore, the role of carbohydrate quality and food processing in blood glucose control should be explored. This section provides an overview of dietary carbohydrates and resistant starch and their potential effects on glycaemic response.

6.3.1 **Dietary Carbohydrates**

Dietary carbohydrates are compounds consisting of carbon, hydrogen and oxygen and are categorised based on their chain length into mono-, di-, oligo- and polysaccharides (Aller et al., 2011). Other differentiating characteristics are their glycosidic bond type and the characteristics of the monomers that form the carbohydrate polymer (Cummings & Stephen, 2007). Although classifying carbohydrates by chemical characteristics provides some insight into their potential availability and digestibility within the body, this is often insufficient to capture their metabolic impact.

The classification of carbohydrates also considers changes during processing, such as solubility, hydration properties, cell wall structure and ability to form crystalline structures, gels or complexes with lipids and proteins (Cummings & Stephen, 2007). By grouping carbohydrates by both their chemical and physical properties, a more comprehensive insight into their availability for digestion and effects on metabolism can be formed. Total

carbohydrate includes both digestible and indigestible forms of carbohydrates in a food whereas available carbohydrate excludes non-digestible proportions of foods like plant cell wall polysaccharides (Cummings & Stephen, 2007).

Monosaccharides, such as fructose, glucose and galactose, are the simplest units of carbohydrates (Aller et al., 2011). Disaccharides, like sucrose, maltose and lactose, consist of two monosaccharides bound by glycosidic bonds (Aller et al., 2011). There are two types of glycosidic bonds. Alpha-glycosidic bonds that are easily hydrolysed by α-amylases produced in the saliva and by the pancreas, or β-glycosidic bonds which cannot be broken down by endogenously-produced enzymes present in humans, which the exception of the monosaccharide bonds in lactose (Cummings & Stephen, 2007). Both monosaccharides and disaccharides are quickly and easily digested, though disaccharides must be hydrolysed into their composite monosaccharides before absorption (Aller et al., 2011). The mechanical and chemical digestion of carbohydrates begins via oral processing (i.e. chewing) and amylase enzymes present in the mouth. The primary site of carbohydrate digestion is the small intestine, where enzymes such as α-amylase and α-glucosidase breakdown carbohydrate polymers into their constituent monomers glucose, sucrose and galactose (Gray, 1975). These monosaccharides are transported from the lumen through enterocytes in the duodenum and into the circulation shortly after food intake. The rapid elevation in plasma glucose leads to rapid insulin secretion. These peaks in insulin and glucose often result in a period of compensatory hypoglycaemia shortly after, which is followed by increased hunger and food intake (Aller et al., 2011).

Polysaccharides, which often take longer to digest, include starch, cellulose and chitin and are composed of 10 or more monosaccharides. Resistant polysaccharides are categorised as either starch or non-starch polysaccharides (NSPs). NSPs, like cellulose and beta-glycan, contain β-glycosidic linkages meaning that they are resistant to digestion by human digestive enzymes (Lovegrove et al., 2017). Cellulose, the principle NSP consumed by humans, is abundant in plant cell walls. Cellulose has the β-glycosidic linkages between glucose monomers and hydrogen bonds that bind these chains contributing to its strong and dense structure (Lovegrove et al., 2017). Therefore, cellulose reaches the colon intact and could be fermented by colonic bacteria. However, there is a low abundance of cellulolytic bacteria in the human colon and evidence suggests that less than 5% of cellulose is fermented in non-ruminants (Topping & Clifton, 2001).

6.3.2 **Starch structure and digestion**

Starch, the main source of energy in seeds and tubers of plants, accounts for approximately ≥ 30% of the human diet (Public Health England, 2020). Factors influencing starch digestion in the gastrointestinal tract include plant cell wall encapsulation, branching of glucose polymers, starch granule morphology, gelatinisation and retrogradation capacity (Birt et al., 2013; Lovegrove et al., 2017; Mishra et al., 2012). Gelatinisation occurs when starch molecules are heated in an excess of water (Ratnayake & Jackson, 2008). Certain botanical sources of starch, like tubers, gelatinise at lower temperatures than starch in cereals (Lovegrove et al., 2017). During gelatinisation, starch molecules swell and change shape, losing their semi-crystalline structure and enhancing digestibility (Mishra et al., 2012). When starch in this form cools, starch structures are altered once again and form a novel crystalline structure. The process of the formation of a new crystalline structure is known as retrogradation.

At molecular level, starch is composed of two glucose polymers, amylose and amylopectin. Although most starch consists of 70% amylopectin and 30% amylose, proportions can vary depending upon the botanical source, genetics and the environment (Lovegrove et al., 2017). Amylose consists of straight chains of glucose monomer bonded with α-D-(1,4)-glycosidic linkages whereas amylopectin has a highly branched structure due to the presence of α-D-(1,6)-linkages and α-D-(1,4)-linkages (Mishra et al., 2012). The α-D-(1,6)-linkages create an amorphous structure within the starch molecule (Lovegrove et al., 2017). This branched structure of amylopectin lowers the molecular density, increases surface area and water retention making it more prone to gelatinisation (Mishra et al., 2012). In contrast, the α-D-(1,4)-linkages in amylose create densely-packed, highly-organised semi-crystalline helices which are more difficult to gelatinise and hydrolyse for digestion (Lovegrove et al., 2017).

Amylose and amylopectin chains are packed into starch granules for storage. The size and shape of the starch granule is dependent upon botanical origin. Starch granule size and shape alter their characteristics and the effects that cooking has on their structure (Kovrlija et al., 2020). For example, larger granules, often found in tubers, tend to be more prone to gelatinisation and retrogradation whereas smaller granules do not gelatinise as readily. At starch macrostructural level, starch digestion is dependent upon the ratio of amylose to amylopectin, morphology of the starch granule, starch–lipid interactions and starch–protein interactions within the plant cell (Cai et al., 2021). out.

Industrial processing methods like milling can damage cell walls and cause loss of starch crystallinity making starch more prone to gelatinisation (Donald, 2004).

6.3.3 **Resistant Starch**

Previously, starch has been categorised into three types, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), based on the rate of starch digestion and absorption in order to predict glucose responses to these different sources of carbohydrates (Englyst & Hudson, 1996). The rate of starch digestion is not only determined by natural characteristics like botanical source, starch solubility and starch granule shape, but also by industrial, biotechnical or domestic processes such as milling, boiling or the suppression of starch branching enzymes (Birt et al., 2013).

RS remains largely undigested in the upper gastrointestinal tract and is delivered to the colon where it can be utilised as a substrate for bacterial fermentation (defined in **Chapter 1**) (Topping & Clifton, 2001). Resistant starch is found in legumes, intact grain kernels, fruits and vegetables and as it is largely indigestible, it is considered to have a low GI (Birt et al., 2013). Resistant starch is categorised based on source, formation method and physical characteristics. There are currently five different types of resistant starch which are described in **Table 6.1**.

Table 6.1: Descriptions and examples of resistant starch types

Type	Description	Food types
1	Starch encapsulated within plant cell walls	Legumes and intact grain kernels
2	Dense, ungelatinised starch granules	Starches with high-amylose content, raw bananas or potatoes
3	Retrograded starch	Cooked and cooled pasta, potatoes, rice
4	Chemically modified starches	Cross-linked starch
5	Amylose-lipid complexes	Steric acid-complexed starch with high amylose content

Adapted from (Birt et al., 2013; Raigond et al., 2019)

6.3.3.1 RS 1

RS 1 is found in cereal grains and seeds and is surrounded by plant cell walls that prevent contact with digestive enzymes (Birt et al., 2013). These plant cell walls are complex matrices consisting of cellulose, hemicellulose, pectin, lignin, proteins and water, though composition and the presence of some components, like lignin, varies based on species

(Holland et al., 2020). Depending on the plant type and maturity, the strength and porosity of a plant cell wall will vary (Holland et al., 2020). Plant cell walls contain several NSPs including cellulose that are resistant to digestion in the human gastrointestinal tract (Lovegrove et al., 2017). Intact plant cell walls limit bioaccessibility and α-amylase ingress, lower gelatinisation and swelling of starch granules and contain compounds that may inhibit enzyme activity (Xiong et al., 2022).

Notedly, structures of plant cell walls in pulses, like chickpeas and beans, differ from those in grains. Pulses have type 1 cell walls which are higher concentrations of pectin, which weakens on cooking and allows the cell to separate, rather than rupture during domestic processing and hemicelluloses like xyloglucan which improve structural integrity (Edwards et al., 2021; Tovar et al., 2003). For example, Noah and colleagues recovered approximately 16% of starch within legume cell walls in ileum samples from healthy participants (Noah et al., 1998). In contrast, cereal grain cell walls tend to have higher proportions of arabinoxylan and beta-glucans (Edwards et al., 2021). Therefore, cell wall composition and NSP ratio may also affect digestion. As a result, RS 1 may be better preserved in legumes and legume and cereal grain cell walls may affect metabolic responses differently (Edwards et al., 2021).

Food processing can alter food structure to modulate digestion (Cai et al., 2021). RS content can be diminished through industrial, mechanical, or domestic processing. RS 1 content is reduced through mechanical processes, such as milling, which ruptures plant cell walls. Therefore, starch within plant cell matrices may produce a lower glycaemic response compared with ruptured cells.

6.3.3.2 RS 2

RS 2 is native crystalline starch found in raw potatoes or bananas (Birt et al., 2013). Most raw starch is resistant to digestion due to its dense, semi-crystalline structure (Snelson et al., 2019). However, the crystalline structure of these starch types can be disrupted during thermal processing where gelatinisation of the starch granule occurs. For example, Langkilde and colleagues observed that three times more raw banana starch was preserved in the effluents of ileostomy subjects than cooked banana starch (Langkilde et al., 2002). There are foods which still contain raw starch, such as foods produced at in high-temperature, low-moisture conditions, to prevent gelatinisation (Baptista et al., 2024). Examples include oats, biscuits and crackers.

6.3.3.3 RS 3

RS 3 is formed through retrogradation where the gelatinised molecule forms an atypical crystalline structure which is harder for amylase enzymes to bind to and hydrolyse (Mishra et al., 2012). For example, cooked and cooled rice and potatoes contain significantly less digestible starch than their freshly cooked constituents (Riva et al., 2000; Sonia et al., 2015). Interestingly, pasta that has been cooked, chilled and reheated tended to lower glycaemic response to a greater extent than cooked and chilled pasta alone (Robertson et al., 2021).

6.3.3.4 RS 4 and 5

Limited research has been conducted on RS 4 and 5. RS 4 is any starch that has been chemically modified to increase resistance to digestion (Mishra et al., 2012). Chemical modification can take the form of acetylation, oxidation, cross-linkage or esterification (Mishra et al., 2012). These bonds may also make starch more resistant to swelling and gelatinisation as well as chemical or thermal degradation and digestion (Birt et al., 2013; Lovegrove et al., 2017). RS 5 is produced when amylose or amylopectin is bound to lipid molecules through cooking or chemical modification. The starch-lipid binding results in the formation of single helical complexes which are difficult for amylases to bind and hydrolyse (Birt et al., 2013). Evidence of the effect of starch-lipid complexes is less conclusive. One *in vitro* study suggested RS 5 is digested slower but still completely digested (Holm et al., 1983). In contrast, Guraya and colleagues reported that RS type 5 was 33% less digestible than a high-amylopectin control (Guraya et al., 1997).

6.3.4 **Resistant starch as a fermentable carbohydrate**

Resistant starch reaches the colon where it can act as a substrate for microbial fermentation (Topping & Clifton, 2001; van Munster et al., 1994). As mentioned in **Chapter 1**, microbial fermentation of indigestible carbohydrates leads to the production of SCFAS. These molecules have been associated with metabolic processes involved in glucose homeostasis (Birt et al., 2013). Studies in both humans and rodents provide evidence that RS can modulate SCFA production and gut microbiota composition in a manner that may positively affect glycaemic control.

In rodent models, prolonged resistant starch intake, in the form of raw potato starch, enhanced butyrate production over 6 months, whilst lowering acetate production ratios (Le Blay et al., 1999). This was reinforced in another study using stable isotopes to detect changes in SCFA production (Herrmann et al., 2018). However, the effect of butyrate on glycaemic control is limited as butyrate is primarily metabolised within the colon and little reaches the peripheral circulation (Bloemen et al., 2009). Other rodent studies have

indicated that resistant starch supplementation enhanced production of acetate and propionate (Guice et al., 2020; Rosas-Pérez et al., 2020). However, this did not necessarily lead to changes in glycaemic control (Rosas-Pérez et al., 2020). Overall, enhanced resistant starch intake appears to be associated with positive alterations in the microbiota, metabolite and SCFA profiles (Kadyan et al., 2023).

In humans, resistant starch is fermentable in large intestine, indicated by observed elevations in breath hydrogen and increases in SCFA production (Muir et al., 1995; Olesen et al., 1994; van Munster et al., 1994). Similar to animal studies, resistant starch has been shown to enhance production of butyrate (Alfa et al., 2018; Verbeke et al., 2010). For example, SCFA production increased by two-fold in ileostomy patients and ileal 'dry matter effluent' significantly increased meaning that starch was reaching the colon undigested (Nordgaard et al., 1995). The microbiota responds to and ferments RS 2 from cornflakes, high-amylose maize starch and raw potato starch differently, as indicated by disparate breath hydrogen responses to each intervention (Olesen et al., 1994). Similarly, different resistant starch types may promote proliferation of different microbial populations. For example, RS 4 significantly increased *Actinobacteria* and *Bacteroidetes* and reduced *Firmicutes* abundance whereas RS 2 enriched species like *Ruminococcus bromii* (Martínez et al., 2010). Additionally, even within RS 4, different botanical sources produced different ratios of SCFAs alongside having a dose-dependent effect, this response plateaued at 35g resistant starch per day (Deehan et al., 2020).

This evidence highlights the potential of resistant starch in manipulating the gut microbiota and its subsequent fermentation products, like SCFA. However, it also highlights the variability of response dependent on RS type, dose and botanical source on microbial response.

6.3.5 **Resistant starch and glycaemic control**

Research indicates that resistant starch has the potential to improve glycaemic control in both healthy and metabolically unhealthy individuals. However, the current body of evidence is still limited, and the metabolic effects may differ dependent upon resistant starch types. This section will focus on findings from animal models and studies involving healthy human subjects. The specific impacts of resistant starch on individuals with pre-diabetes and T2D will be explored in **Chapter 7** and **8**.

6.3.5.1 RS 1

The positive effect of RS 1 on glycaemic control in animals and healthy individuals is relatively well-established. The importance of limiting processing methods to maintain larger food particle size to enhance cell wall integrity was underscored in a recent systematic review where intact and large food particles lowered postprandial glucose and insulin responses in comparison with smaller food particles (Cai et al., 2021). Furthermore, higher levels of RS 1 intake from wheat kernel porridge was associated with lower postprandial glucose, insulin, c-peptide and GIP concentrations, compared to a milled wheat kernel control (Edwards et al., 2015). In another study, wheat breads with larger wheat particle sizes had lower postprandial glucose responses (Eelderink et al., 2017). However, another study using whole kernel rye bread had no effect on glucose and insulin response until resistant starch 2 was added to the bread product (Sandberg et al., 2017).

In legume studies, pulse flours with intact cell structures were compared with traditionally-milled flours with ruptured cell structures (Edwards et al., 2020). The intact cell wall structure flours had lower observed glycaemic indices and iodine staining showed evidence that RS 1 could be found within the structures after thermal processing (Edwards et al., 2020). Similarly, black bean intake lowered glycaemic responses compared to a matched available carbohydrate dose in the form of glucose (Sánchez-Tapia et al., 2020). The effects of RS 1 on insulin sensitivity have not been widely explored. However, the same group reported that black bean intake in rodents was shown to improve insulin sensitivity via positive alterations to the gut microbiota (Sánchez-Tapia et al., 2020).

6.3.5.2 RS 2

RS 2 has been studied more extensively for its metabolic effects because it is easily isolated and can be used as a flour substitute. In some studies, RS 2 has been administered in doses exceeding 40g per serving which may have unpleasant gastrointestinal effects, though side effects are often unreported.

In rodent models, acute RS 2 intake lowered postprandial glucose when delivered in the form of high-amylose bread and rice (Brites et al., 2011; Zhu et al., 2012). RS 2 intake also increased GLP-1 concentrations and increased transcription of genes related to proglucagon production (Keenan et al., 2006; J. Zhou et al., 2008). Chronic supplementation of RS 2 has been shown to modulate pathways involved in hepatic gluconeogenesis and glycolysis (Sun et al., 2018). Another study demonstrated that pancreatic β-cell mass, insulin secretion, GLP-1 concentrations, levels of butyrogenic bacterial and caecal short-chain fatty acids were increased after RS 2 feeding (Shen et al., 2011). Furthermore,

resistant starch intake improved β-cell sensitivity and preserved β-cell mass in rodent models of T2D (J. Zhou et al., 2008; Zhu et al., 2012). However, some of these effects mentioned may be attributed to energy intake dilution from replacing digestible starch with resistant starch to alter energy balance and lower adipose deposition. For example, one study in rodents reported lowered abdominal fat deposition after an RS 2 intervention (Keenan et al., 2006).

In humans with normal glycaemic control, acute RS 2 intake led to attenuated postprandial glucose responses in some studies (Hallström et al., 2011; Raben et al., 1994; Zenel & Stewart, 2015). However, others showed no effect or even reported an increase in postprandial plasma glucose (Al-Mana & Robertson, 2018; Corrado et al., 2020; García-Vázquez et al., 2019; Marchini et al., 1998). Lowered glucose response was also accompanied by lower insulin, GIP and GLP-1 in one study (Raben et al., 1994), whereas others reported no changes in GLP-1 concentrations (Bodinham et al., 2013; Maziarz et al., 2017).

Chronic intake of RS 2 lowered fasting or postprandial glucose concentrations (K. Ang et al., 2020; Behall et al., 1989; Ble-Castillo et al., 2010; de Roos et al., 1995; Haub et al., 2010; Hughes et al., 2021; Jiménez-Domínguez et al., 2015; M. D. Robertson et al., 2005) potentially via improved glucose uptake into muscle tissue (M. D. Robertson et al., 2005). Although, some studies did not report any effect of RS 2 on glucose homeostasis (D. J. Jenkins et al., 1998; Noakes et al., 1996; Penn-Marshall et al., 2010). Several studies reported that chronic RS 2 intake lowered insulin concentrations and enhanced insulin sensitivity (Ble-Castillo et al., 2010; Hughes et al., 2021; Jiménez-Domínguez et al., 2015; M. D. Robertson et al., 2005). Interestingly, sex-specific differences were noted, insulin sensitivity improved in men but not women after a 15 or 30 gram resistant starch intervention (Maki et al., 2012). Furthermore, improvements in insulin sensitivity also appeared to differ based on baseline metabolic health, Gower and colleagues reported that insulin sensitivity improved in insulin resistant participants but not those with normal insulin sensitivity (Gower et al., 2016).

6.3.5.3 Resistant starch type 3

In rodent studies, chronic RS 3 has a more pronounced effect on reducing weight gain, triglycerides, and blood glucose levels in rodents compared to RS 2 over six weeks (Xu et al., 2020). In two studies using rodent models of T2D, RS 3 improved insulin sensitivity and glycaemic control (C. Zhang et al., 2020, 2020).

In humans, acute RS 3 intake improved postprandial glucose and insulin, potentially associated with an increase in insulin sensitivity (M. D. Robertson et al., 2003; T. M. Robertson et al., 2021; Sonia et al., 2015).

Chronic intake of RS 3 lowered insulin production, potentially linked to improvements in insulin sensitivity as no reduction in glucose concentrations was detected (de Roos et al., 1995). However, some studies found no significant difference in glycaemic control after chronic intake of RS 3, particularly when compared to a standard weight loss diet (D. J. Jenkins et al., 1998; Lobley et al., 2013).

6.3.5.4 RS 4 and 5

Studies on RS 4 and 5 are limited. However, the available evidence appears encouraging. Chronic RS 4 intake in rodents reportedly improved insulin sensitivity independent of the gut microbiota (Bindels et al., 2017). In humans, intake of RS 4 resulted in lower postprandial glucose (Al-Tamimi et al., 2010; Haub et al., 2010; Stewart et al., 2018, 2018; Stewart & Zimmer, 2017) and insulin responses (Al-Tamimi et al., 2010; Stewart et al., 2018; Stewart & Zimmer, 2017, 2018). However, Nichenametla and colleagues reported no significant effects of RS 4 on glycaemia (Nichenametla et al., 2014).

In summary, the background literature indicates that resistant starch-rich foods may improve glycaemic control in humans when compared with the commonly consumed version of the food. However, effects likely vary depending on RS type. RS 1 more consistently lowers postprandial glucose and insulin and improves insulin sensitivity. Chronic RS 2 lowers fasting glucose and insulin. However, more mixed results were observed in acute responses to RS 2. RS 3 improves acute insulin sensitivity and postprandial glycaemic response, but evidence is sparse. Studies also suggest RS 4 lowers glucose and insulin responses, but more research is needed. Outcomes appear to vary based on RS type, dose size and participant characteristics, like sex or metabolic health.

6.4 Rationale and general aims

Part two of this thesis will explore how glycaemic control in individuals with T2D may be improved through increased resistant intake within the habitual diet. The objectives of this part are:

To explore the current evidence for the effect of resistant starch types on glycaemic response in individuals with T2D or pre-diabetes in **Chapter 7.**

To investigate the effect of resistant starch as an easily accessible and adaptable tool for improved blood glucose control in individuals with T2D in **Chapter 8**.

6.4.1 **Thesis Hypothesis**

I hypothesised that resistant starch intake would improve glycaemic control in individuals with T2D.

6.4.2 **Thesis Aims**

The aims of this section of the thesis are:

- To understand whether RS has any significant effects on fasting or postprandial glycaemia in individuals with impaired glucose tolerance.
- To determine whether meals with resistant starch, co-designed through patient and public involvement (PPI) groups with individuals with T2D, could lower postprandial glucose responses compared with a low-RS control diet in participants with T2D.

Chapter 7: The effects of resistant starch types on glycaemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis.

Research outputs from the chapter:

Pugh, J. E., Cai, M., Altieri, N., & Frost, G. (2023). A comparison of the effects of RS types on glycemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis. Frontiers in Nutrition, 10. https://www.frontiersin.org/articles/10.3389/fnut.2023.1118229

7.1 Summary of key findings

- Studies investigating the effects of RS types on glycaemic response are limited and searches only identified studies investigating the acute effects of RS 1,2 and 3 and the chronic effects of RS 1, 2 and 4 on glycaemia in individuals with impaired glucose homeostasis
- 78% of studies had a high risk of bias or some concerns
- Meta-analyses of acute resistant starch studies indicated that resistant starch intake lowered postprandial glucose and insulin
- RS 2 appeared to mostly drive this effect
- Meta-analyses of chronic RS 1 and 2 intake revealed lowered fasting and postprandial glucose, as well fasting insulin concentrations.
- Chronic RS 2 supplementation appeared to drive the effect on fasting glucose and insulin

7.2 Introduction

T2D is a state of chronic hyperglycaemia resulting in elevated insulin secretion, impaired glucose tolerance and augmented endogenous glucose production (Cerf, 2013). T2D currently affects 8.5% of the global population and diagnosis rates are rising (World Health Organization, 2023a). Individuals diagnosed with T2D have a 6-year loss in predicted life expectancy associated with T2D comorbidities such as CVD and chronic kidney disease (Kianmehr et al., 2022; Nowakowska et al., 2019). Dietary fibre intake is associated with improved glycaemic outcomes in individuals with diabetes. Specifically, lower HbA1c, fasting plasma glucose, insulin and homeostatic model assessment of insulin resistance (HOMA IR) in those with intakes of 35g dietary fibre per day (Reynolds, Akerman, et al., 2020). Processing methods like milling, can lower dietary fibre content and modify food structure, raising postprandial glucose and insulin in healthy volunteers to a greater extent than their unprocessed counterparts (Cai et al., 2021). Currently, T2D is managed through a combination of lifestyle and pharmacological interventions, these interventions are described in **Chapter 6, Section 1.3.** Accessible nutrition interventions for T2D that do not rely on individual responsibility and can complement existing drug therapies are needed. One such approach could be to increase RS intake.

Starch-rich foods like pasta, bread and rice contribute to 45% of UK dietary intake (Public Health England, 2020). Therefore, these foods could represent a potential therapeutic target for glycaemic control. A large proportion of RS is not digested by endogenous enzymes, like α-amylase, in the gastrointestinal tract and travels to the colon intact where it can be used as a substrate for microbial fermentation (Englyst & Cummings, 1985; Nordgaard et al., 1995). RS can alter the colonic environment, promote the growth of certain bacteria populations such as *Bifidobacterium adolescentis*, *Ruminococcus bromii* and *Eubacterium rectale* (Martínez et al., 2010; Walker et al., 2011) and enhance the production of SCFAs (Topping & Clifton, 2001).

RS is categorised into five types based on physical properties and production methods, which are described in **Chapter 6, Section 3.2.** In brief, RS 1 is starch encapsulated within plant cell walls. RS 2 comprises naturally-existing compact raw starch granules. RS 3 is formed when starchy foods are subjected to a cooking and cooling process and their starch granules re-crystallise (retrogradation). RS 4 is chemically modified starch with increased bonding or esterification and is often found commercial food products (Topping & Clifton, 2001). RS 5 is generated through the formation of bonds between starch and lipids during the cooking process (Wang et al., 2020). The cell wall surrounding RS 1 provides a physical

barrier to digestion, whereas RS 2 through 5 are resistant to α-amylase hydrolysis due to starch molecule characteristics. Resistant starch has been shown to affect glycaemia in animal models and studies in healthy volunteers, these studies have been described in **Chapter 6, Section 3.4**.

Since the gastrointestinal tract is largely inaccessible and difficult to sample from, gaining a comprehensive understanding of the effects of RS on glycaemia remains challenging. One proposed mechanism is that RS replaces available carbohydrate content in a meal and dilutes starch available for digestion in the small intestine. Consequently, glucose concentrations are lower after a meal and less insulin is required to maintain glycaemic homeostasis. Further, as indicated from chronic studies, RS may affect fasting glycaemia and insulin sensitivity. The use of RS to augment specific bacterial populations and enhance SCFA production may have favourable outcomes for both gut and metabolic health (Singh et al., 2022). SCFAs binding to enteroendocrine L-cells enhances GLP-1 secretion, subsequently promoting insulin release (De Silva & Bloom, 2012; Psichas, Sleeth, et al., 2015). Additionally, SCFAs can have direct effects on the pancreas, by elevating glucose-stimulated insulin secretion or the skeletal muscles, by promoting glucose transport via the GLUT4 receptor. These effects have been comprehensively reviewed elsewhere (Frampton et al., 2020; Morrison & Preston, 2016).

Current literature suggests that RS affects glucose homeostasis. However, animal models and studies conducted in healthy volunteers do not necessarily reflect outcomes in individuals with impaired glucose control. The current evidence for the effect of RS on glycaemia in humans with T2D and prediabetes is yet to be systematically reviewed. The findings from this study may help to elucidate the effects of acute and chronic RS intake on markers of glycaemic control in individuals with T2D and prediabetes.

7.3 Purpose of the Study

7.3.1 **Hypothesis**

I hypothesised that RS 1-5 would lower glucose and insulin (postprandial and fasting), when consumed in chronic and acute interventions and differential effects may be observed, dependent upon RS type and the population group, i.e., individuals with prediabetes versus T2D.

7.3.2 **Aims**

The primary aim of this systematic review and meta-analysis was to understand whether RS has any significant effects on fasting or postprandial glycaemia in individuals with impaired glucose tolerance.

7.3.3 **Outcomes**

The outcomes investigated in this systematic review and meta-analysis were:

- Postprandial glucose and insulin
- Fasting glucose and insulin
- HOMA-IR, -%B, -%S
- HbA1c
- Hormones: GLP-1, GIP

7.4 Methods

7.4.1 **Registration**

This systematic review and meta-analysis was registered on PROSPERO on 1st February 2021, reference number CRD42021233918.

7.4.2 **Criteria**

The population, intervention, comparator, outcome and study design (PICOS) criteria were predefined before conducting the literature search to conceptualise the intention and strategy of the systematic review.

Population: Adults diagnosed with T2D, prediabetes, insulin resistance or metabolic syndrome were included in this systematic review. Animal studies, studies on healthy adults or on individuals with other conditions alongside T2D, were excluded.

Intervention: Naturally occurring or purified sources of RS 1 to 5 and foods that have undergone minimal processing to preserve intact plant cell walls and RS 1 content were included. Interventions that were described as 'high fibre' were excluded.

Comparator: Rapidly or slowly digestible starches, foods which have undergone processing, e.g., milling, which may rupture plant cell walls were included. Control foods with incomparable nutrient compositions were excluded.

Outcomes: Fasting and postprandial glucose and insulin, Homeostatic model assessment (HOMA) HOMA-IR, HbA1c, HOMA-%B, HOMA-%S, GLP-1, GIP.

Study design: Parallel or crossover randomised controlled trials were included. Reviews, abstracts, lectures, study registrations and *corrigendums* were excluded.

7.4.3 **Search Strategy and Search Terms**

The literature search was conducted via PubMed, SCOPUS, Ovid MEDLINE, Cochrane, and Web of Science databases for any study-related documents published prior to 5th May 2022. Specific search terms are found in **Appendix 2**.

7.4.4 **Study selection**

The study selection was performed using the Covidence systematic review software (Veritas Health Innovation; www.covidence.org), which allowed researchers to select 'no', 'maybe' or 'yes' for inclusion in the systematic review. The title and abstract screening was conducted by at least two reviewers independently via the Covidence software. Then a full-text screening was conducted by three reviewers independently to determine whether studies met the PICOS criteria. Any conflicts that arose during study selection process were

discussed between the reviewers until a consensus was reached. On the rare occasion that an agreement could not be reached, the issue was discussed with a researcher uninvolved in the review process.

7.4.5 **Data extraction**

When the data was contained within figures, means and variances were extracted using WebPlotDigitizer 4.4 (Ankit Rohatgi, USA) (https://apps.automeris.io/wpd/). In the case of postprandial glucose and insulin, data was extracted from figures for each time point and the AUC was calculated using the trapezoidal rule (Wolever, 2006).

7.4.6 **Data processing and analysis**

Descriptive data (e.g., age, BMI) was displayed as the mean. Measurements of glycaemic control included postprandial glucose and insulin area under the curve or incremental area under the curve, fasting blood glucose or insulin, HbA1c and measurements of insulin sensitivity. Studies were separated based on study duration and RS type. Data was documented in an Excel Spreadsheet. Means and standard deviations (SD) were calculated if they were not found in the results. If standard errors of means or 95% confidence intervals were converted to SD. If there were multiple relevant intervention groups, the means and SD were pooled according to the Cochrane method (Deeks et al., n.d.). Review Manager Version 5.4 (the Cochrane Collaboration, Software Update. Oxford, UK) was used for meta-analyses using the random effects model, under generic inverse variance. Pooled standard mean differences (SMDs) with 95% confidence intervals (CIs) were calculated for outcomes of interest listed above. A random effects model and SMDs were used due to differences in the type, duration and design of the included studies. P-values of <0.05 were deemed statistically significant. Heterogeneity was assessed using the I^2 statistic. I^2 values ranged from 0% to 100%, with values of 25% to 49%, 50% to 74%, and ≥75% classified as low, moderate, and high, respectively (Higgins et al., 2003). Where possible, subgroup analysis was used to explore differences in effect between different types of resistant starch and between subjects with T2D versus prediabetes. Sensitivity analyses were conducted by omitting studies with a high risk of bias. Publication bias was assessed using funnel plots and the regression test for Funnel plot asymmetry (“Egger’s test”), in JASP (Eric-Jan Wagenmakers, Amsterdam, Netherlands). When there were fewer than 3 studies for any outcome category a narrative review was conducted (Deeks et al., n.d.).

7.4.7 **Risk of Bias Assessment**

Risk of bias was assessed by three reviewers and any discrepancies between categorisation were discussed with an independent party. The Cochrane revised risk of bias tool (v2.0) was

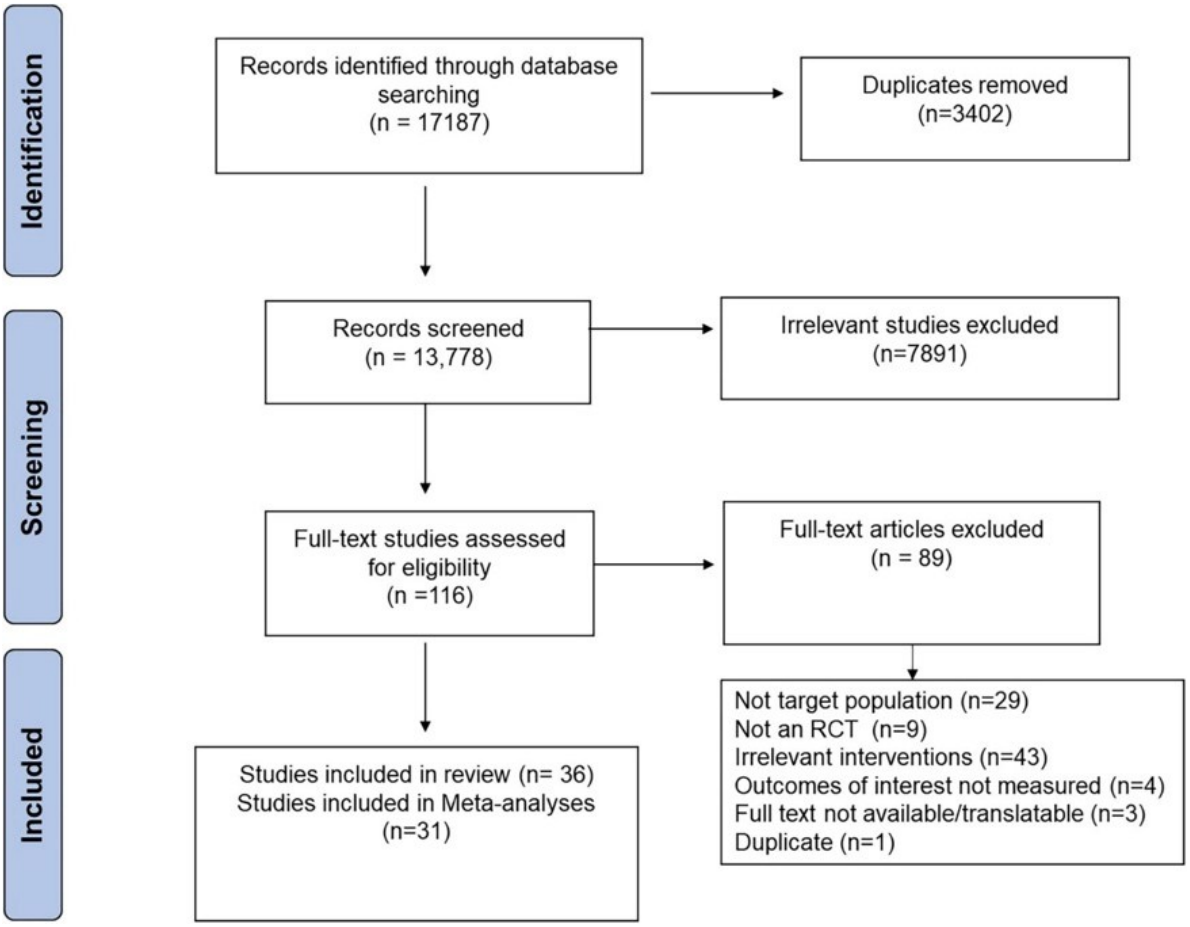
used to assess the risk that aspects of the methodology or measurement of outcomes will result in spurious results (Higgins et al., 2021). The risk of bias was assessed in the following domains: randomisation process, assignment to intervention, intervention adherence, effect of missing outcome data, measurement of outcome and selection of reported result. Using the algorithms provided in the risk of bias tool, the risk of bias for each domain was assessed to be low, of some concerns or high. If the study had any high risk of bias domains or more than 3 domains with some concerns, the study was deemed to have a high risk of bias.

7.5 Results

7.5.1 **Study Search**

A total of 17,187 references were identified in the literature searches. SCOPUS searches yielded 6,871, OVID yielded 5,783, Web of Science yielded 4,060, PUBMED yielded 378 and Cochrane yielded 95 references. A full-text review was conducted on 116 references and 89 were excluded after this review. The researchers who were contacted for access to full data sets failed to respond. In total, 36 papers and 982 participants were included in this systematic review and 31 papers could be included in the meta-analyses. Five studies could not be included in the meta-analysis because they did not report measures of variability (e.g. standard deviation) or they reported difference from baseline for outcomes of interest. None of the studies identified investigated the effect of RS 5 on glycaemia. A summary of the study selection process is found in **Figure 7.1.**

Figure 7.1: **A flow diagram of the study selection process adapted from the PRISMA 2020 statement.**



7.5.2 **Summary of identified studies**

Five acute studies with 57 participants and three chronic studies with 83 participants investigated the effect of RS 1 on glycaemia. Six acute studies with 65 participants and 11 chronic studies with 384 participants investigated the effect of RS 2 on glycaemia. Two

studies with 29 participants investigated the effect of acute RS 3 intake on glycaemia. One study with 27 participants investigated the effect of chronic RS 4 intake on glycaemia. No studies were identified investigating acute RS 4 and 5 intake nor chronic RS 3 or 5 intake on glycaemia.

7.5.3 **Study and study participant characteristics**

The study and study participant characteristics are described in the table below (**table 7.1**).

Table 7.1: Description of study participants characteristics, interventions and outcomes

Study	Participant characteristics	Intervention characteristics	Outcomes Analysed
Acute RS			
Behall and Schofield 2005a	6 males, 6 females with insulin resistance Age: 46 BMI: 25.6	RCT, crossover Duration: 180 min Intervention: RS 2 crisps RS content: 11.9g Carbohydrate content: 50g Available carbohydrate matched Control: high-amylopectin crisps	Glucose, insulin
Behall and Schofield 2005b	6 males, 6 females with insulin resistance Age: 46 BMI: 25.6	RCT, crossover Duration: 180 min Intervention: RS 2 muffins RS content: 24.7g Carbohydrate content: 70g Available carbohydrate matched Control: high-amylopectin muffins	Glucose, insulin
Canfora et al. 2021	12 males with prediabetes Age: 59 BMI: 29.2	RCT, crossover Duration: 240 min Intervention: inulin-RS 2 RS content: 7.5g Carbohydrate content: NR Control: inulin	Glucose, insulin
Giacco et al. 1998	6 males. 4 females with T2D Age: 50 BMI: 27	RCT, crossover Duration: 480 min Intervention: RS 2 cheesecake	Glucose, insulin

		RS content: 16g Carbohydrate content: 116g Control: high-amylopectin starch cheesecake	
Hartvigsen et al. 2014	7 males, 8 females with metabolic syndrome Age: 62.8 BMI: 31.1	RCT, crossover Duration: 270 min Intervention: intact kernel rye bread RS content: NR Carbohydrate content: NR Control: wheat bread	Glucose, insulin, GLP-1 and GIP
Jarvi et al. 1995 (study 2)	2 males, 8 females with T2D Age: 61 BMI: 27	RCT, crossover Duration: 360 min Intervention: low GI meal RS content: NR Carbohydrate content: 84.1g* Control: high GI meal	Glucose, insulin
Krezowski et al. 1987 (study 2)	9 males with T2D Age: 63 BMI: NR	RCT, crossover Duration: 300 min Intervention: RS 2 muffins RS content: 18g* Carbohydrate content: 50g Control: low-amylose starch muffins	Glucose, insulin
Larsen et al. 2000	6 males, 3 females with T2D Age: 60 BMI: 26	RCT, crossover Duration: 180 min Intervention: pressure-parboiled rice containing RS 3 RS content: 1g* Carbohydrate content: 50g Available carbohydrate matched Control: traditionally parboiled and non-parboiled rice	Glucose, insulin
MacNeil et al. 2013	5 males, 7 females with T2D Age: 60 BMI: 33	RCT, crossover Duration: 300 Intervention: RS 2 bagel RS content: 25.1g Carbohydrate content: 64.6g	Glucose, insulin, GLP-1 and GIP

		Available carbohydrate matched Control: wheat bagel	
Mete et al. 2021	8 participants with impaired glucose tolerance Age: NR BMI: NR	RCT, crossover Duration: 180 min Intervention: coarse, stoneground flour RS content: NR Carbohydrate content: 34.5g* Available carbohydrate matched Control: fine roller-milled flour	Glucose, insulin
Panlasigui et al. 2006	5 males, 4 females with T2D Age: 55.9 BMI: NR	RCT, crossover Duration: 60 min Intervention: brown rice RS content: NR Carbohydrate content: 87.7g* Available carbohydrate matched Control: milled rice	Glucose
Reader et al. 2002	7 males, 3 females with T2D Age: NR BMI: NR	RCT, crossover Duration: 300 min Intervention: RS 2 energy bar RS content: 7.25g Carbohydrate content: 50g Control: commercial energy bar	Glucose, insulin
Reynolds et al. 2020	10 males, 5 females with T2D Age: 64 BMI: 32	RCT, crossover Duration: 180 min Intervention: Roller-milled wholegrain flour (40%), intact whole grains (30%), kibbled whole grains (30%) RS content: NR Carbohydrate content: 50g Available carbohydrate matched Control: roller-milled, wholegrain flour	Glucose
Tucker et al. 2014	11 males, 1 female with T2D Age: 63.8 BMI: 32	RCT, crossover Duration: 300 min Intervention: wholegrain sourdough bread RS content: NR	Glucose, insulin

		Carbohydrate content: 50g Available carbohydrate matched Control: white sourdough bread	
Yamada et al. 2005	9 males, 11 females with insulin resistance Age: 50.5 BMI: NR	RCT, crossover Duration: 120 min Intervention: RS 3 bread RS content: 6g Carbohydrate content: 65.2g* Control: wheat bread	Glucose, insulin
Chronic RS			
Ble-Castillo et al. 2010	4 males, 24 females with T2D Age: 51.7 BMI: 34.9	RCT, crossover Duration: 28 days Intervention: RS 2 RS content: 8.2g Carbohydrate content: NR Control: soymilk powder	Fasting blood glucose, fasting insulin, HOMA-IR, HbA1c
Bodinham et al. 2012	8 males, 4 females with insulin resistance Age: 37 BMI: 28.2	RCT, crossover Duration: 28 days Intervention: 60% RS 2, 40% rapidly digestible starch RS content: 40g Carbohydrate content: NR Control: rapidly digestible starch	Fasting and postprandial blood glucose, fasting insulin
Bodinham et al. 2014	12 males, 5 females with T2D Age: 55 BMI: 30.6	RCT, crossover Duration: 84 days Intervention: 60% RS 2, 40% rapidly digestible starch RS content: 40g Carbohydrate content: NR Control: rapidly digestible starch	Fasting and postprandial blood glucose, fasting and postprandial insulin, HOMA-%S, HOMA-%B, HbA1c
Dainty et al. 2016	16 males, 8 females with insulin resistance Age: 55.3 BMI: 30.2	RCT, crossover Duration: 57 days Intervention: RS 2 bagel RS content: 25g Carbohydrate content: 60.9 Control: wheat bagel	Fasting and postprandial blood glucose, fasting and postprandial insulin, HOMA-IR

Dodevska et al. 2016	19 males, 31 females with prediabetes Age: I: 58.36; C: 56.96 BMI: I: 33.06; C: 31.07	RCT, parallel Duration: 365 days Intervention: RS diet RS content: 14g Carbohydrate content: 270.2g Available carbohydrate matched Control: high fibre diet	Fasting blood glucose, fasting insulin, HOMA-IR
Eshghi et al. 2019	8 males, 13 females with prediabetes Age: 35 BMI: 32	RCT, crossover Duration: 84 days Intervention: RS 2 RS content: 8.1 g Carbohydrate content: NR Control: maltodextrin	Fasting blood glucose, fasting insulin, HOMA-IR
Gower 2016	14 females with insulin resistance Age: 48.3 BMI: 29.8	RCT, crossover Duration: 28 days Intervention: RS 2 cookies RS content: 15g + 30g Carbohydrate content: 39g Control: waxy corn starch cookies	Fasting blood glucose, fasting insulin, insulin sensitivity
Jarvi 1999	15 males, 5 females with T2D Age: 66.5 BMI: 25.3	RCT, crossover Duration: 24 days Intervention: low GI food menu RS content: NR Carbohydrate content: 247g* Control: high GI food menu	Fasting and postprandial blood glucose, fasting and postprandial insulin, HbA1c
Johnston 2010	12 males, 8 females with insulin resistance Age: I: 45.2; C: 50.1 BMI: I: 31.3; C: 30.4	RCT, parallel Duration: 84 days Intervention: RS 2 RS content: 40g Carbohydrate content: NR Control: rapidly digestible starch	HOMA-%S, HOMA-%B
Karimi 2015	56 females with T2D Age: I: 49.5; C: 48.6 BMI: I: 31.5; C: 31.0	RCT, parallel Duration: 56 days Intervention: RS 2 RS content: 10g Carbohydrate content: NR	Fasting blood glucose, fasting insulin, HbA1c, HOMA-IR

		Available carbohydrate matched Control: maltodextrin	
Kwak 2012	47 males, 38 females with T2D or prediabetes Age: I: 51.7; C: 49.4 BMI: I: 25; C: 24.5	RCT, parallel Duration: 28 days Intervention: high RS 2 rice RS content: 6.5g Carbohydrate content: NR Available carbohydrate matched Control: refined rice	Fasting blood glucose, fasting insulin, HOMA-IR
Lin 2015	22 males, 22 females with T2D Age: 52.1 BMI: 26.1	RCT, crossover Duration: 2 days Intervention: RS 2 (20%) and RS 3 (10%) rice/noodles RS content: 6g* Carbohydrate content: NR Control: commercially-available rice/noodles	Postprandial blood glucose
Lobley 2013	14 males with metabolic syndrome Age: NR BMI: NR	RCT, crossover Duration: 21 days Intervention: RS-enriched diet RS content: NR Carbohydrate content: NR Control: NSP-enriched diet	Fasting and postprandial blood glucose, fasting and postprandial insulin, HOMA-IR
Malin 2019	3 males, 10 females with insulin resistance Age: 37.2 BMI: 33.6	RCT, crossover Duration: 56 days Intervention: whole-grain diet RS content: NR Carbohydrate content: NR Control: refined-grain diet	Fasting and postprandial blood glucose, fasting and postprandial insulin
Meng 2015	47 males, 54 females with T2D Age: I (A): 52.5; I (B): 51.4 ± 7.8; C: 53.2 BMI: I (A): 25.21; I (B): 25.44; C: 25.1	RCT, parallel Duration: 84 days Intervention: A:RS fortified stream bun; B: Herbal Steamed bun RS content: 30g Carbohydrate content: NR Control: commercially available steamed bun	Fasting blood glucose, fasting insulin

Nichenametla 2014	13 males, 14 females with metabolic syndrome Age: 55.7 BMI: 33.7	RCT, crossover Duration: 84 days Intervention: RS 4 flour RS content: 24g Carbohydrate content: NR Available carbohydrate matched Control: wheat flour	Fasting blood glucose, HbA1c
Peterson 2018	20 males, 39 females with prediabetes Age: I: 54; C: 55 BMI: I: 35.5; C: 35.7	RCT, crossover Duration: 84 days Intervention: RS 2 RS content: 45 g Carbohydrate content: NR Available carbohydrate not matched Control: rapidly digestible starch	Fasting and postprandial blood glucose, fasting and postprandial insulin, HbA1c
Robertson 2012	8 males, 7 females with insulin resistance Age: 48.9 BMI: 33.8	RCT, crossover Duration: 56 days Intervention: RS 2 RS content: 40 g Carbohydrate content: NR Available carbohydrate matched Control: rapidly digestible starch	Fasting blood glucose, fasting insulin, HOMA-%B HOMA-IR
Sandberg 2018	30 males, 8 females with prediabetes Age: 63.6 BMI: 24.6	RCT, crossover Duration: 3 days Intervention: RS 2 rye bread RS content: 14.3 Carbohydrate content: 50g Available carbohydrate matched Control: white bread, rye bread	Fasting and postprandial blood glucose, fasting and postprandial insulin, HOMA-IR, GLP-1
Wang 2017	42 males, 39 females with T2D Age: I: 60.5; C: 60.4 BMI: I: 26.4; C: 23.4	RCT, parallel Duration: 84 days Intervention: RS 2 rice RS content: 20-35g Carbohydrate content: NR Control: commercially available rice	Fasting and postprandial blood glucose, fasting insulin

Zhang 2007	17 males, 23 females with T2D Age: 51.7 BMI: 27	RCT, crossover Duration: 28 days Intervention: RS 2 RS content: 30g Carbohydrate content: NR Control: wheat	Fasting blood glucose, fasting insulin
C, control; GIP, Gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; ; HbA1c, glycated haemoglobin; HOMA-%B, Homeostatic model assessment of β-cell function; HOMA-%S, Homeostatic model assessment of insulin sensitivity; I, intervention; NR, not reported; RS 2, RS 2; RS 3, RS 3; RCT, randomized control trial; T2D, type 2 diabetes, *, calculated by researchers			

7.5.4 **Risk of Bias Assessment**

Some risk of bias was identified in half of the studies included in this systematic review, 28% of studies were assessed to have a high risk of bias and 22%, a low risk of bias. Domains where risk of bias was more common were risk of bias arising from the randomisation process (D1) and due deviations from intended interventions (D2.2). The risk of bias assessment for each publication can be found in **Figure 7.2.**

Figure 7.2: **Risk of bias assessment for included publications**

Study name	D1	D2.1	D2.2	S	D3	D4	D5	Overall
Arias-Cordova 2021	-	+	+	+	+	+	+	-
Behall 2005	-	+	+	-	+	+	+	-
Ble-Castillo 2010	-	+	+	+	+	-	+	-
Bodinham 2012	+	+	+	+	+	+	+	+
Bodinham 2014	-	+	+	+	+	+	+	-
Canfora 2021	+	+	+	+	+	+	+	+
Dodevska 2016	-	+	-		+	-	+	-
Dainty 2016	-	+	+	+	+	+	+	-
Eshghi 2019	+	+	+	+	+	+	+	+
Giacco 1998	-	-	x	+	+	+	+	x
Gower 2016	-	+	+	-	+	+	+	-
Hartvigsen, 2014	+	+	+	+	+	+	+	+
Järvi 1995	-	-	+	+	-	+	+	x
Järvi 1999	-	-	-	+	-	+	+	x
Johnston 2010	-	+	+		+	+	+	-
Karimi 2015	+	+	+		+	+	+	+
Krezowski 1987	-	+	+	-	+	+	+	-
Kwak 2012	-	-	-	-	+	+	+	x
Larsen 2000	-	-	+	+	+	+	-	x
Lin 2015	-	+	-	+	+	+	+	-
Lobely 2013	-	-	x	x	+	+	+	x
MacNeil 2013	-	-	+	+	+	+	+	-
Malin 2019	-	+	+	+	+	+	+	-
Meng 2015	-	-	x		+	+	+	x
Mete 2020	+	+	+	+	+	+	-	-
Nichenametla 2014	-	+	+	+	+	+	+	-
Panlasigui 2006	-	+	+	-	+	+	+	-
Peterson 2018	+	+	+		+	+	+	+
Reader 2002	+	+	+	+	+	+	+	+
Reynolds 2020	+	+	+	+	+	+	+	+
Robertson 2012	-	-	+	+	-	+	+	x
Sandberg 2018	-	+	+	+	+	-	+	-
Tucker 2014	-	+	+	-	+	+	+	-
Wang 2017	-	-	x		+	+	+	x
Yamada 2005	-	+	+	+	+	+	+	-
Zhang 2007	-	-	x	x	+	+	+	x

Judgement	
	Not applicable
+	Low
-	Some Concerns
x	High

Risk of bias...	
D1	...arising from randomisation process
D2.1	...due to deviations from intended interventions (asssignment to the intervention)
D2.2	...due to deviations from intended interventions (effect of adhering to intervention)
S	...arising from period and carryover effects of crossover studies
D3	...due to missing outcome data
D4	...in measurement of outcome
D5	...in selection of reported result

7.5.5 The effect of acute RS interventions on glycaemic response

The effect of acute RS intake on glycaemic response was investigated using RS 1,2 and 3.

7.5.5.1 The effect of acute RS interventions on postprandial glucose

A total of 13 studies (n=151) were included in the systematic review of RS on postprandial blood glucose. Five studies were included for RS 1 (Hartvigsen et al., 2014; Järvi et al., 1995; Mete et al., 2021; Panlasigui & Thompson, 2006; Reynolds, Mann, et al., 2020). Six studies were included for RS 2 (Behall & Scholfield, 2005; Canfora et al., 2021; Giacco et al., 1998; Krezowski et al., 1987; MacNeil et al., 2013; Reader et al., 2002) and two studies were included for RS 3 (H. Larsen et al., 2000; Yamada et al., 2005). Overall acute RS intake lowered postprandial blood glucose response [SMD (95% CI) = -0.65 (–0.98, -0.32)]. In subgroup analyses, RS 1 and 2 also had a significant effect on postprandial blood glucose [-0.54 (-1.00, -0.07)] and [-0.96 (-1.61, -0.31)], respectively. Further subgroup analyses were conducted to compare the effect of RS on individuals with T2D versus prediabetes. Acute RS intake influenced individuals with T2D and prediabetes [-0.79 (-1.31, -0.27)] and [-0.51 (-0.90, -0.11)], respectively. Interstudy heterogeneity was significant (I^2 = 68%; P = 0.001). Results are presented in **Figure 7.3** and **7.4**.

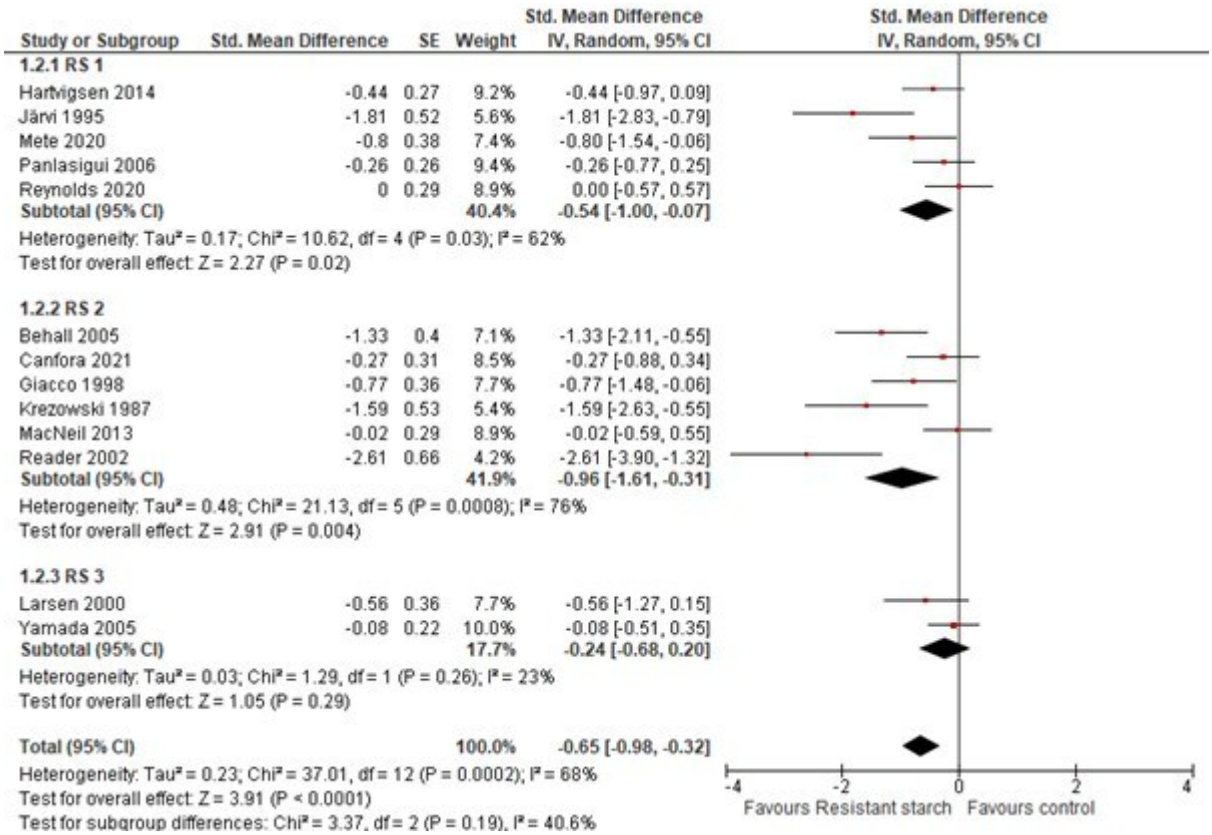


Figure 7.3: Forest plot of studies assessing the effect of acute RS intake on postprandial glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, Resistant starch.

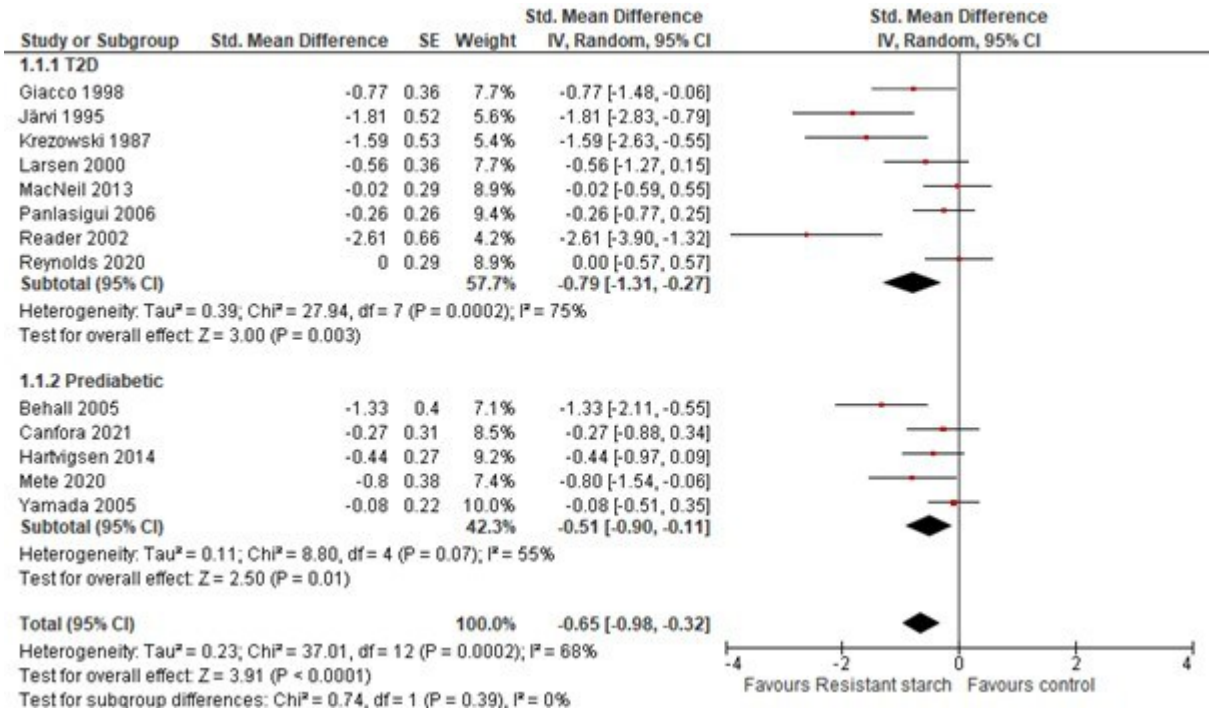


Figure 7.4: Forest plot of studies assessing the effect of acute RS intake on postprandial glucose concentrations with subgroups for T2D versus prediabetes.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. T2D, Type 2 diabetes

7.5.5.2 The effect of acute RS interventions on postprandial insulin

A total of 11 studies (n=119) were included in the systematic review of RS on postprandial blood glucose. Four studies were included for RS 1 (Hartvigsen et al., 2014; Järvi et al., 1995; Mete et al., 2021; Tucker et al., 2014). Five studies were included for RS 2 (Behall & Scholfield, 2005; Canfora et al., 2021; Krezowski et al., 1987; MacNeil et al., 2013; Reader et al., 2002) and two studies were included for RS 3 (H. Larsen et al., 2000; Yamada et al., 2005). Overall, acute RS intake lowered postprandial insulin response [-0.41 (–0.72, -0.10)]. In subgroup analyses, RS 2 also had a significant effect on postprandial insulin [-0.71 (-1.31, -0.11)]. Further subgroup analyses were conducted to compare the effect of RS on individuals with T2D versus prediabetes, no significant effect on postprandial insulin after this subgroup analysis. Interstudy heterogeneity was significant (I^2=65%; p=0.009). Results are presented in **Figure 7.5** and **7.6**.

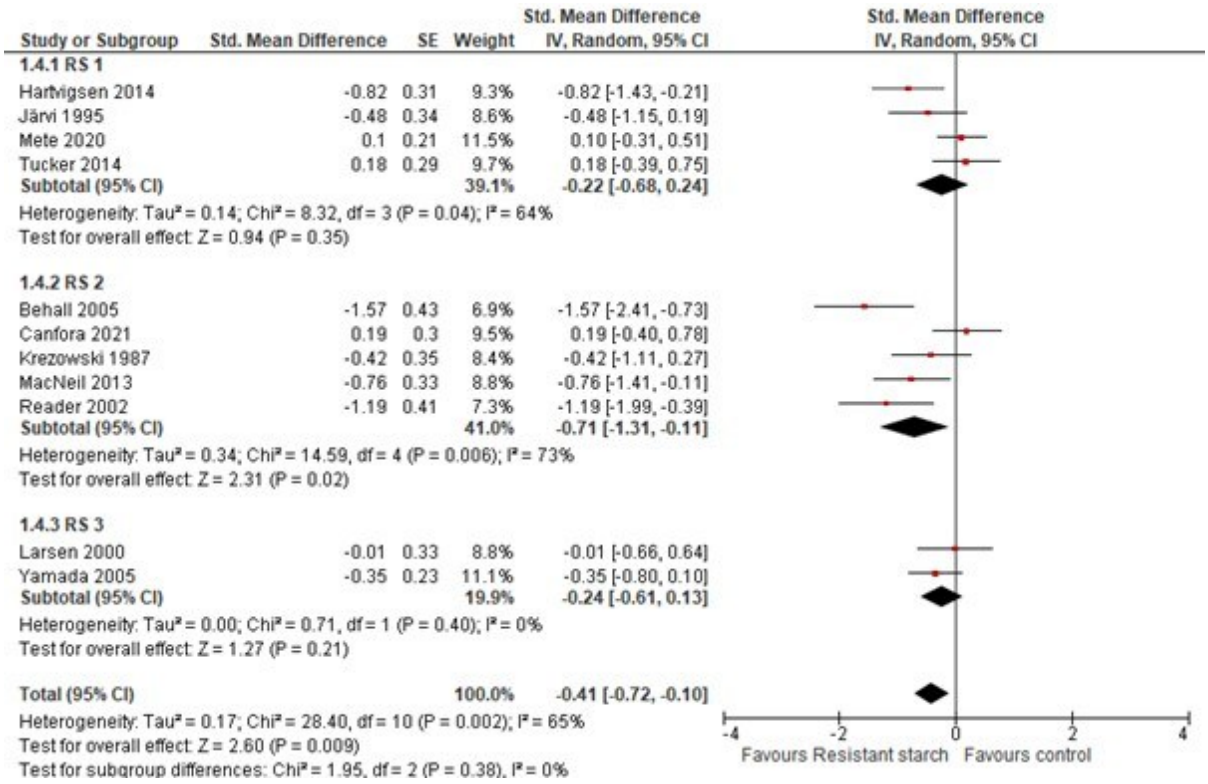


Figure 7.5: Forest plot of studies assessing the effect of acute RS intake on postprandial insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, resistant starch.

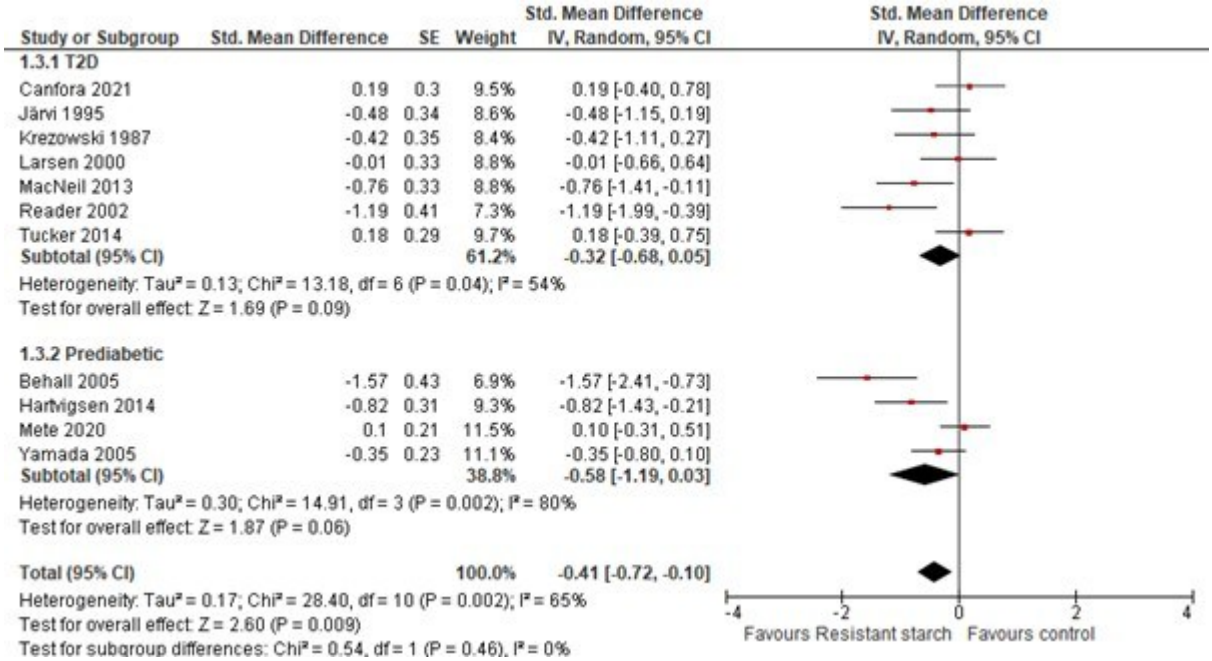


Figure 7.6: Forest plot of studies assessing the effect of acute RS intake on postprandial insulin concentrations with subgroups for T2D versus prediabetes.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. T2D, Type 2 diabetes

7.5.6 The effect of chronic RS interventions on glycaemic response

The effect of chronic RS intake on glycaemic response was investigated in RS 1, 2 and 4.

7.5.6.1 The effect of chronic RS interventions on glucose response

Seven studies (n=168) investigated the effect of chronic RS intake on postprandial blood glucose response. Two studies used RS 1 (Jarvi et al., 1999; Malin et al., 2019) and five studies used RS 2 (Bodinham et al., 2012, 2014; Dainty et al., 2016; C.-H. Lin et al., 2015; Sandberg et al., 2018). Overall, chronic RS supplementation affected postprandial glucose response [-0.31 (-0.50, -0.13). Subgroup analyses indicated that neither RS 1 nor RS 2 affected this outcome individually. Results are presented in **Figure 7.7.**

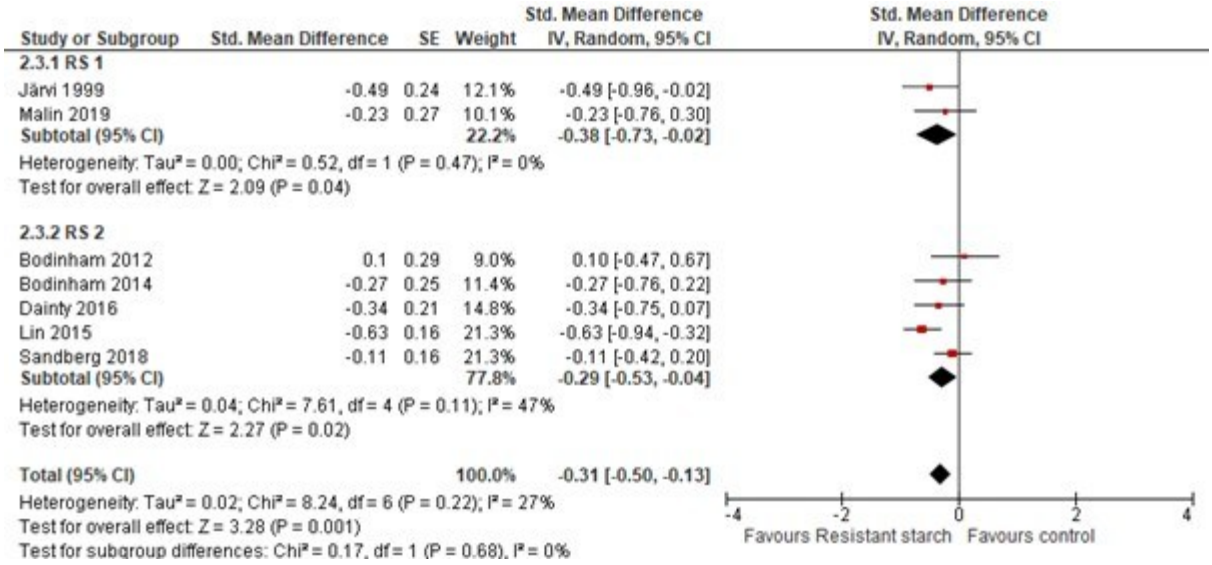


Figure 7.7: Forest plot of studies assessing the effect of chronic RS intake on postprandial glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, resistant starch.

14 studies (n=168) investigated the effect of chronic RS intake on fasting blood glucose response. Three studies used RS 1 (Dodevska et al., 2016; Jarvi et al., 1999; Malin et al., 2019), 10 studies used RS 2 (Bodinham et al., 2012, 2014; Dainty et al., 2016; Eshghi et al., 2019; Gower et al., 2016; Karimi et al., 2016; C.-H. Lin et al., 2015; Meng & Chen, 2015; M. D. Robertson et al., 2012; Sandberg et al., 2018; Y. Zhang et al., 2007). Overall, chronic RS supplementation affected fasting blood glucose [-0.31 (-0.51, -0.11). Subgroup analyses indicated that RS 2 had the greatest effect on fasting blood glucose [-0.39 (-0.66, -0.13)]. Further subgroup analyses were conducted to compare the effect of RS on individuals with T2D versus prediabetes. Chronic RS intake influenced individuals with T2D and prediabetes [-0.46 (-0.85, -0.08)] and [-0.15 (-0.3, 0.00)], respectively. Heterogeneity was significant (I^2 = 67%; p=0.002). Results can be seen in **Figure 7.8 and 7.9**.

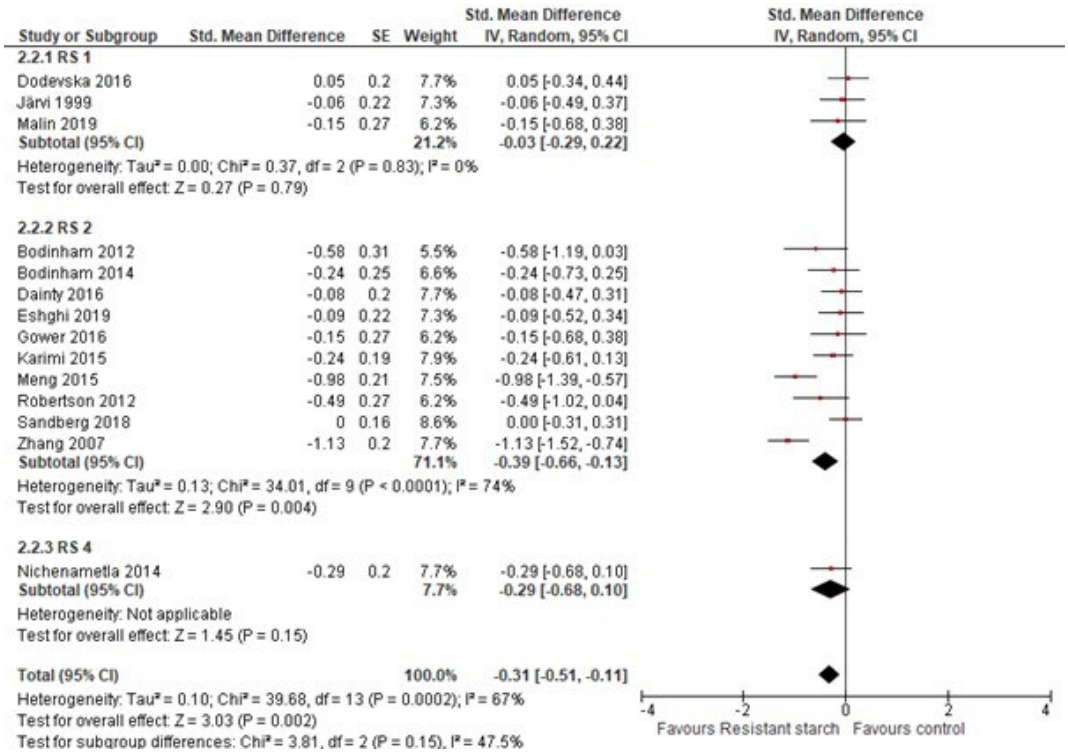


Figure 7.8: Forest plot of studies assessing the effect of chronic RS intake on fasting blood glucose concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, Resistant starch.

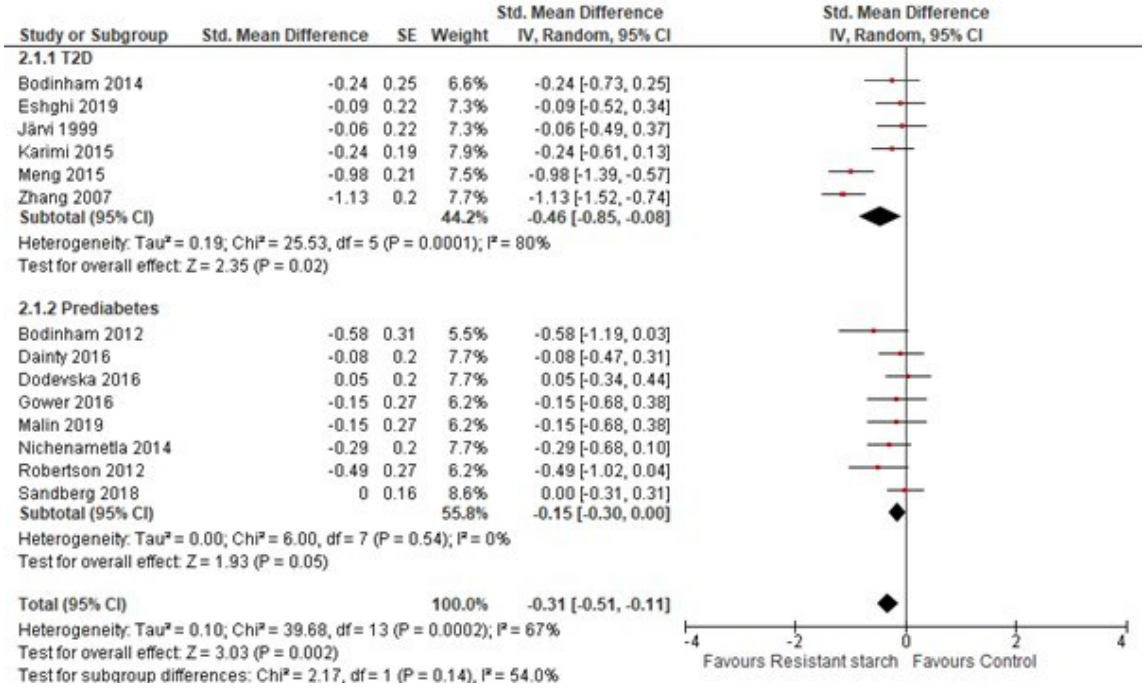


Figure 7.9: **Forest plot of studies assessing the effect of chronic RS intake on fasting blood glucose concentrations with subgroups for T2D versus prediabetes.**

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. T2D, Type 2 diabetes

Five studies investigated the effect of RS on fasting blood glucose, four of which reported that RS had no significant effect on fasting glucose (Ble-Castillo et al., 2010; Lobley et al., 2013; Peterson et al., 2018; Wang et al., 2020). One study reported a significant reduction in fasting blood glucose after chronic RS supplementation (Arias-Córdova et al., 2021).

7.5.6.2 The effect of chronic RS interventions on insulin response

Five studies (n=107) investigated the effect of chronic RS intake on postprandial insulin response. Two studies used RS 1 (Jarvi et al., 1999; Malin et al., 2019) and three studies used RS 2 (Bodinham et al., 2012; Dainty et al., 2016; Sandberg et al., 2018). Overall, chronic RS supplementation did not affect postprandial insulin response and subgroup analyses indicated that neither RS 1 nor RS 2 affected this outcome individually. Results can be seen in **Figure 7.10.**

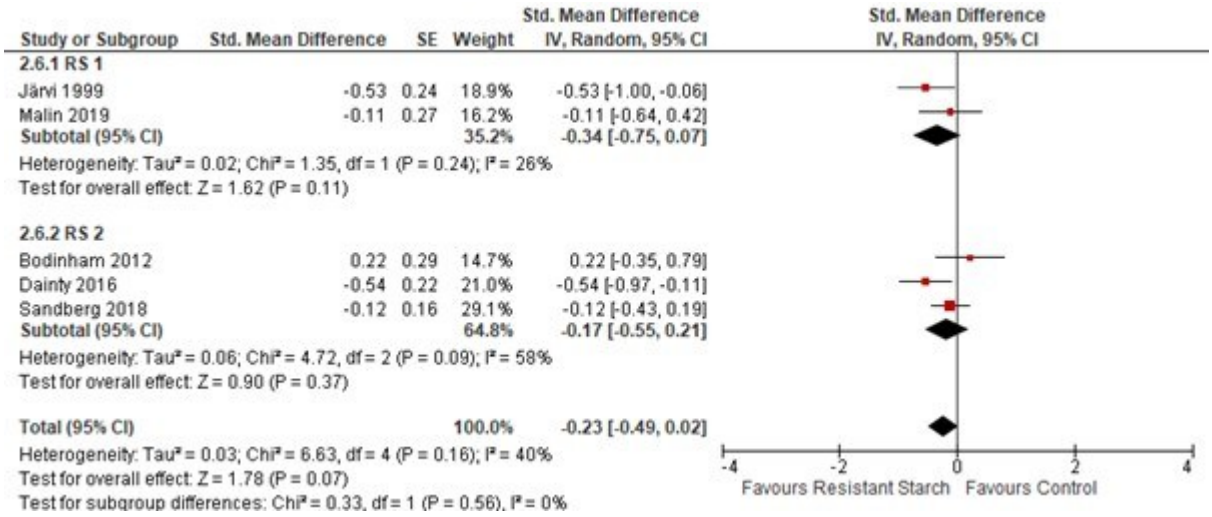


Figure 7.10: Forest plot of studies assessing the effect of chronic RS intake on postprandial insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, Resistant starch.

13 studies (n=124) investigated the effect of chronic RS intake on fasting insulin response. Three studies used RS 1 (Dodevska et al., 2016; Jarvi et al., 1999; Malin et al., 2019), nine studies used RS 2 (Bodinham et al., 2012, 2014; Dainty et al., 2016; Eshghi et al., 2019; Gower et al., 2016; Karimi et al., 2016; Meng & Chen, 2015; M. D. Robertson et al., 2012; Sandberg et al., 2018; Y. Zhang et al., 2007). Overall, chronic RS supplementation affected fasting insulin [-0.29 (-0.49, -0.10). Subgroup analyses indicated that RS 2 lowered fasting insulin [-0.40 (-0.60, -0.21)] and RS 1 did not. Further subgroup analyses were conducted to compare the effect of RS on individuals with T2D versus prediabetes. Chronic RS intake influenced fasting insulin in individuals with T2D only [-0.46 (-0.79, -0.13)]. Heterogeneity was significant (I^2 = 62%; p=0.002). Results can be seen in **Figure 7.11 and 7.12**.

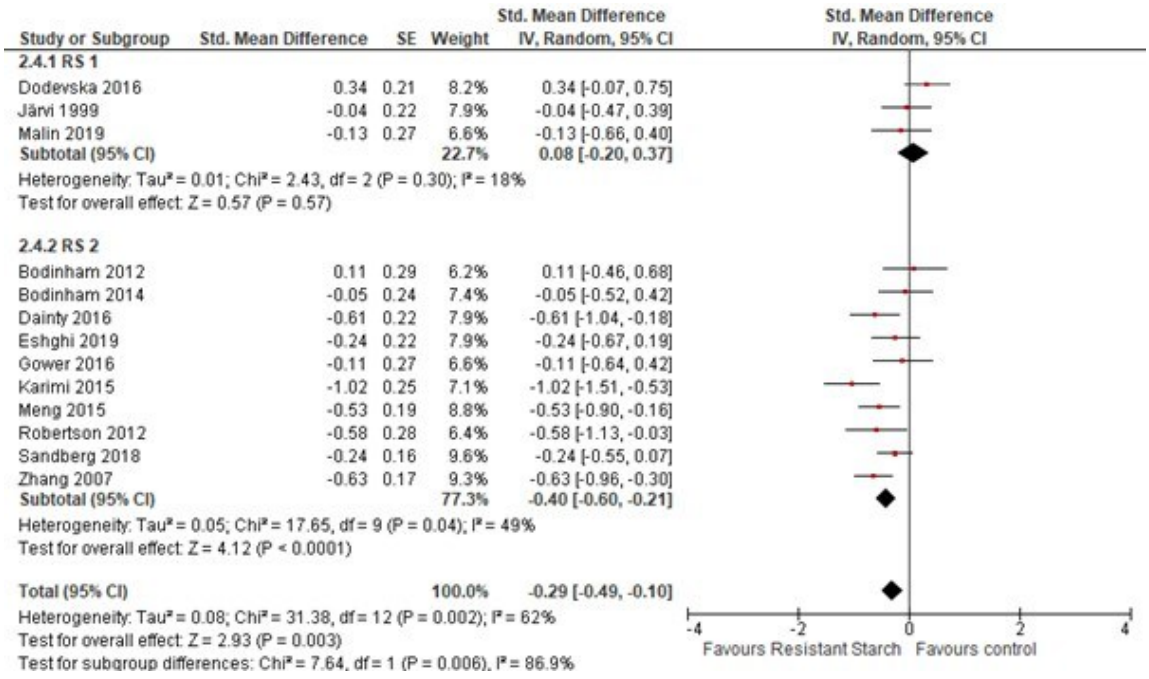


Figure 7.11: Forest plot of studies assessing the effect of chronic RS intake on fasting insulin concentrations on individuals with T2D and prediabetes with subgroups based on RS type.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. RS, Resistant starch.

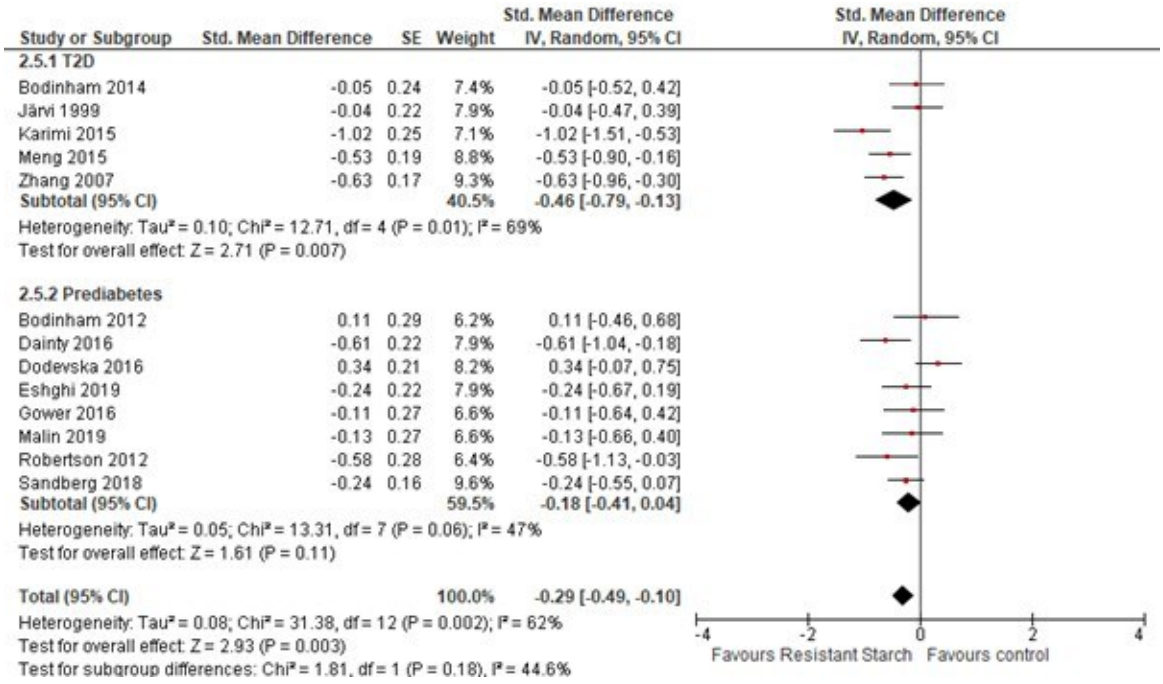


Figure 7.12: Forest plot of studies assessing the effect of chronic RS intake on postprandial insulin concentrations with subgroups for T2D versus prediabetes.

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant. T2D, Type 2 diabetes

Five studies investigated the effect of RS on fasting insulin, four of which reported that RS had no significant effect on fasting insulin (Arias-Córdova et al., 2021; Lobley et al., 2013;

Peterson et al., 2018; Wang et al., 2020). One study reported a significant reduction in fasting blood glucose after chronic RS supplementation (Ble-Castillo et al., 2010).

7.5.6.3 RS and incretins

Five studies (n=95) measured the effect of acute and chronic RS intake on GLP-1 secretion (Bodinham et al., 2014; Hartvigsen et al., 2014; MacNeil et al., 2013; Malin et al., 2019; Sandberg et al., 2018). RS did not significantly affect GLP-1 [0.01 (–0.24, 0.27)]. Three studies (n=40) measured the effect of acute and chronic RS intake on GIP concentrations (Hartvigsen et al., 2014; MacNeil et al., 2013; Malin et al., 2019). RS did not significantly affect GIP concentrations [–0.64, (–1.57, 0.30)]. These results can be seen in **figure 7.13** and **7.14**.

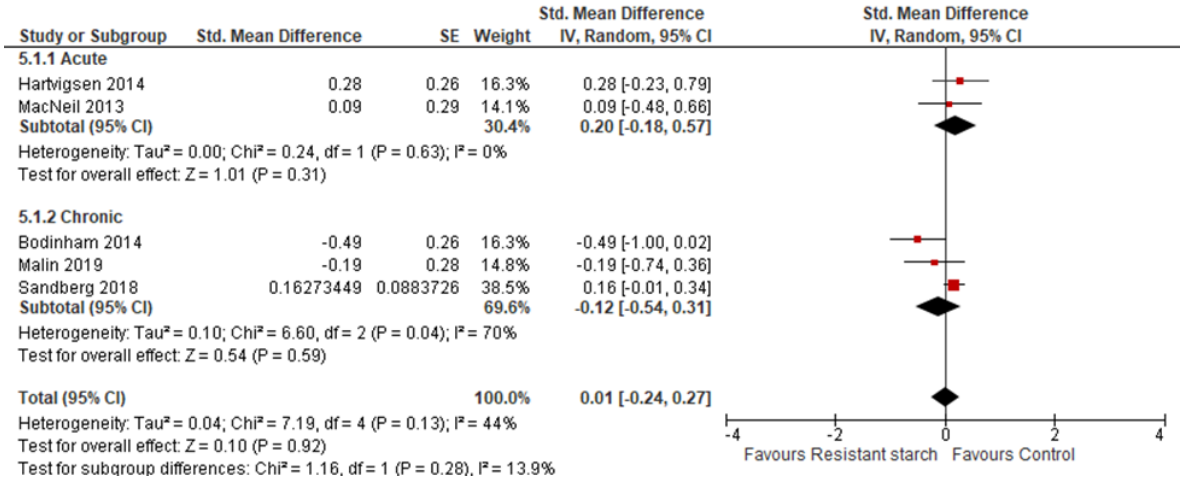


Figure 7.13: **Forest plot of studies assessing the effect of acute and chronic RS intake on GLP-1 concentrations with subgroups for length of study intervention.**

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

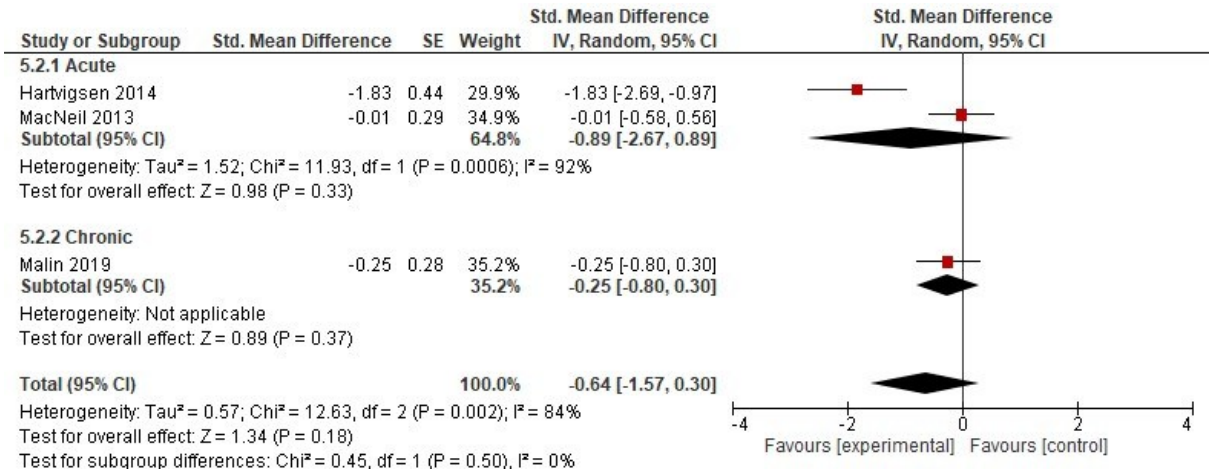


Figure 7.14: **Forest plot of studies assessing the effect of acute and chronic RS intake on GIP concentrations with subgroups for length of study intervention.**

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

7.5.6.4 RS and measures of insulin sensitivity

A meta-analysis of seven studies (n=222) was conducted to investigate the effect of chronic resistant start intake on HOMA-IR (Dainty et al., 2016; Dodevska et al., 2016; Eshghi et al., 2019; Karimi et al., 2016; Kwak et al., 2012; M. D. Robertson et al., 2012; Sandberg et al., 2018). RS did not affect HOMA-IR [–0.25 (–0.52, 0.03)].

A meta-analysis of four studies (n=66) investigated the effect of chronic RS intake on HOMA-%B (Bodinham et al., 2014; Dainty et al., 2016; K. L. Johnston et al., 2010; M. D. Robertson et al., 2012). RS did not affect HOMA-%B [–0.31 (–0.82, 0.21)].

A meta-analysis of three studies (n = 61) was conducted to investigate the effect of chronic RS intake on HOMA-%S (Bodinham et al., 2014; Dainty et al., 2016; K. L. Johnston et al., 2010). RS had no effect on HOMA-%S [0.32, (–0.06, 0.69)].

A meta-analysis of four studies (n = 101) investigated the effect of RS on HbA1c (Bodinham et al., 2014; Jarvi et al., 1999; Karimi et al., 2016; Nichenametla et al., 2014). RS did not affect HbA1c [–0.18, (–0.54, 0.17)]. These results can be seen in **Figures 7.15** to **7.18.**

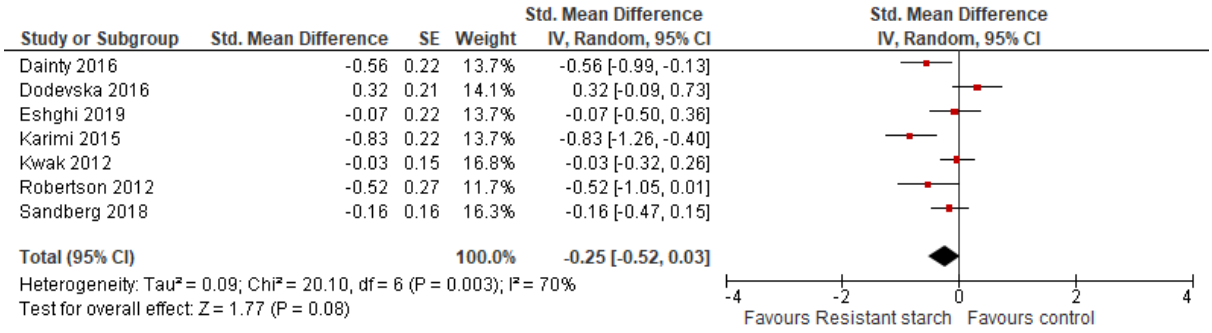


Figure 7.15: Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of insulin resistance (HOMA-IR).

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

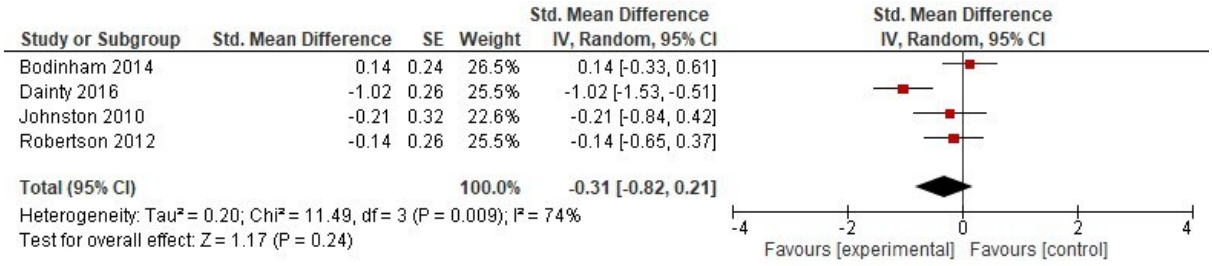


Figure 7.16: Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of β-cell function (HOMA-%B).

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

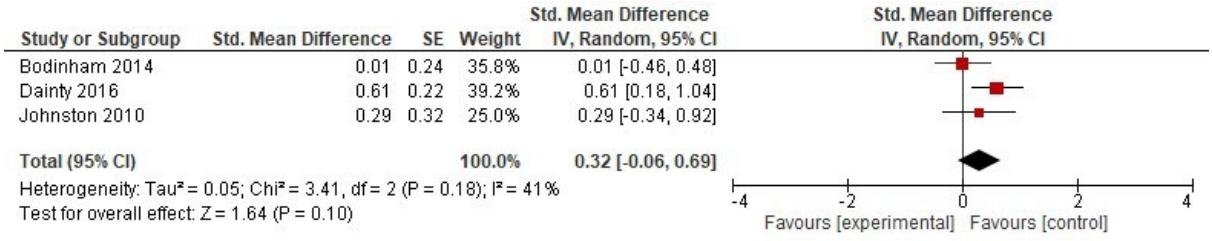


Figure 7.17: **Forest plot of studies assessing the effect of chronic RS intake on homeostatic model of insulin sensitivity (HOMA-%S).**

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

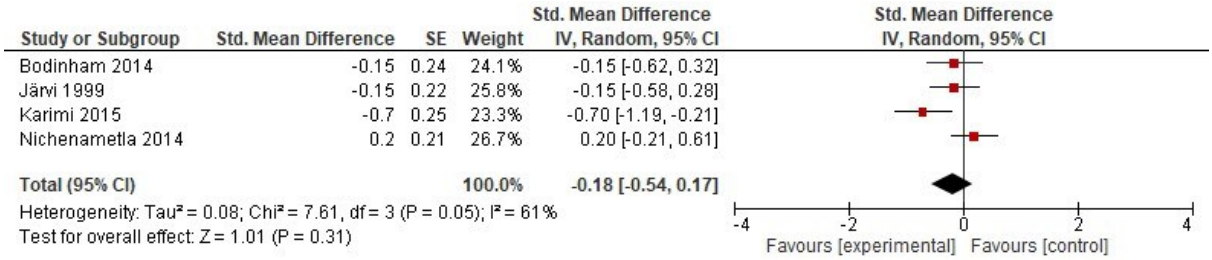


Figure 7.18: **Forest plot of studies assessing the effect of chronic RS intake on glycated haemoglobin (HbA1c).**

Data are presented as SMD ± 95% CI. Random-effects model. Heterogeneity was calculated using the I^2 statistic. P values ≤ 0.05 were considered statistically significant.

Four studies could not be included in the meta-analysis but investigated the effect of RS on measures of insulin sensitivity (Arias-Córdova et al., 2021; Ble-Castillo et al., 2010; Lobley et al., 2013; Peterson et al., 2018). One study reported a significant reduction in HbA1c after supplementation of 45g RS 2 (Peterson et al., 2018). Ble-Castillo and colleagues found that HOMA-IR was elevated after RS supplementation (Ble-Castillo et al., 2010). Two studies found no significant differences in HOMA-IR (Arias-Córdova et al., 2021; Lobley et al., 2013) or HOMA-%B and -%S (Arias-Córdova et al., 2021).

7.5.6.5 Studies identified post-publication

Since the publication of this systematic review and meta-analysis there have been three new studies published investigating RS and glycaemic response in individuals with impaired glucose control. These are summarised below.

Acute RS 4 intake was explored in a study where participants were provided with a bagel containing 15g RS 4 versus a standard bagel and postprandial glucose response was measured using a continuous glucose monitor. RS 4 significantly lowered both the postprandial AUC (0.017) and blood glucose peak (0.015) compared with the standard bagel (Cesur et al., 2023)

Two types of RS 2 were explored in a chronic study conducted over 4 days. Banana starch lowered fasting glycemia, glycemia peak and insulinemic response during a mixed-meal tolerance test, whereas high-amylose RS had no effect (García-Vázquez et al., 2023).

Another study using high RS rice demonstrated lower postprandial glucose iAUC after intake of high RS rice, compared to standard rice. The type of RS present within this rice is not specified (Tan et al., 2022).

7.6 Discussion

7.6.1 **Summarised discussion**

This systematic review and meta-analysis included 36 studies and a total of 982 participants. Acute intake of RS 1 and 2 significantly lowered postprandial blood glucose and acute intake of RS 2 significantly lowered postprandial insulin. Sub-group analyses indicated that the effect on glucose was consistent for both participants with T2D and prediabetes. Chronic intakes of RS lowered fasting and postprandial blood glucose and fasting insulin. Sub-group analyses indicated that the effect of chronic RS intake on fasting blood glucose was consistent in participants with T2D and prediabetes. However, fasting insulin only improved in participants with T2D. Meta-analyses for chronic RS intake found no significant effect on

postprandial glucose or insulin, concentrations of GLP-1, GIP, HbA1c, HOMA-IR, HOMA-%B or HOMA-%S. Searches did not identify any studies exploring the effect of acute RS 4 and 5 or chronic RS 3 and 5 intakes on glycaemia that met the PICOS criteria.

7.6.2 **Acute RS and glycaemic response**

Acute RS 1 and 2 were found to ameliorate glycaemic response in individuals with T2D and prediabetes. No studies that investigated acute glycaemic responses using RS 4 and 5 in our target population group were identified. Further studies are required to gain a complete understanding of the effects of different RS types on metabolic responses in those with impaired glucose control.

RS 1 interventions consisted of studies using 'whole grains' (Hartvigsen et al., 2014; Panlasigui & Thompson, 2006; Tucker et al., 2014) or grains that were subjected to less milling, resulting in larger grain particle size (Järvi et al., 1995; Mete et al., 2021; Reynolds, Mann, et al., 2020). Incorporating whole grains or whole kernels into the intervention product lowered their glycaemic index (Hartvigsen et al., 2014). Plant cell wall rupture, which may occur during milling and other processing methods, allows for the ingress of digestive enzymes, and increases the rate of starch hydrolysis and postprandial glucose rise. Intact plant cell walls, like those in whole grains or un-milled starchy foods, protect starch from hydrolysis and contain bioactive compounds, such as phytates, that may slow starch digestion and lower nutrient bioaccessibility (Hartvigsen et al., 2014; Panlasigui & Thompson, 2006; Tucker et al., 2014; Yoon et al., 1983). Moreover, the damaged starch structures of finely-milled foods enables water ingression during cooking thereby elevating gelatinisation and digestibility (Panlasigui & Thompson, 2006). Lowering postprandial glucose rise tends to result in a lower postprandial insulin excursion (Hartvigsen et al., 2014; Järvi et al., 1995). However, one research group reported that coarsely ground flour with larger particles elevated insulin responses in participants with impaired glucose tolerance (Mete et al., 2021). Further, some studies reported greater effects in participants with compromised glucose control. For example, a blood glucose reduction of 35.2% versus 19.8% in participants with T2D and healthy volunteers, respectively (Panlasigui & Thompson, 2006). Available carbohydrates were matched in several studies, despite this, several still report RS caused an attenuated effect on glycaemia (Panlasigui & Thompson, 2006; Reynolds, Akerman, et al., 2020). However, in one study, not RS had no significant effect (Tucker et al., 2014). The results of this meta-analysis indicate that reducing processing of grains, to preserve cell wall structures, lowers glucose responses in participants with impaired glucose control. However, the effect on postprandial insulin

remains inconclusive. The impact of RS on insulin may be associated with SCFA production in the colon, which subsequently stimulates insulin secretion, as discussed in **Chapters 1** and **2** of this thesis. Detecting these effects would require a study duration long enough for non-digestible carbohydrates to reach the colon and promote SCFA production. In some instances, the short study duration may have prevented the detection the effects of RS on insulin response. However, these results still support one research group's argument that the definition of whole grains for maximal health benefit should be based the level of processing that occurs (Reynolds, Mann, et al., 2020).

RS 2 was incorporated into crisps (Behall & Scholfield, 2005), muffins (Behall & Scholfield, 2005; Krezowski et al., 1987), cheesecake (Giacco et al., 1998), bagels (MacNeil et al., 2013) and energy bars (Reader et al., 2002). The use of RS 2 in many commonly consumed foods underscores its versatility as a digestible starch substitute. One study reported that 13g or more of RS is required to have a beneficial effect on metabolic response (Behall & Scholfield, 2005). However, the dose of RS must be tolerable to the participants. Arguably it is more clinically relevant to use moderate amounts of RS that can realistically be incorporated into the diet (Giacco et al., 1998). Substituting digestible starch for RS 2 may lower postprandial glucose responses (MacNeil et al., 2013; Reader et al., 2002). However, Giacco and colleagues argue that the 16% reduction in available carbohydrates does not account for the observed 30% blood glucose reduction (Giacco et al., 1998). Further, glucose response was lower in an RS 2 intervention when available carbohydrate contents were matched with the control (Behall & Scholfield, 2005). The second-meal effect, where there is an improved glycaemic response to subsequent meals after a high RS meal was demonstrated when insulin secretion increased after the second meal in one study using RS 2 (MacNeil et al., 2013). This reinforces the suggestion that longer studies are potentially required to detect the effects of RS on insulin secretion.

The effect of RS 3 was explored using bread containing retrograded flour (Yamada et al., 2005) and parboiled rice (H. Larsen et al., 2000). Evidence for RS 3 is inconclusive, at least partly due to the lack of studies investigating this RS type in participants with impaired glycaemic control. Yamada and colleagues reported that postprandial insulin responses were significantly reduced in volunteers with prediabetes with no effect on glucose reported (Yamada et al., 2005). However, RS 3 content was only measured in the flour content, so whether RS 3 was preserved in the cooked bread is unclear. Conversely, the pressure parboiled rice (high RS 3) intervention elicited lower postprandial glucose response than the

non-parboiled rice when available carbohydrate content was matched (H. Larsen et al., 2000).

7.6.3 **Chronic RS and glycaemic response**

Chronic RS intake lowered fasting and postprandial glucose and fasting insulin response. Subgroup analyses indicated that RS lowered fasting glucose in individuals with prediabetes and T2D but did not affect fasting insulin in participants with prediabetes. We did not identify any studies that investigated chronic glycaemic responses using RS 3 and 5 in our target population group.

Although chronic RS intake resulted in lower fasting blood glucose and fasting insulin concentrations, subgroup analyses indicated that RS 1 alone had no effect. The limited number of studies using RS 1 (Dodevska et al., 2016; Jarvi et al., 1999; Malin et al., 2019), may have contributed in part to the observed lack of significance effect. Dodevska and colleagues compared high-RS and high-fibre diets and found no significant differences in glucose and insulin metabolism over 12 months (Dodevska et al., 2016). However, they did see a greater decrease in body weight in the RS group, -4.6% versus -2.7% (Dodevska et al., 2016). Järvi and colleagues also reported significant reductions in body weight, alongside lower, albeit non-significant, fasting blood glucose after a diet of lower glycaemic index foods (Jarvi et al., 1999). This could suggest that changes in glycaemia may be driven by improvements in energy balance. Malin and colleagues reported increased first phase insulin secretion and lowered postprandial GLP-1 after a wholegrain diet compared with a refined grain diet (Malin et al., 2019). Weight loss was also seen in both intervention groups but greater fat loss of roughly 0.5 kg was reported after the wholegrain diet, this could partially explain the improved insulin sensitivity (Malin et al., 2019). Furthermore, increased insulin sensitivity may be attributed to the inflammation-lowering effects of components within plant cell wall structures. Both the low-GI, high-GI, wholegrain and refined grain diets followed dietary guidelines, potentially improving the participants' original diets, which may explain the lack of significant differences between the intervention and control groups (Jarvi et al., 1999; Malin et al., 2019). As these studies were conducted in free-living conditions, it's possible that other dietary changes could have influenced the results, as highlighted by Dodevska and colleagues, who observed a significant reduction in fat intake in the RS diet group (Dodevska et al., 2016). Furthermore, as RS 1 is delivered within food, it is more challenging to provide in quantities comparable to RS 2 which may contribute to the inconsistent impact on glycaemia (Dodevska et al., 2016).

RS 2 is shown to have the most substantial and consistent effect on glycaemia. As it is normally used as a supplement or to substitute readily digestible starch, it can be consumed in higher doses. In the included studies, RS 2 intakes ranged from 6 to 45g per day. RS is delivered to the colon where it is utilised as an energy source for bacteria. This prebiotic effect of RS may lower pathogenic bacteria populations and concentrations of proinflammatory molecules like lipolysaccharides (LPS) (Sandberg et al., 2018), potentially reducing chronic low-grade inflammation (K. L. Johnston et al., 2010; Karimi et al., 2016; Kwak et al., 2012). Some studies reported reductions in low-grade inflammation, indicated by lower concentrations of tumour necrosis factor-α (TNF-α) and interleukin-1ß (Bodinham et al., 2014; Peterson et al., 2018; Sandberg et al., 2018). Further, decreased markers of oxidative stress and elevations in oxidative enzymes were also reported (Eshghi et al., 2019; Kwak et al., 2012). Inflammation and oxidative stress are associated with insulin resistance, reinforced by the strong positive correlations between malondialdehyde and blood glucose concentrations (Kwak et al., 2012).

SCFAs are also produced via microbial fermentation and acetate, butyrate, and propionate may have several direct and indirect downstream effects on glucose metabolism. However, inconsistent SCFA responses were reported in the included studies (Bodinham et al., 2014; Sandberg et al., 2018). SCFAs can bind to enteroendocrine L-cells and promote the secretion of GLP-1 and PYY (as reported in **Chapter 1**). One study reported elevated postprandial GLP-1 but no change to fasting GLP-1 and another reported elevated PYY concentrations (Bodinham et al., 2014; Sandberg et al., 2018). Another study reported no effect on GLP-1 (Ble-Castillo et al., 2010). Several studies suggested SCFAs may improve first-phase insulin secretion but none managed to demonstrate this (Ble-Castillo et al., 2010; Bodinham et al., 2012, 2014; Karimi et al., 2016; Kwak et al., 2012). Enhanced first-phase insulin secretion would lower postprandial glucose response (Bodinham et al., 2014; Kwak et al., 2012; C.-H. Lin et al., 2015) and improve skeletal muscle glucose uptake (M. D. Robertson et al., 2012). One study suggested that RS may affect whole body insulin sensitivity by upregulating genes involved in lipid transport and metabolism (Gower et al., 2016).

The effect of chronic RS 4 on glycaemia was explored in one study (Nichenametla et al., 2014). This study reported RS 4 had no significant effect on glycaemia however, it did have a favourable effect on plasma lipid parameters. The study group argued part of the reason for the lack of significant results could be due to the high variability in the fasting glucose concentrations and because participant diets were not controlled (Nichenametla et al.,

2014). However, prior studies on healthy volunteers have shown that RS 4 more effectively lowered postprandial blood glucose (Haub et al., 2010) and distinctly altered colonic microbial populations when compared with RS 2 (Martínez et al., 2010).

Changes in body weight may have a confounded the effects of RS on glucose control. Some studies reported changes in body weight or composition (Ble-Castillo et al., 2010; Dodevska et al., 2016; Jarvi et al., 1999; Malin et al., 2019; Meng & Chen, 2015; Y. Zhang et al., 2007). Whereas others did not (Bodinham et al., 2012, 2014; K. L. Johnston et al., 2010; M. D. Robertson et al., 2012). Furthermore, when comparing a weight loss versus a high-RS diet, the former had a more favourable impact on glycaemic homeostasis (Lobley et al., 2013). However, it could be an oversimplification to attribute the impact of RS on insulin sensitivity solely to reductions in ectopic fat stores (K. L. Johnston et al., 2010).

A few of the studies compared the effects of a resistant starch intervention on a metabolically healthy cohort versus a cohort with impaired glucose control. A study with an hyperinsulinemic cohort resistant starch lowered postprandial glucose response to a greater extent than in the healthy cohort (Freeland & Wolever, 2010). Another study indicated that the insulin-resistant group had more favourable responses to higher RS intake than the insulin sensitive group (Gower et al., 2016). However, as noted by the study team, the insulin-resistant group were consuming less fibre at baseline (23-33%) (Gower et al., 2016).

The disparate results among studies were due to variations in RS intervention dose, type, duration, glycaemic indices used and level of glycaemic deterioration (Dainty et al., 2016; Karimi et al., 2016). Furthermore, there appears to be differing effects of supplemented RS versus RS within food upon metabolic response (C.-H. Lin et al., 2015; Lobley et al., 2013).

7.6.4 **Limitations**

There are several limitations to this study. Few studies investigated GLP-1, GIP, and insulin sensitivity, contributing to the non-significant results in the meta-analysis and highlighting the need to explore the role of incretins in glycaemic response after RS supplementation. High heterogeneity was identified in meta-analyses that investigated the effect of acute RS intake on postprandial glucose and insulin and chronic RS intake on fasting blood glucose and insulin response. The heterogeneity can partly be attributed to variability in dose, types and durations of RS intake. While this review shows that RS improves glycaemic response, it is debatable as to whether these reductions are clinically significant.

Additionally, many of the RCTs did not report digestible starch content. This made it difficult to conclude whether the effect of RS on glucose response is due to the substitution of RDS

for RS. Furthermore, RS 1 studies did not quantify resistant starch because there are currently no suitable analytical techniques for measuring RS 1, making it hard to compare with types 2-5.

As there were few studies which used RS types 3 to 5, it is difficult to distinguish whether the lack of significant effect was simply due to limited data or if there was no effect. Differences in metabolic effects of RS 1 may depend on the botantical source of RS (grains versus legumes). However, legume RS 1 interventions could not be included in this review as they did not meet the PICOS criteria. However, RS 1 source could differentially affect glycaemia in individuals with impaired glucose control and this is a potential avenue for further investigation. Study adherence was often unreported in chronic interventions, potentially increasing risk of bias (Domain 2.1). Additionally, side effects from significant resistant starch intake were only reported in one study. Gastrointestinal tolerance data are needed to assess the feasibility of RS as an intervention for improving glycaemic response.

Conclusions from this systematic review and meta-analysis should be interpreted cautiously. Significant heterogeneity exists in several meta-analyses and many included studies have a high risk of bias. Sensitivity analyses show that when studies with a high risk of bias were omitted, one outcome (acute RS 1 and postprandial insulin) was no longer significant. Publication bias was also detected in acute postprandial glucose, GIP, and HbA1c meta-analyses.

7.6.5 **Summary**

This systematic review and meta-analysis found that acute RS 1 and 2 lowered postprandial glucose and this effect was consistent in all study populations. Acute RS 2 intake lowered postprandial insulin. However, when sub-group analyses were performed, no significant effect was observed after separating participants with T2D and prediabetes. Chronic RS 2 intake lowered fasting glucose and insulin, although differences were observed between in participants with T2D and prediabetes. Fasting glucose was reduced in both groups but lowered fasting insulin was only found in participants with T2D. Chronic RS intake was found to have no effect on postprandial glucose or insulin, HOMA-IR, HOMA-%B, HOMA-%S or HbA1c. Further, no significant effects were seen on gut hormone concentrations. Meta-analyses could not be conducted for acute RS 4 and 5 and chronic RS 3 and 5 intakes as no studies matched the PICOS criteria. Interestingly, some studies reported more substantial effects on glycaemia in response to a resistant starch intervention in a group with impaired glycaemic control, compared with a healthy cohort. Therefore, further research exploring the role of RS on metabolic effects in individuals with T2D is warranted. The findings of one such study are explored in **Chapter 8**.

Chapter 8: The effect of an 8-hour intervention of high RS and intact food structures on glycaemic response in individuals with type 2 diabetes

8.1 Summary of key findings

- Feedback from four PPI panels steered study design, writing of study materials and intervention menus
- An intervention of three high-resistant starch meals (>8g) had no significant difference to glucose iAUC compared with low-resistant starch controls.
- An exploratory analysis did not identify any significant differences in characteristics of glycaemic control between those who exhibited a lower postprandial glycaemic iAUC to high-RS meals and those who did not.

8.2 Introduction

T2D is characterised by continuous elevations in blood glucose caused by insulin resistance or impaired insulin production. T2D is one of the most prevalent health concerns of our time and the ninth leading cause of death worldwide (World Health Organization, 2023a). The diagnostic criteria, pathogenesis and burden of T2D is outlined in **Chapter 6, Section 1.1.**

Modulating rises in glucose post-meal is a key aspect of T2D management. Postprandial hyperglycaemia is a major predictor of CVD risk which is one of the main causes of mortality for individuals with T2D (Cavalot et al., 2011). Treatment strategies for T2D include dietary changes, exercise and pharmacological interventions (described in **Chapter 6, Sections 1.3-1.5**). A recent umbrella review of dietary strategies for T2D remission indicated that weight loss interventions lowered HbA1c and contributed towards T2D remission (Szczerba et al., 2023). However, questions remain regarding patient adherence to more extreme dietary or exercise interventions and their efficacy as long-term therapeutic solutions. Additionally, pharmacological interventions for T2D are effective in lowering the risk of T2D-related illnesses and deaths but are not without side effects, most commonly including gastrointestinal effects or increased risk of hypoglycaemia (Shi et al., 2023). Considering these challenges, prioritising simple and effective lifestyle modifications to prevent prolonged hyperglycaemia is crucial.

Lowering the GI of the habitual diet is a potential strategy for minimising blood glucose fluctuations, GI is defined as the rise in blood glucose following food intake compared with a standard (glucose or white bread) containing equivalent quantities of carbohydrate (D. J. Jenkins et al., 1981). Definitions of GI and its effect on T2D management are described in **Chapter 6**, **Section 1.5**. The GI of a food can be influenced by the amount, type and proportion of carbohydrate, the food structure and meal composition.

RS is the portion of starch which remains largely undigested in the upper gastrointestinal tract and reaches the colon intact, thereby limiting available starch for conversion to glucose (Englyst & Cummings, 1985) and providing a substrate for fermentation by colonic bacteria (Wyatt & Horn, 1988). Substituting proportions of available starch for resistant starch may lower GI and promote euglycemia to complement other methods of T2D management.

Factors affecting RS content, such as the presence of plant cell walls, amylose to amylopectin ratio, food particle size and cooking or processing methods can influence the glycaemic index of the food and the subsequent metabolic response. Food structure and food processing can modulate concentrations of RS within food (Alsaffar, 2011). Processing

methods can alter the digestibility. Milling, for example, reduces the starch particle size and increases surface area accelerating hydrolysis by amylases (Mackie et al., 2017). Milling and other processing methods can also rupture plant cell walls, making starch more accessible to digestive enzymes and reducing RS 1 content (Tian et al., 2019). Cooking techniques such as boiling, increase starch gelatinisation which weakens the bonds within a starch molecule, facilitating starch hydrolysis and the release of glucose (Parada & Aguilera, 2012). However, if starch is cooled after cooking, retrogradation occurs whereby the starch forms a novel structure with stronger bonds, impinging upon enzyme hydrolysis and slowing starch breakdown (Haralampu, 2000). The effects of natural variations and processing on starch availability is discussed in further detail in **Chapter 6, Section 3.2 – 3.4**.

A meta-analysis (**Chapter 7**) indicated that acute and chronic intake of RS 1 and 2 lowered postprandial glucose responses in individuals with T2D and prediabetes. Further, RS 2 lowered acute postprandial and fasting glucose response and fasting insulin concentrations. In another meta-analysis, chronic RS supplementation reportedly improved insulin resistance and fasting insulin levels in participants with type 2 diabetes (C. Gao et al., 2019). However, the present meta-analysis, identified no effect on insulin sensitivity. The other systematic review included fewer studies, indicating the search criteria utilised may have differed. RS 2 appears to have the most pronounced effect on glycaemia. This may be driven large doses used in many of the studies as RS 2 can be easily isolated from natural sources and incorporated into widely consumed foods like bread, crackers and muffins. However, RS 2 products are also not available to the general public. A more feasible approach would involve identifying readily available food items rich in resistant starch that can be incorporated into the habitual diet.

The mechanisms by which RS influences glycaemic response and insulin sensitivity remain unclear. RS may lower postprandial glucose response by lowering the GL compared with foods containing greater available carbohydrates. However, in studies where interventions have been matched for available carbohydrates, RS has lowered postprandial glucose response (Behall & Scholfield, 2005; H. Larsen et al., 2000; Panlasigui & Thompson, 2006; Reynolds, Mann, et al., 2020). RS could impact fasting glycaemia and insulin sensitivity through mechanisms beyond lowering GL or GI. For example, via enhanced microbial fermentation and the production of SCFA, which may lead to enhanced secretion of GLP-1 and PYY (J. Zhou et al., 2008)and improved insulin sensitivity (Bindels et al., 2017; Nordgaard et al., 1995; van Munster et al., 1994).(Bindels et al., 2017; Nordgaard et al.,

1995; van Munster et al., 1994; J. Zhou et al., 2008). This is explored in greater detail in **Chapter 6, Section 3.3-3.4**.

Research exploring the effects of food processing and structure on glycaemic response in individuals with T2D remains limited. The therapeutic potential of manipulating food structures to enhance RS content for T2D management warrants further investigation. Further, foods with a high fibre or RS content are not widely consumed within the UK diet and despite public health messaging, 9% of individuals reaching target intakes of dietary fibre per day (Public Health England, 2020). Therefore, the PPI aspect of this study was important in providing feedback on foods that were acceptable to a diverse group of individuals with T2D. The aim of this study was to design an 8-hour long dietary intervention using available and acceptable foods to lower postprandial glycaemia in participants with T2D.

8.3 Purpose of the Study

8.3.1 **Hypothesis**

I hypothesised that common high-resistant starch foods would lower postprandial glucose response over an 8-hour study period measured through a continuous glucose monitor (CGM) and assessed via the difference in the incremental area under the curve.

8.3.2 **Aims**

The primary aim of this small-scale, randomised controlled study was to determine whether meals with intact food structures and RS, co-designed through PPI groups with individuals with T2D, could lower postprandial glucose responses compared with a low-RS diet.

8.3.3 **Outcomes**

The outcomes investigated in this randomised controlled study were:

- Postprandial glucose
- Postprandial insulin
- HOMA measurements (HOMA-%B, HOMA-IR and HOMA-%S)
- Subjective appetite
- Breath hydrogen
- Meal acceptability
- Gastrointestinal symptoms

The following outcomes were outlined in the protocol but were not measured due to time constraints and funding limitations:

- Postprandial GLP-1 and PYY
- Postprandial c-peptide and glucagon
- Postprandial free fatty acids
- Postprandial SCFA concentrations
- Urinary metabolomics
- 7-day food diaries

8.4 Methods

8.4.1 **Study design**

This study was a single-blind randomised controlled crossover study. The participants acted as their own controls.

8.4.2 **Ethical approval**

This study received ethical approval from the Stanmore Research Ethics Committee (REC) on 14th June 2021 (REC reference no: 21/PR/0563).

8.4.3 **Participants**

8.4.3.1 Inclusion criteria

Participants were required to have the following characteristics to be eligible for the study:

- BMI of 20-35 kg/m^2
- Age between 18-75 years (inclusive)
- Clinical diagnosis of T2D (see **Chapter 6, Section 6.1.1**)
- Stable oral diabetes medication three months prior

8.4.3.2 Exclusion criteria

Participants were excluded from the study if they met any of the following conditions:

- Weight change of >3 kg in last 3 months
- Use of Insulin treatment
- COVID-19 onset symptoms
- Positive COVID-19 test result within 14 days of study visit
- Self-isolation and quarantine period, according to the latest government guidelines.
- Cold or flu-like symptoms
- A recent change in taste and smell
- Inability to eat or chew certain foods including recent dental surgery
- HbA1c exceeding 69 mmol/mol (8.5%)
- Bowel reconstruction surgery
- Food allergies
- Current smokers
- Substance abuse
- Excess alcohol intake
- Pregnancy or breastfeeding
- Cardiovascular disease

- Cancer
- Kidney failure
- Abnormal electrocardiogram (ECG)
- Participation in another research study in the past 12 weeks
- Diagnosed gastrointestinal conditions
- Use of antibiotics in the past three months
- New medication in the past three months
- Any other reason in the opinion of the investigator

8.4.3.3 Acceptable levels of adherence

Participants who met the following criteria were withdrawn from the study:

- Meal consumption during the study of <85%
- Incomplete food diary or 24hr food recall shows dietary changes
- Overnight fasted capillary blood glucose upon arrival on study visits 3 and 4 ≥ 8.5 mmol/L
- Oral medications changes
- New medical diagnosis

8.4.4 **Study visits**

8.4.4.1 Patient and Public involvement panels

Individuals with T2D were invited to participate in virtual public involvement panels, recruited via the ICRF volunteer database. During each 90-minute session, participants were asked about their own challenges around food and managing their diabetes alongside questions pertaining to the study. There were three overarching aims of conducting the PPI panels. However, the panels were free flowing conversations with limited structure, so all three aims were not always expected to be achieved. The aims were to:

- Assess confidence of individuals with T2D in managing their blood glucose through their diet
- Gain feedback on the study methods and materials for accessibility and clarity
- Discuss high-RS foods to determine acceptability, compliance and guide menu design for the main study

Participants were asked to provide feedback on the study methodology, using the following discussion points:

- Were the visits too demanding?

- Do you think you would participate?
- What parts do you think participants might find challenging?
- What do you like or dislike about the study?

Participants were then taken through the current ideas for menus and asked to provide feedback or suggestions for improvements based on their own experiences. Discussion points were mostly guided by the panel conversation. The questions below were used to guide conversation when necessary:

- Why does this meal look appealing or unappealing to you?
- Do you feel this cooking method would be practical?
- Is this healthy swap acceptable and appropriate compared with [unhealthier option]?

8.4.4.2 Screening visit

Participants were sent the participant information sheet and asked to complete a pre-screening questionnaire to ensure they understood the study and met the eligibility criteria.

Participants were invited to the NIHR Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital for the screening and all other visits. They had the opportunity to ask any questions, the study was explained in detail and informed consent was obtained. Measurements such as body weight (kg) and height (m) were recorded to calculate BMI. Body composition (fat mass, water weight, lean body mass) and waist/hip circumference were also measured. Blood samples were taken to assess full blood count and HbA1c. An electrocardiogram (ECG) was conducted and blood pressure was monitored. All women of child-bearing age were asked to provide a urine sample for a pregnancy test. The participants were asked about their medical history, current medications and physical activity (using the international physical activity questionnaire (IPAQ). Lastly, participants were interviewed about any eating difficulties, food dislikes or allergies before they were given the study menu options.

8.4.4.3 Continuous glucose monitor implementation

Approximately 24 hours before their first study visit, participants visited the CRF to have a Dexcom CGM implemented to allow for a 24-hour calibration period. The CGM was intended to function for both study visits, if both were within the 10 days of CGM function. Participants were given the choice to have the CGM implemented by a study team member at the facility or have the CGM posted to them with remote guidance for self-implementation. The latter aimed to reduce participant burden by minimising travel and COVID-19 infection risk. The CGM was removed after the second visit. The CGM was blinded so participants did not manipulate their diet according to changes in their blood glucose. Following study

completion, participants could request access to their blood glucose results. A second CGM was implemented if a participant had to reschedule a visit or had to attend their visits more than 10 days apart.

8.4.4.4 Randomisation

To limit order effects, the order of study treatments was randomly assigned. Participants were allocated Treatment A (intervention meals) or Treatment B (control meals) using the Randomisation software Sealed Envelope (Open-source software, www.sealedenvelope.com).

8.4.4.5 Study visits

For seven days before the study visit participants were asked to keep a diet diary to assess any changes in diet over the study period. The day before the study visit, participants were reminded to avoid caffeine, alcohol, diet changes, and intense exercise for 24 hours. Participants fasted for 12 hours before attending the CRF for their visit the following morning. During this period, they were allowed to drink water.

As CGM receivers were blinded, capillary blood glucose using a finger-prick glucose test was conducted at the beginning of the study day to ensure blood glucose was not above 8.5 mmol/l before the study had begun. If blood glucose is above 8.5 mmol/l, participants were asked to return home and come for their visit another day.

Once blood glucose was confirmed to be within range, participants were asked to void their bladder so urine collection could commence. For women of child-bearing age, a urine sample was requested for a pregnancy test. Then the participant's weight, body composition, BMI, waist to hip ratio and blood pressure was measured and a cannula was placed in the antecubital forearm vein so blood sample collection could begin. Between the first and second study visit, there was a minimum wash-out period of seven days. At the end of the second visit, the CGM was removed.

8.4.5 Study interventions and measurements

8.4.5.1 Interventions

Participants received two controlled diets, one at each study visit. For clarity, the high-RS, intact food structures diet will be referred to as **Treatment A** and the low-RS, processed food structure diet will be referred to as **Treatment B**. Participants selected one breakfast from the **two** breakfast options and a lunch and dinner option from the **five** lunch and dinner options. The meal options were informed by the preferences of PPI panel of individuals with T2D and commonly consumed UK foods.

The meal options for Treatment A and B were designed to be as similar as possible and nutritionally comparable, with all meals differing by no more than 10% in their nutritional content. However, the RS content over the course of the day for Treatment A was approximately ≥ 24 g (8g per meal) and the processing and cooking methods of the meals varied dependent on the Treatment. The food preparation SOP for all meals can be found in **Appendix 3**.

Resistant starch content was calculated from several published RS databases (Brighenti et al., 1998; Chen et al., 2010; Moongngarm, 2013; M. M. Murphy et al., 2008; Patterson et al., 2020). Glycaemic index of individual meal ingredients were mostly taken from the University of Sydney glycaemic index database (University of Sydney, n.d.). If GI values were not found on this database, some other publications were used to find this information (Goletzke et al., 2016; Sagili et al., 2022). The GIs for blended raspberries and blackberries, crunchy peanut butter and blended red lentils were unavailable so the most similar ingredient was used (i.e. unsweetened fruit jams, smooth peanut butter and whole, cooked red lentils).

Glycaemic index of meal was calculated using the following equation validated by Dodd et al. (Dodd et al., 2011):

GI of meal = Sum of GI of each ingredient (GI per ingredient = CHO content per portion of ingredient (g) * GI of ingredient) / total CHO content (g)

The nutrient composition of the meals for males and females can be found in **Table 8.1** and **Table 8.2** respectively. Breakfast was served at time point (T) 0, lunch at T 180 min and dinner at T 360 min. For consistency, participants were asked to consume each meal within 15 minutes.

Table 8.1: Macronutrient composition of meals for men

Meal	Treatment	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	RS (g)	Fibre (g)	GI
Breakfast 1	A	726	93.2	26.9	26.0	9.7	12.0	44
Breakfast 1	B	759	97.0	27.5	25.3	0.3	10.9	48*
Breakfast 2	A	744	89.8	32.1	22.1	11.7	15.7	59
Breakfast 2	B	711	93.4	31.4	23.9	0.7	3.7	71**
Mean	**A**	**607.0**	**64.1**	**28.3**	**18.9**	**8.8**	**11.8**	**53**
Mean	**B**	**598.5**	**72.7**	**27.5**	**19.6**	**1.0**	**7.2**	**60**
Main meal 1	A	865	86.9	40.3	33.5	8.8	16.5	50
Main meal 1	B	880	87.0	40.4	33.7	1.9	2.9	69
Main meal 2	A	858	80.9	39.3	38.5	13.1	13.7	46
Main meal 2	B	828	76.4	38.9	36.9	4.7	11.7	72
Main meal 3	A	839	86.6	40.8	39.9	10.2	17.8	51
Main meal 3	B	834	81.8	40.6	39.9	6.7	8.9	77
Main meal 4	A	859	76.2	43.0	37.5	12.6	16.5	43
Main meal 4	B	879	77.5	38.3	37.7	6.1	11.1	56
Main meal 5	A	878	91.5	36.8	37	12	16.5	45
Main meal 5	B	869	86.3	38.7	34.5	5.1	14	60
Mean	**A**	**859.9**	**84.4**	**40.0**	**37.3**	**11.3**	**16.2**	**47**
Mean	**B**	**858.0**	**81.8**	**39.4**	**36.5**	**4.9**	**9.7**	**67**

Abbreviations: CHO, carbohydrates; GI, glycaemic index; RS, Resistant starch

*Unsweetened jam used for blended fruit.

**Smooth peanut butter GI value used for crunchy peanut butter.

Table 8.2: Macronutrient composition of meals for women

Meal	Treatment	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)	RS (g)	Fibre (g)	GI
Breakfast 1	A	607	62.5	27.4	19.2	8.1	10.0	43
Breakfast 1	B	612	74.2	25.4	18.8	0.2	8.4	48*
Breakfast 2	A	607	65.7	29.1	18.5	9.4	13.5	50
Breakfast 2	B	585	71.2	29.6	20.3	1.8	5.9	80**
Mean	**A**	**607**	**64.2**	**28.3**	**18.9**	**8.8**	**11.8**	**47**
Mean	**B**	**599**	**72.2**	**27.5**	**39.1**	**1.0**	**7.2**	**65**
Main meal 1	A	705	66.9	35.3	27.5	8	19	41
Main meal 1	B	728	73.8	32.8	27	2.1	6.5	71
Main meal 2	A	695	62.9	32.1	32	10.2	12.1	48
Main meal 2	B	683	65.8	31.7	29.2	3.6	9.1	67
Main meal 3	A	685	74.4	35.1	30.6	8.7	13.5	51
Main meal 3	B	695	71	33.3	31.8	6.5	8.3	76
Main meal 4	A	709	68.8	35.1	30.4	12	14.9	39
Main meal 4	B	685	69.8	30.1	27.4	6.7	10.8	53
Main meal 5	A	708	66.9	31.5	31.2	8.4	13.4	42
Main meal 5	B	692	69.2	30.1	28.1	4.2	11.9	61
Mean	**A**	**700.4**	**68.0**	**33.8**	**30.3**	**9.5**	**14.6**	**44.2**
Mean	**B**	**696.6**	**69.9**	**31.6**	**28.7**	**4.6**	**9.3**	**65.6**

Abbreviations: CHO, carbohydrates; GI, glycaemic index; RS, Resistant starch

*Unsweetened jam used for blended fruit.

**Smooth peanut butter GI value used for crunchy peanut butter.

8.4.5.2 Continuous Glucose Monitor

The Dexcom CGM measured the participant's blood glucose every 5 minutes throughout the study visit. The data was stored within the Dexcom receiver and pseudonymised data was uploaded onto the Dexcom Clarity Database.

8.4.5.3 Blood sample collection

Blood samples were taken for glucose, insulin, c-peptide, glucagon, GLP-1, FFA and SCFAs at the following time points: T 0, 30, 60, 120, 180, 210, 240, 270, 300, 360, 390, 420, 450, 480 minutes.

8.4.5.4 Visual Analogue Scales for Appetite

Subjective appetite measurements were taken using a standardised 100 mm VAS to assess subjective hunger, fullness, desire to eat, appetite for a meal, snack, something sweet or something savoury, thirst, anxiety, nausea, sleepiness and stress. These 12 measurements were taken at time points 0, 30, 60, 120, 180, 210, 240, 300, 360, and 420 minutes. Participants were asked to draw a vertical line on the scale at the place corresponding to their current state. The scale ranged from 0 = not at all to 100 = extremely. The placement of the lines was measured post-study visit so composite appetite scores could be calculated.

8.4.5.5 Breath sampling

Breath hydrogen measurements using a Gastrolyser (Bedfont Scientific Ltd., Maidstone, UK) were also taken at -30, 60, 120, 180, 240, 300, 360 and 420 minutes.

8.4.5.6 Urine Sample Collection

After voiding their bladders at the baseline timepoint, participants collected their urine throughout the study day in a single 24-hour urine collection container. Total urine volume was recorded. The sample was thoroughly mixed before aliquoting 500 uL samples for storage in freezers at -80ºC for metabolomic analysis upon study completion.

8.4.5.7 Gastrointestinal symptoms

Gastrointestinal symptoms were measured using a standardised 100 mm VAS to assess stomach discomfort, nausea, bloating, flatulence, heartburn, belching and frequency of toilet use. Four measurements were taken at T 0, 150, 330 and 480 minutes. Participants were asked to draw a vertical line on the scale at the place corresponding to their current state. The scale ranged from 0 = not at all to 100 = extremely. The placement of the lines was measured post-study visit so composite appetite scores could be calculated.

8.4.5.8 Meal acceptability

Immediately after participants finished their meals, they were asked to complete questionnaires to assess meal acceptability based on the following criteria: appearance,

smell, flavour, texture and overall appeal. Participants were asked to score the meal from 1 = dislike extremely to 9 = like extremely. They were also given the opportunity to provide further feedback through the open-ended question “did you have any additional comments about the meal?”

8.4.5.9 Compliance measures

Participants were asked to complete a food diary for seven days prior to the first study visit and then again between the first and second study visit, to ensure there were no changes to the diet during the study period. Baseline, visit 1 and visit 2 physical activity levels were compared to ensure no major changes occurred during the study visit. Weight and body composition measurements were conducted to ensure that in the unlikely event that there were major changes in body composition, they could be accounted for.

Figure 8.1: **Study flow chart for this study**

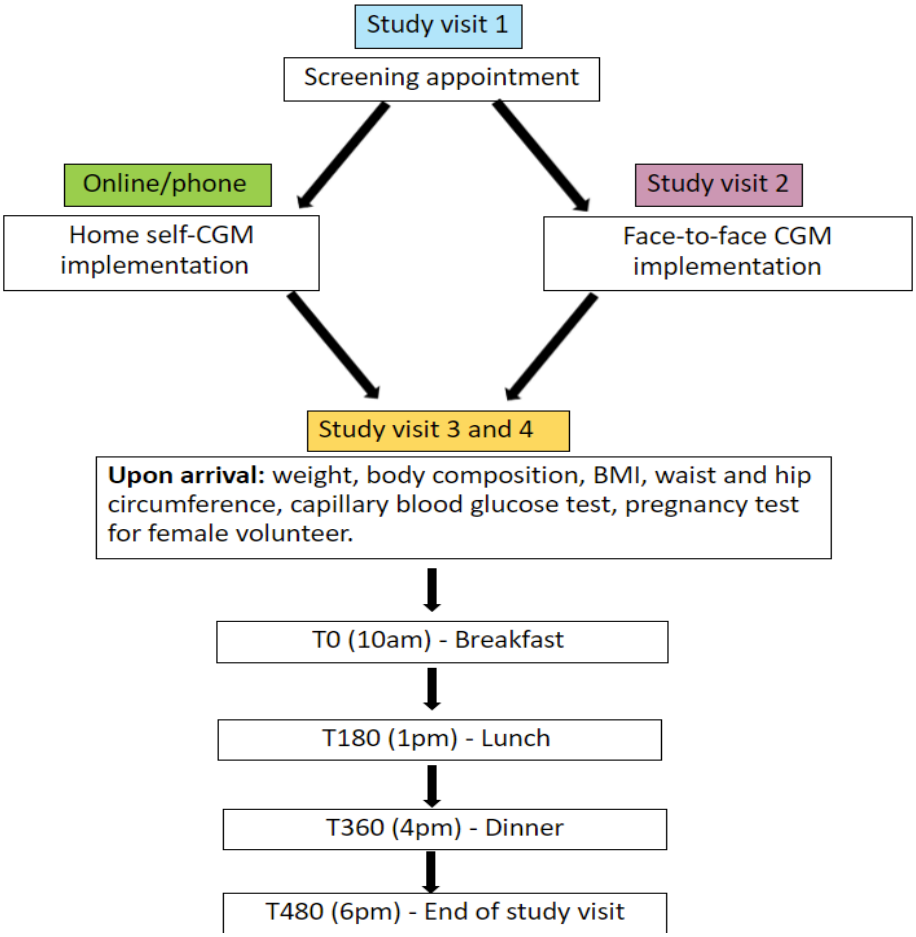
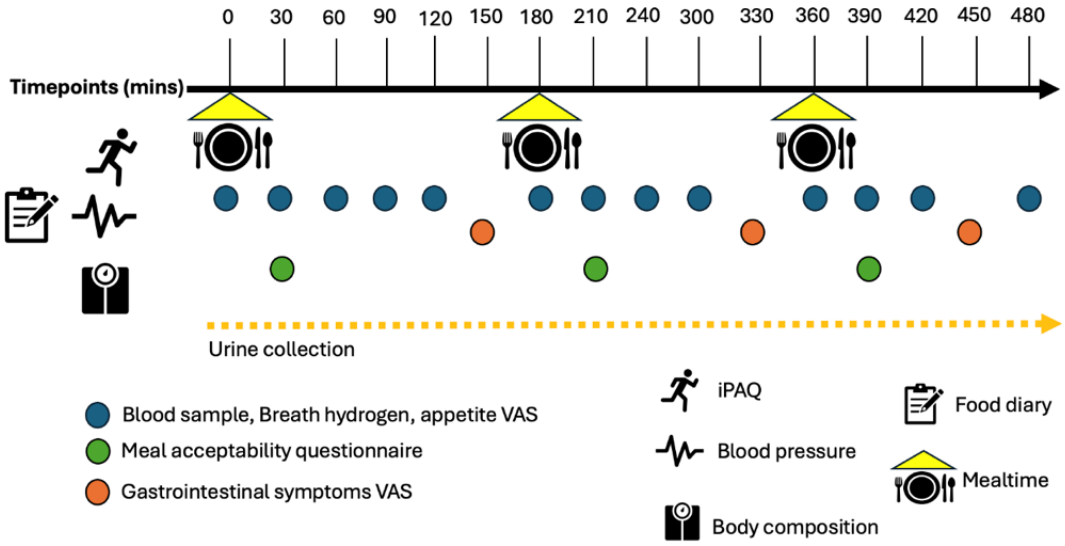


Figure 8.2: **Study visit schedule for the type 2 diabetes food study**



8.4.6 **Sample Processing and Analysis**

8.4.6.1 Glucose analysis

The interstitial blood glucose data were uploaded to the Dexcom clarity software where it was converted into csv files. Blood glucose measurements for baseline (fasting) and every 30 minutes after were extracted.

8.4.6.2 Insulin Analysis

Insulin was measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) (DY8056-05, Biotechne, USA). Assay was conducted in line with the manufacturers' instructions.

8.4.6.3 Subjective appetite

Subjective appetite for hunger, fullness, desire to eat and feeling sick, were measured from the left end of the line using a 100 mm ruler. This value was used as their score for that timepoint.

8.4.6.4 Gastrointestinal effects analysis

Stomach discomfort, nausea, bloating, flatulence, heartburn, belching and frequency of toilet use were measured from the left end of the line using a 100 mm ruler. This value was used as their score for that timepoint. As increased instance of any of the gastrointestinal effects reported would be considered a negative effect and because of individual variability in gastrointestinal effects scores at baseline, this score was calculated as a change from baseline.

8.4.6.5 Meal acceptability

The sum of scores for meal acceptability were totalled to provide an overall score for each meal for Treatment A and Treatment B. Qualitative responses from the open question were sorted to identify any common themes.

8.4.7 **Safety reporting**

8.4.7.1 Adverse events (AEs) and severe adverse events (SAEs)

Definition

Adverse events: An AE is any untoward medical occurrence in a clinical trial subject. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), or symptom, whether it is considered related to the trial protocol or intervention.

Severe adverse events: Any untoward medical occurrence that requires hospitalisation, results in a disability or incapacity, or is life-threatening, a congenital abnormality or medically important or an adverse event that results in death.

8.4.7.2 Side effects

Expected side effects of increased fibre intake are increased gastrointestinal events such as bloating, cramping and gas. Any unexpected side effects were documented as adverse events.

8.4.8 **Statistical analysis**

Statistical analyses were conducted in JASP (JASP Team (2024). JASP (Version 0.19.0) [Computer software]). Graphs were prepared using GraphPad Prism (version 10.3, for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). Statistical significance was determined at $P \leq 0.05$.

8.4.8.1 Area under the Curve Analysis

For this study, iAUCs were deemed the most appropriate measure of postprandial glycaemia as they offer more precise GI values than other AUC measurements (Wolever, 2004). . The iAUC was used for all outcomes unless indicated otherwise, for clinical relevance and compatibility with previous research. Therefore, iAUC and time course data were used to summarise responses at each visit. Paired t-tests or Wilcoxon's signed rank tests were used to determine differences between interventions depending on the normality of the data. Normality was assessed using a Shapiro-Wilk test.

8.4.8.2 HOMA Analysis

HOMA-%B, HOMA-IR and HOMA-%S were calculated using the Oxford University HOMA2 assessment calculator (Version 2.2.3, for Windows, HOMA calculator, Oxford University, Oxford, UK, https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-units/DTU/software/homa)

8.4.8.3 Exploratory analyses

In a post-hoc exploratory analysis, participants were separated into non-responders and responders. Responders were participants whose postprandial iAUC lowered after intake of treatment A and non-responders were those who had higher postprandial iAUC after Treatment A meals. To identify any key differences between β-cell function, insulin resistance, BMI or glycaemic deterioration between the two groups, the mean HOMA-%B, HOMA-IR, HOMA-%S, BMI, HbA1c and metformin dose were compared between non-responders and responders using Student's t-tests.

8.4.9 **Sample Size Calculation**

The primary outcome of this study was change in blood glucose iAUC throughout the 8-hour study period. Similar randomised, controlled acute crossover studies on the impact of a menu of intact food structures or high RS foods to improve glucose control were investigated for significant results.

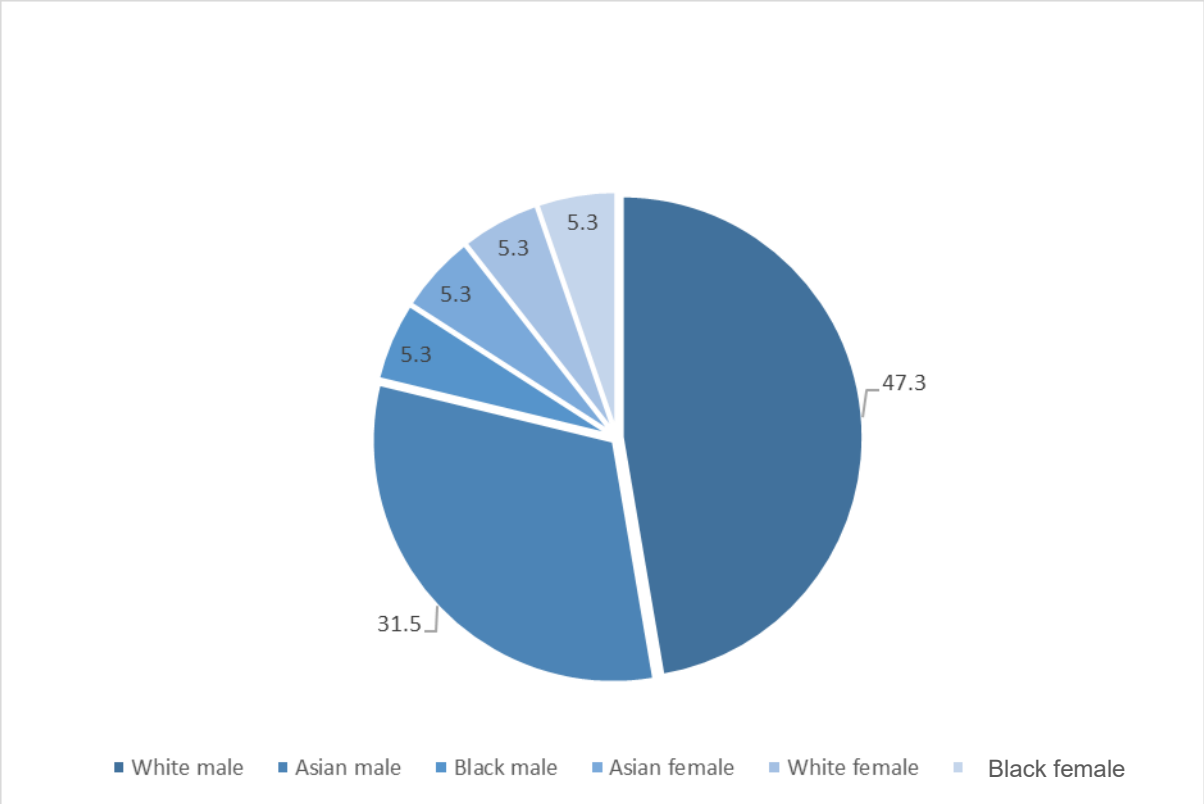
A similar study by Järvi and colleagues detected a -35% ($p < 0.05$) difference in glucose AUC between a low-GI and high-GI meal in individuals with T2D with reported mean (SD) of 95 ± 51 and 146 ± 48, respectively (Järvi et al., 1995). On this basis, we set a 90% power to detect a significant reduction in the AUC of 35% (effect size = 1.03), calculated from the AUC results above, using a correlation of 0.5. A sample size calculation using G*power software indicated that 13 participants are required. We aimed to recruit 16 participants in total to allow for a 25% dropout rate.

8.5 Results

8.5.1 **Insights from the patient and public involvement panels**

In January and February 2021, 20 individuals with T2D (mean length of diagnosis; 12.7 ± 9.7 years) participated in a 90-minute panel discussion about their understanding of food structure, RS and blood glucose management. Participants were recruited from Diabetes UK noticeboards, Facebook groups, local diabetes support groups and previous participant databases within the CRF. This PPI panel had a broad demographic with men and women from white, black and South Asian backgrounds (**Figure 8.3**).

Figure 8.3: **Ethnicity of patient and public involvement panel participants**



Participants were asked about their experiences managing T2D through dietary modifications and their understanding of food structure, particularly in relation to RS. Most

participants demonstrated limited knowledge of food structure and the impact of food processing. A few participants were aware that whole grains are generally preferable to refined options, such as white bread, and one individual mentioned that whole grains might slow glucose release. Additionally, there was some understanding that cooking and cooling food could influence blood glucose control.

When discussing research methods and materials, participants generally understood that the primary objective of the study was to identify acceptable food substitutions to improve blood glucose management. The participant information sheet was largely considered appropriate; however, based on feedback from the PPI panels, adjustments were made to increase the number of images, simplify the scientific language, and reduce the volume of text where possible. Panel members found participant burden to be acceptable overall, though concerns were raised regarding the potential impact of the blood glucose monitor on daily life and the risk of injury when using it. To quell these concerns, an additional section was added to the participant information sheet, providing more detailed information about the continuous glucose monitor, including images to help participants better understand its function and appearance. Key findings from these discussions are summarised in **Table 8.3**.

Table 8.3: PPI answers to questions relating to study themes and study methodology

Discussion themes	PPI comments
What do you know about food structure?	• White carbs are not good • Breads that contain whole grains • Breaking down food before you eat it makes it more digestible • Foods that release sugar slowly are healthier
What effects do you think cooking methods have on blood sugar?	• Anything is better than fried • Cold and reheated is better than hot
What do you think about our research question?	• Guiding you to easy alternatives • The lay title makes it easy to understand
Did you understand the participant information sheet?	• Lengthy • Some language was complex • More clarity on who can participate • Location was not clear • Make the research question more obvious
How do you feel about the burden on participants	• Mostly acceptable • 8 hours is a long time to expect people to remain in the research facility
What were your thoughts on the images we used to illustrate the study procedures?	• Image of cannula needed • More images needed, people need to understand the procedures so they can make an informed decision

What do you consider when trying new things?	• Taste • Positive effects on blood glucose • General health benefit • Interest in experiencing new foods • Acceptable to other family members • Religious restrictions • Budget • Variety
Is changing your diet a big burden on you?	• Not if there was a chance would benefit health
What aspect you do not wish to compromise on?	• Taste
How do you feel about the continuous glucose monitor?	• Effect on day-to-day behaviour • Concerns about risk of injury

Table 8.4 presents a summary of the insights gathered from our PPI panels. PPI panelists demonstrated greater openness to dietary modifications than anticipated, which may be partially attributable to the extended duration many had been living with T2D. Some of the responses were unexpected, such as the willingness to incorporate foods like porridge, peanut butter, and avocado on toast into their diet. However, there was a reluctance to try vegetarian substitutes. Other responses were as anticipated, including the resistance to replacing crisps with whole nuts or chips with vegetable sticks. Additionally, the panel provided constructive suggestions for menu inclusions, expressing a preference for items such as berries as an alternative to bananas, salted or flavoured nuts, and unprocessed vegetarian options like legumes or tofu rather than vegetarian meat alternatives. These comments and preferences fed into the menu design for this study.

Table 8.4: PPI comments on specific foods that may improve glycaemic response

Food type or swap	PPI Comments
Porridge for generic cereal	• Widely accepted
Unripe banana for a ripe banana	• Did not understand why it was better • Berries preferred
Semi-skimmed milk	• Whole milk is believed to be better for blood sugar • Some have dairy alternatives, “higher in fibre.”
Peanut butter/avocado for jam	• More accepted than jam • “More filling.” • Sugar content of peanut butter
Added seeds	• 25% of PPI panel consume regularly

Nuts	• 50% accepted instead of crisps • salted or flavoured preferred • crisps: convenient and cheaper • "Sweet" snack desired
Retrograding method	• Not very convenient, has to be prepared in advance • Some had tried, one person did not think it lowered blood sugar
Regular bread for bread made from low GI flour	• Acceptable to all
Whole fruit for fruit juice	• Whole fruit "more filling." • Fruit more "slow-release carbs."
Potatoes combined with pulses	• Acceptable to all
Vegetarian option for meat	• "Tasteless" to meat-eaters • Concerns about additives and carbohydrate quantities • Vegetarians prefer unprocessed options, e.g., tofu • Easier swap for meat-eaters
Half meat swapped for vegetables/pulses in sauces	• Texture unappetising • Some did this already
Chips for other options	• Unacceptable: vegetable sticks Acceptable: Baked or fried sweet potato and potato salad

8.5.2 **Recruitment and Baseline Characteristics**

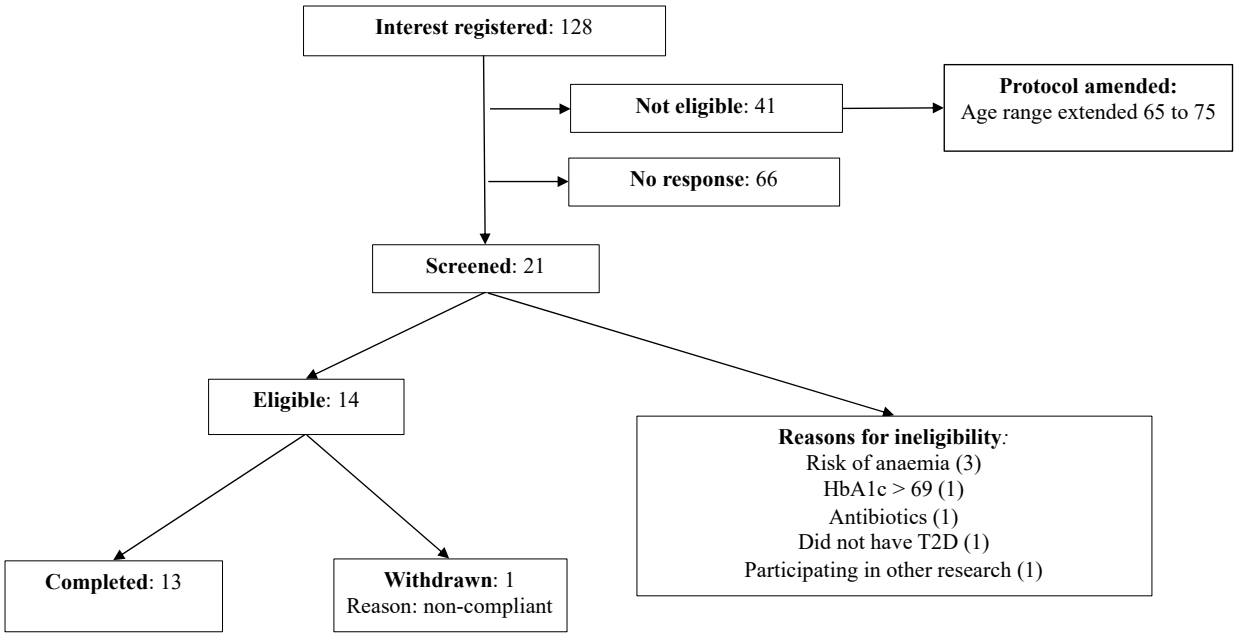
A total of 128 participants registered their interest in the study after being recruited through the following methods, listed in order of productivity:

- Volunteer databases from CRF studies
- Cardiac clinic at Hammersmith Hospital
- Study posters
- DESMOND UK (NHS training course for individuals with T2D)
- Imperial College Newspaper
- Diabetes UK newsletters
- Diabetes forums via Diabetes UK or Facebook

Participants for this study were recruited from January 2022 to March 2024, 14 participants were found to be eligible after screening 21 volunteers. A substantial amendment to the protocol was submitted to the REC in March 2022 to extend the age range from 65 to 75 years old. Participants were excluded due to risk of anaemia, high HbA1c, recent antibiotic

intake, no clinical T2D diagnosis and participation in another research study at time of screening. One participant was withdrawn from the study because they failed to comply with the protocol (< 85% meals consumed). A flow diagram of participant recruitment can be seen in **Figure 8.4**.

Figure 8.4: Recruitment flow diagram



Most participants were either of White British or South Asian background **(Table 8.5).** Doses of Metformin or other anti-diabetic pharmaceuticals prescribed to the participants are described in **Table 8.5**. Participants who were enrolled and completed the study had a mean (SD) age of 62.3 (6.12) years, HbA1c of 52.62 (5.98) mmol/mol and c-peptide concentration of 666.17 (220.40) pmol/L. There were no significant differences in weight, fat mass, body water, fat-free mass, BMI and fasting glucose between Treatment A and Treatment B visits (**Table 8.6**).

Table 8.5: Demographic characteristics of participants

	N (%)
Sex	
Male	8 (62)
Female	5 (38)
Ethnicity	
White	7 (54)

Black	0 (0)
South Asian	5 (38)
Any other ethnic group	1 (8)
Metformin Dose per day (mg)	
0	1 (8)
500	1 (8)
1000	4 (31)
1500	1 (8)
2000	6 (46)
Other anti-diabetic medication	
Meglitinides	1 (8)
DPP-4 inhibitors	2 (16)
SGLT2 inhibitors	2 (16)
Sulphonylureas	1 (8)

Table 8.6: Baseline characteristics by visit

	Screening		Treatment A		Treatment B		Cond A – Cond B* (p-value)
	Mean	**SD**	**Mean**	**SD**	**mean**	**SD**	
Age (years)	62.31	6.12	62.31	6.12	62.31	6.12	
Weight (kg)	76.34	18.69	75.73	18.40	75.72	18.20	0.02 (0.923)
Fat Mass (kg)	23.44	7.07	22.55	7.19	23.48	9.21	-0.92 (0.575)
Water Mass (kg)	37.25	9.25	36.58	8.48	36.90	8.92	-0.32 (0.345)
Fat Free Mass (kg)	54.70	13.73	53.18	13.22	52.24	13.12	0.94 (0.567)
BMI (kg/m^2)	27.66	4.08	27.27	4.27	27.37	4.16	-0.10 (0.459)
HbA1c* (mmol/mol)	52.62	5.98	52.62	5.98	52.62	5.98	
C-peptide* (pmol/L)	666.17	220.40	666.17	220.40	666.17	220.40	
HOMA-IR			1.89	1.66	1.89	1.66	
HOMA-%B			63.35	32.05	63.35	32.05	
HOMA-%S			92.08	67.37	92.08	67.37	
Fasting glucose (mmol/L)			7.19	1.02	7.25	0.81	-0.05 (0.977)
Fasting Insulin (pmol/L)			85.31	70.22	115.33	119.40	-30.02 (0.164)

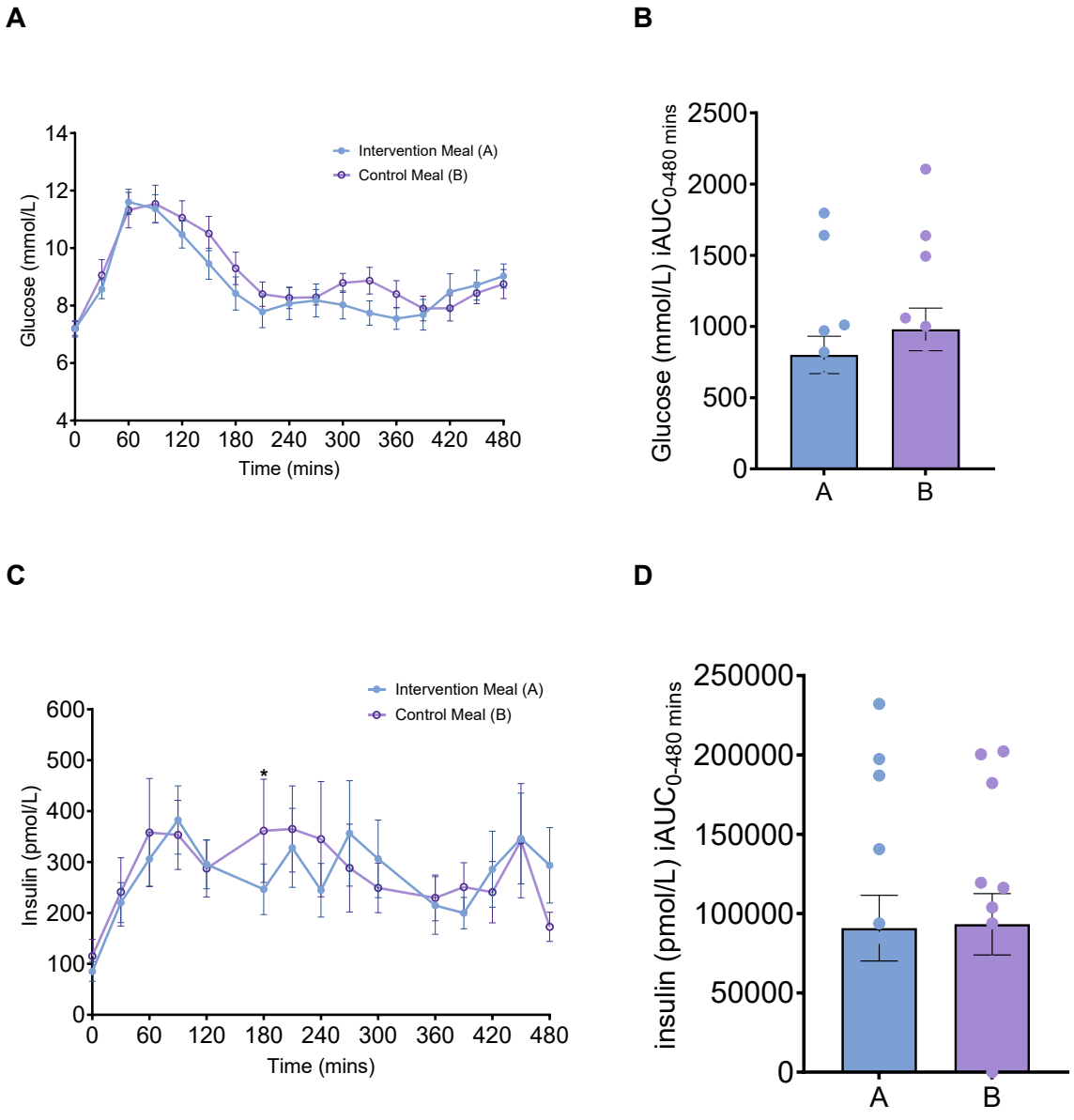
*measured at baseline only

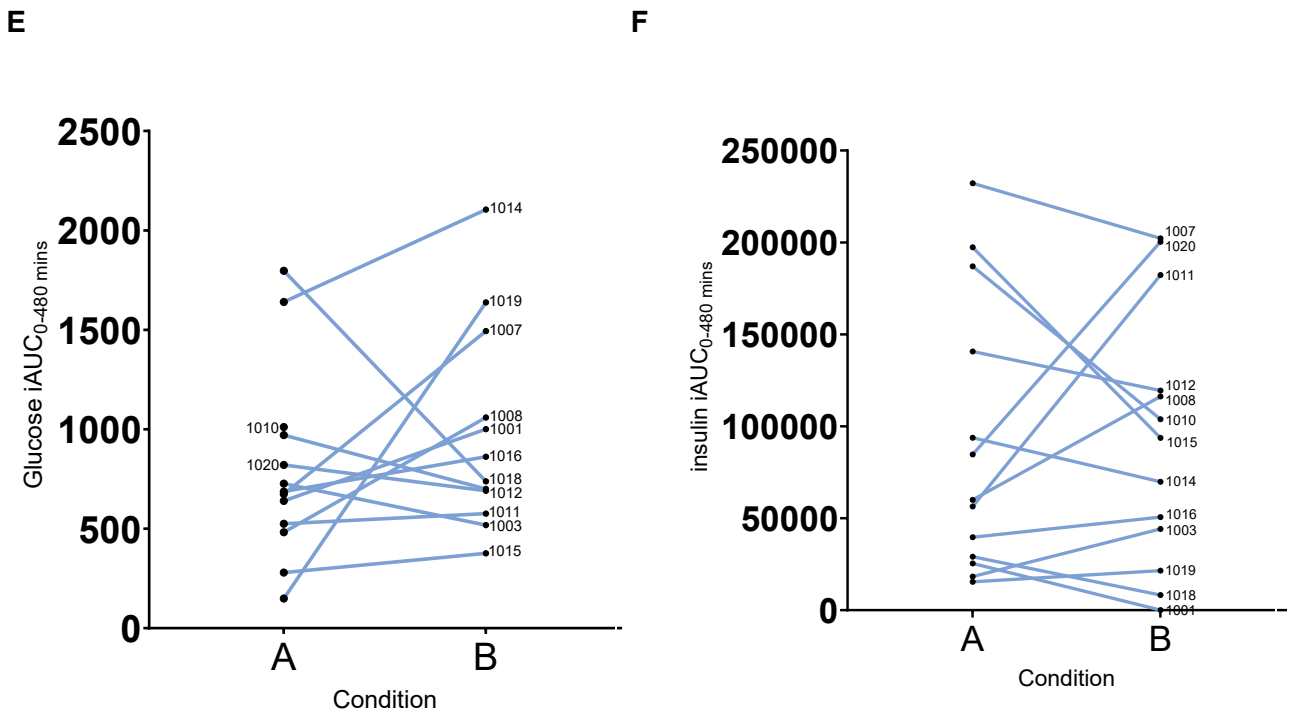
Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; Homeostasis Model Assessment β-cell function, HOMA-%B, Homeostasis Model Assessment insulin resistance, HOMA-IR; Homeostasis Model Assessment insulin sensitivity, HOMA-%S; SD, standard deviation

8.5.3 **Interstitial fluid glucose and serum insulin responses**

Figure 8.5 shows interstitial fluid glucose concentrations over the study visit day (0 to 480 mins). There were no significant differences in glucose concentrations at each timepoint or between $iAUC_{0\text{-}480\ mins}$ for Treatment A and B (**A & B**). For Treatment B, the continuous glucose monitors malfunctioned in two participants, reducing the sample size to 11 participants. There was a difference in insulin concentrations at timepoint 180 (p=0.024) (**C**). There were no significant differences in insulin $iAUC_{0\text{-}480\ mins}$ (**D**). **Figure 8.5 E** and **F** show individual glucose and insulin responses, respectively, with participant numbers annotated. These participant numbers can be cross-referenced to individual meal order and meal choices captured in **Table 8.7.**

Figure 8.5: Interstitial fluid glucose and serum insulin concentrations following the consumption of the intervention and control meals





(A) Postprandial interstitial glucose concentrations after intervention and control meals (n=13). (B) $iAUC_{0\text{-}480}$ glucose responses. (Treatment A; n=13, Treatment B; n=11). (C) Serum insulin postprandial concentrations after intervention and control meals (n=13). (D) $iAUC_{0\text{-}480}$ insulin responses (n=13). (E) Individual $iAUC_{0\text{-}480}$ glucose responses to Treatment A and Treatment B (n=11) (F) Individual $iAUC_{0\text{-}480}$ insulin responses to Treatment A and Treatment B (n=13). Data represent mean ± SEM. Paired t-tests or Wilcoxon signed-rank test used to detect differences in the postprandial data and iAUCs. * = $p<0.05$. Abbreviations: iAUC, incremental Area Under the Curve.

Table 8.7: Sequence of meals selected by participants separated into responders and non-responders

Participant No.	Responder?	Breakfast*	Lunch**	Dinner**
1001	Y	1	4	5
1007	Y	1	1	4
1008	Y	2	1	4
1010	Y	1	5	1
1011	Y	1	5	1
1014	Y	1	2	4
1015	Y	1	1	4
1016	Y	1	3	5

1019	Y	1	2	4
1003	N	1	3	4
1012	N	1	3	4
1018	N	2	4	1
1020	N	1	4	3

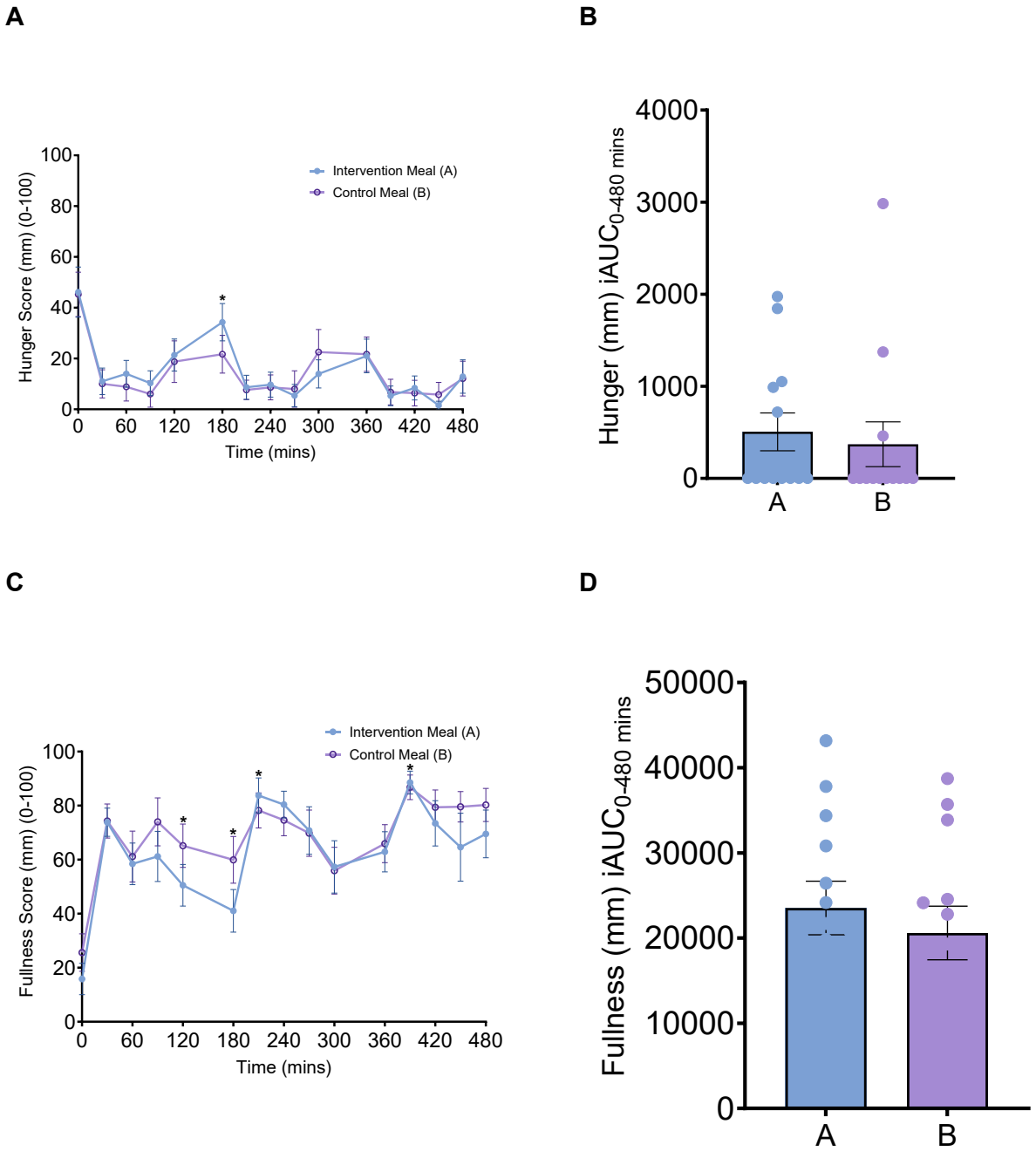
***1, porridge and berries; 2, toast with peanut butter and banana**

****1, fish and potatoes; 2, cheese sandwich; 3, beans on toast; 4, gnocchi with lentil ragu; 5, sausage, mash, beans**

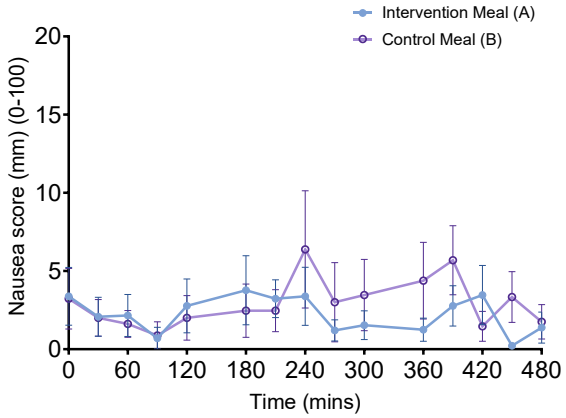
8.5.4 **Subjective appetite scores**

Figure 8.6 shows subjective appetite for hunger per study timepoint and iAUC. There was a significantly higher score for subjective hunger for Treatment A at timepoint 180 mins (p = 0.017) but no significant differences in hunger iAUC (**A & B**). Subjective fullness was significantly greater in Treatment B at timepoints 120 (p = 0.045), 180 (p = 0.034) and 240 (p = 0.017) mins (**C**). Subjective fullness was significantly greater in Treatment A at timepoint 390 mins (p = 0.007) (**C**). There was no significant difference in fullness iAUC (**D**). No significant differences in nausea were detected in the time course data or iAUC (**E & F**).

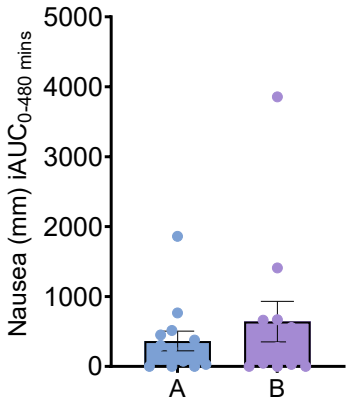
Figure 8.6: Subjective appetite scores following the consumption of the intervention and control meals



E



F

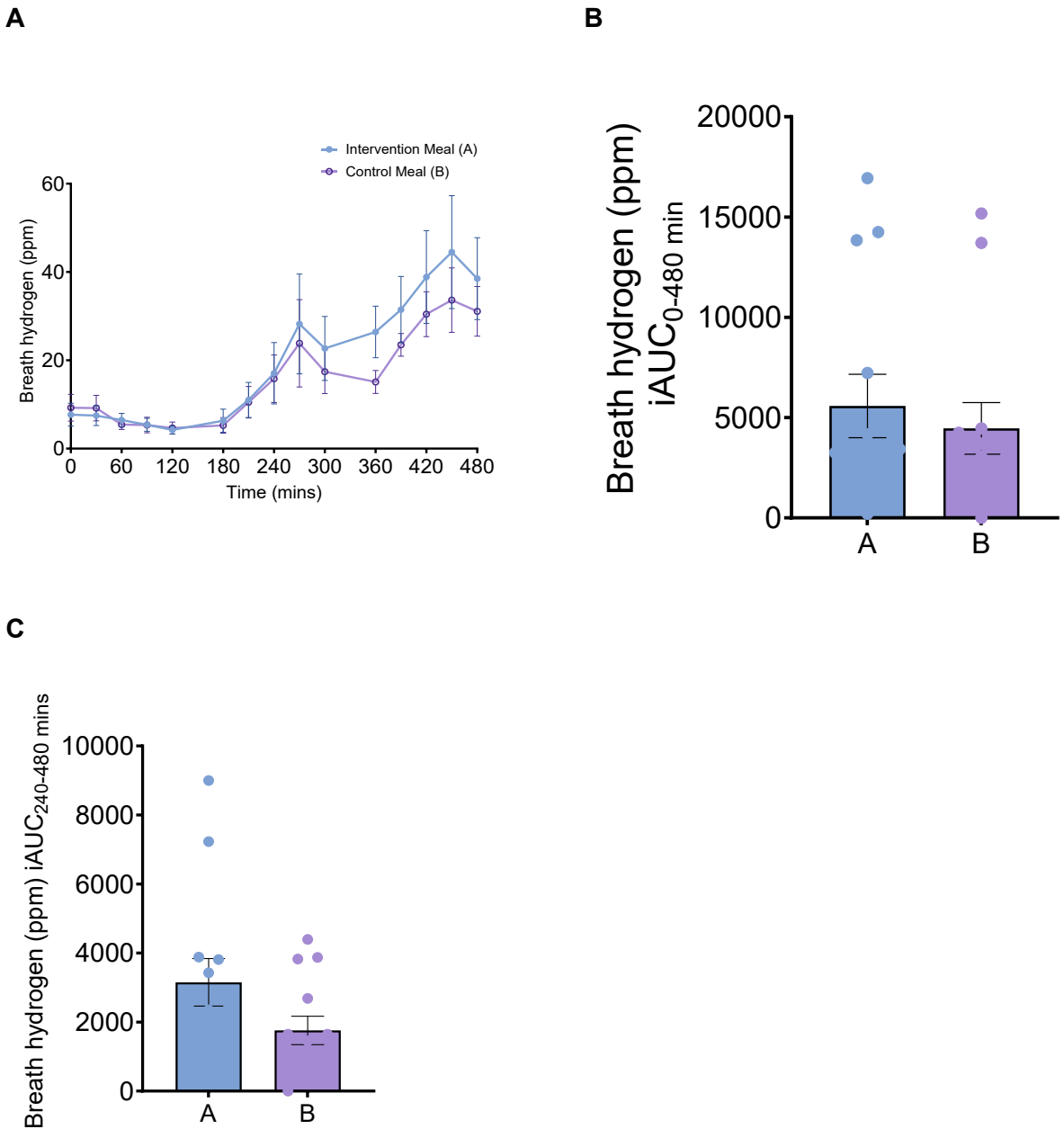


(A) Subjective hunger from timepoint 0 to 480 minutes between Treatment A and B. (B) $iAUC_{0\text{-}480}$ subjective hunger. (C) Subjective fullness from timepoint 0 to 480 minutes between Treatment A and B. (D) $iAUC_{0\text{-}480}$ subjective fullness. (E) Subjective nausea from timepoint 0 to 480 minutes between Treatment A and B. (F) $iAUC_{0\text{-}480}$ subjective nausea. Data represent mean ± SEM (n=13). Paired t-tests or Wilcoxon signed-rank test used to detect differences in the postprandial data and iAUCs. * = $p<0.05$. Abbreviations: iAUC, incremental Area Under the Curve.

8.5.5 **Breath Hydrogen**

Figure 8.7 shows Breath hydrogen concentrations (ppm) in Treatments A and B. There were no significant differences in breath hydrogen concentrations at any timepoint or in the iAUC (A & B). Further, no differences in breath hydrogen iAUC were detected between 240 and 480 minutes (C).

Figure 8.7: Breath hydrogen following the consumption of the intervention and control meals

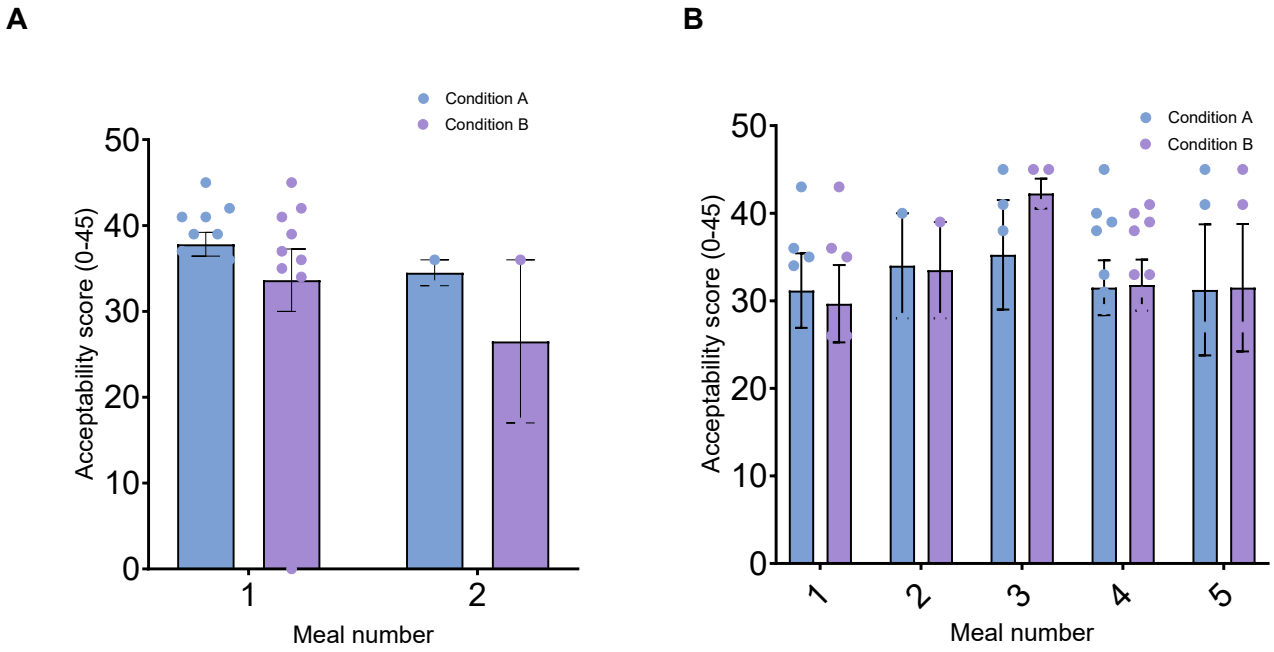


(A) Postprandial breath hydrogen (ppm) after intake of Treatment A and Treatment B meals. (B) $iAUC_{0\text{-}480}$ breath hydrogen responses. (C) $iAUC_{240\text{-}480}$ breath hydrogen responses. Data represent mean ± SEM (n=13). Paired t-tests or Wilcoxon signed-rank test used to detect differences in the postprandial data and iAUCs. * = $p<0.05$. Abbreviations: iAUC, incremental Area Under the Curve.

8.5.6 **Meal acceptability**

Figure 8.8 shows meal acceptability for breakfast (A) and main meals (B) for Treatments A and B. There were no significant differences in meal acceptability scores between Treatment A and B for any of the breakfasts or main meals.

Figure 8.8: Meal acceptability of Treatment Meals A and B



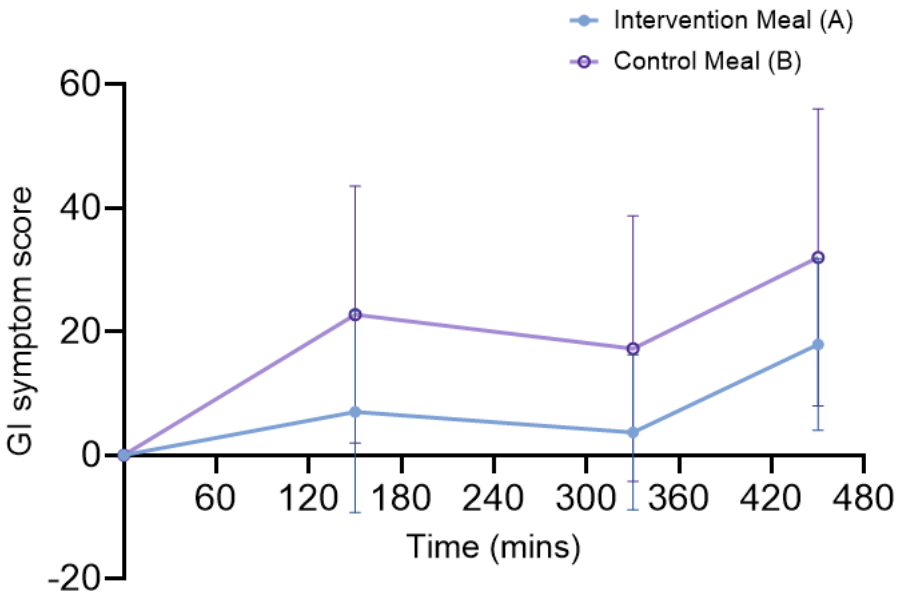
(A) Meal acceptability ratings for breakfast 1 (n=11) and breakfast 2 (n=2). (B) Meal acceptability ratings for meal 1 (n=6), meal 2 (n=2), meal 3 (n=4), meal 4 (n=10) and meal 5 (n=4). Data represent mean ± SEM (n=13). Paired t-tests or Wilcoxon signed-rank test used to detect differences in the postprandial data and iAUCs.

8.5.7

8.5.8 **Gastrointestinal effects**

Figure 8.9 shows the gastrointestinal effects of Treatments A and B as a change from baseline. There were no significant differences in gastrointestinal effects at any timepoint.

Figure 8.9: Gastrointestinal effects following the consumption of the intervention and control meals



Data represent mean ± SEM (n=13). Paired t-tests or Wilcoxon signed-rank test used to detect differences in the postprandial data. * = p<0.05. Abbreviations: GI, gastrointestinal.

8.5.9 **Exploratory Analyses**

Table 8.8 shows the mean (SD) in HOMA-%B, HOMA-%S, HOMA-IR, HbA1c, BMI and metformin dose between individuals who responded (n=7) and non-responders (n=4) to the Treatment A intervention. There were no significant differences in these characteristics of glycaemia between the two groups. Two participants could not be included in this analysis because the blood glucose measurements for Treatment B were incomplete.

Table 8.8: Student's t-tests to determine significant differences in glycaemic characteristics between responders and non-responders

Characteristic	Group	N	Mean	SD	t	p-value
HOMA-B	Non-responder	4	48.90	20.86	-1.15	0.28
	Responder	7	71.60	35.71		
HOMA-S	Non-responder	4	101.40	37.46	0.33	0.75
	Responder	7	86.76	82.29		
HOMA-IR	Non-responder	4	1.11	0.47	-1.21	0.26
	Responder	7	2.34	1.95		
HbA1c	Non-responder	4	52.00	3.92	0.33	0.75
	Responder	7	50.86	6.23		
BMI	Non-responder	4	26.00	2.73	-1.60	0.15
	Responder	7	29.89	4.35		
Metformin dose	Non-responder	4	1625.00	478.71	0.74	0.48
	Responder	7	1357.14	626.78		

8.6 PPI Discussion

The PPI panels offered valuable insight into dietary habits of individuals with T2D and their awareness of how food structure and RS could improve blood glucose control. The participants, who had lived with T2D for an average of 13 years, had a limited understanding of how starch types and food processing affect blood glucose response. Some recognised the benefits of whole grains and cooking and cooling starchy foods to enhance RS. However, further education and awareness-raising is required. The lack of knowledge means this is a potentially novel dietary approach for managing blood glucose control.

Feedback on study materials highlighted the need to simplify the language used and incorporate more images in the participant information sheet for accessibility. The importance of involving research stakeholders to provide feedback and help co-design study materials is increasingly recognised and was particularly beneficial to this study. PPI feedback indicated that a major concern was safety and comfort around CGM use, which was addressed by adding more detailed information about the device onto the participant information sheet.

Dietary preferences and acceptability of certain foods or ingredients from these panels shaped the study's menu design, with the aim of balancing acceptability with potential glycaemic benefits. These panels reinforced the need for practical, culturally sensitive dietary substitutions that align with personal preferences, to ensure long-term adherence to diets for improved glucose control.

8.7 Main Discussion

8.7.1 **Summarised Discussion**

Prolonged postprandial hyperglycaemia substantially contributes to the risk of comorbidity development in individuals with T2D (Blaak et al., 2012; Ceriello, 2005). The aim of this study was to understand whether RS intake (≥ 8g per meal) (Treatment A), in meals co-designed with PPI panels of individuals with T2D, would result in a lower postprandial glucose iAUC, compared with a diet of low RS foods (Treatment B). However, no disparities in glucose response were observed between the two treatments though estimated GI and RS content significantly differed. This is with the exception of the estimated GI of the porridge breakfast (breakfast 1). Previous studies using RS 1 and RS 3 have had mixed effects, with some reporting an attenuated postprandial response (Järvi et al., 1995; Mete et al., 2021) and others showing no significant impact (Hartvigsen et al., 2014; Larsen et al., 2000; Panlasigui & Thompson, 2006; Reynolds et al., 2020; Yamada et al., 2005) in individuals with T2D. Whilst these studies mostly explored the effects of one type of RS in isolation, the present study was the first to explore the effect of incorporating readily available high-RS foods into meals on postprandial glycaemia in individuals with T2D.

8.7.2 **Primary Outcome**

Postprandial blood glucose iAUC was not significantly lower in Treatment A compared with Treatment B. One reason for this may have been the high fibre content of Treatment B at an average of 26.2g over the three meals. This is almost double the current UK average intake (Gressier & Frost, 2022). Fibre content was matched to ensure that the nutrient content of meals in both treatments was similar, with differences kept within 10%. Any effect of RS may have been masked by the high fibre content as both soluble (Cassidy et al., 2018) and insoluble (Kabisch et al., 2019) dietary fibre are associated with lower postprandial glucose response compared with similar low fibre meals. Furthermore, a previous chronic study found no differences in glycaemic markers in individuals with T2D when comparing a high-RS and a high-fibre dietary intervention (Dodevska et al., 2016). Accuracy of fibre content calculations may have been reduced because they were estimated based on existing nutritional data for individual ingredients. Although certain processing methods that could not be accounted for may have reduced fibre content, this effect is likely negligible as domestic food processing has minimal impact on fibre content (Marlett & Cheung, 1997; J. L. Slavin & Lloyd, 2012)

To demonstrate how domestic and industrial processing can affect postprandial glycaemia, various methods such as blending, mashing and boiling were used to disrupt plant cell

structures. However, microscopy was not used to verify the presence of intact plant cells in Treatment A. As a result, it remains unclear whether the plant cell walls were preserved to protect the internal starch. If the cell walls were ruptured, releasing starch from these matrices, this could have contributed to a greater postprandial glycaemic response (Holland et al., 2020).

The highest glucose peak during the study period occurred after breakfast. The peaks were similar for both Treatments. Breakfast 1, consisting of porridge and berries, had a high estimated RS content. However, GI differences between Treatment A and Treatment B were insubstantial, potentially contributing to the lack of difference in glucose response. These observations are inconsistent with a recent meta-analysis of studies on normoglycemic individuals reported a significantly lower postprandial glucose response when whole oats were given, compared with a refined oat control (Musa-Veloso et al., 2021). In the present study, no difference in effect was seen. This could suggest that normoglycemic individuals have different glycaemic responses to oat processing than those with T2D. This cannot be confirmed as no studies have compared the effects of oat processing and glycaemia between healthy volunteers and individuals with impaired glucose control but is a potential avenue for further research.

In the current study, blood glucose concentrations peaked after breakfast. The “second-meal effect” refers to a phenomenon where the metabolic responses to one meal determine physiological responses to subsequent meals. For example, one study demonstrated that a meal with a low GI resulted in a reduced postprandial glucose response to subsequent meals compared to meals with a high GI (Wolever et al., 1988). There is a possibility that the high glucose response at breakfast contributed to the lack of effect seen throughout the rest of the day. Additionally, the RS consumed at breakfast did not lower postprandial glucose responses from the study lunch or dinner either. However, there is limited evidence for the second meal effect, particularly in individuals with impaired glucose control (Jovanovic et al., 2009). Although in one study, RS 2 induced a second meal effect via enhanced insulin secretion at the second meal in individuals with T2D (MacNeil et al., 2013).

There is also the possibility that viscosity played a role in attenuating the glycaemic response of the porridge breakfast, as finely milled oats may form a more viscous substance than whole, steel-cut oats. Viscosity plays an established role in slowing gastric emptying and may have contributed to the observed postprandial glucose response (D. J. Jenkins et al., 1978).

Processing can cause cell rupture, which releases nutrients and other compounds from within cell matrices (Xiong et al., 2022). Blending 'seeded fruit' such as raspberries and blueberries could release polyphenols from plant cells which may attenuate postprandial glucose response (Y. Kim et al., 2016). One study reported that blended fruit produced a significantly lower postprandial glucose response than whole fruit (Crummett & Grosso, 2022). However, *in vitro* digestion and microscopy of crushed fruit identified intact cell clusters in another study but intact cell preservation varied depending on the fruit (Chu et al., 2017). Therefore, there may still be RS 1 present in the blended fruit in Treatment B leading to a lower than estimated glycaemic response. Further research to determine which characteristics of food have the greatest effect on glycaemic response, such as fibre or RS content, viscosity or level of processing is warranted.

Evidence for the appearance of RS 3 during retrogradation of starchy foods such as potatoes and pasta is still relatively limited. In healthy individuals, acute RS 3 intake lowered postprandial glucose response in two studies (T. M. Robertson et al., 2021; Sonia et al., 2015). However, evidence to reinforce the theorised effects in individuals with T2D is even more limited, as indicated in a recent meta-analysis (Pugh et al., 2023). Rice was not used in meals in the present study as food safety concerns were mentioned multiple times during the PPI panels. Therefore, rice was not considered an acceptable intervention, so potatoes and gnocchi were used instead. Evidence from *in vitro* digestion demonstrates increased SDS and RS in cooked and cooled potatoes (Mishra et al., 2008). However, one study in healthy individuals indicated that retrograded potatoes did not significantly affect postprandial glycaemia, without the addition of vinegar (Leeman et al., 2005), which influences postprandial glucose responses in individuals with T2D (Cherta-Murillo et al., 2022). Although no effect was seen in one study in healthy individuals, there is no evidence exploring the effects of retrograded potato starch in individuals with impaired glycaemic control. No differences in postprandial glucose concentrations were detected between meals using retrograded or freshly cooked potatoes. This could suggest that RS 3 does not form as readily in potatoes as in other starchy foods like pasta or rice or induces a different metabolic response.

It is worth noting that participants were able to choose their lunch and dinner from five meal options, meaning no two participants had the same meals in the same order. This variability makes it challenging to compare responses between participants or identify whether one specific meal contributed to the lack of effect. This study design was selected to explore whether a portfolio of foods designed and chosen by the individuals with T2D could improve

blood glucose equally. This potentially further highlights that different foods and food structures have varying effects on glycaemic response even when the nutrient composition is matched.

8.7.3 **Secondary Outcomes**

There were significantly greater insulin concentrations at timepoint 180 in Treatment B compared with Treatment A. However, no significant difference in insulin iAUC between the two treatments was observed. The lack of significant difference in iAUC is expected and aligns with the current literature reporting no significant change to insulin secretion after acute intake of RS 1 and 3 in individuals with T2D (Järvi et al., 1995; H. Larsen et al., 2000; Mete et al., 2021; Tucker et al., 2014; Yamada et al., 2005). Interestingly, when insulin concentrations were lower in treatment A, hunger was significantly greater, potentially reinforcing the role of insulin in satiety signalling (Anika et al., 1980). However, higher postprandial insulin concentrations have been associated with lower satiety in another study, a response associated with rapid starch digestibility and compensatory hypoglycaemia (Holt & Miller, 1995).

Subjective appetite responses differed between Treatment A and B. Treatment A had significantly greater hunger at 180 minutes and significantly greater fullness was detected at 120, 180 and 240 minutes for Treatment B. The greater fullness could also be indicative of slower gastric emptying which could have contributed to the dampened glycaemic response seen. No significant differences were observed for nausea, indicating that appetite responses were not influenced by feelings of discomfort. Hunger, fullness and nausea iAUC were comparable between treatments. Future work should investigate whether anorectic gut hormone response was affected through the analysis of GLP-1 and PYY. However, in the current literature there is little evidence for RS affecting appetite in individuals with pre-diabetes (U. White et al., 2020). However, in a meta-analysis of studies conducted in healthy individuals, RS 2 enhanced satiety, whereas RS 1 had no effect (Amini et al., 2021).

Breath hydrogen concentrations, as a surrogate indicator of microbial fermentation, were elevated in both groups. Given that non-digestible carbohydrates take approximately four hours to reach the colon (Olesen et al., 1994), a separate analysis of breath hydrogen between timepoints 240 to 480 minutes was conducted to better assess any differential effects between the two treatment groups. No significant differences were determined in this separate analysis, potentially because resistant starch has a slower gastrointestinal transit time or a slower fermentation rate than other forms of fermentable carbohydrate, like

oligosaccharides (Olesen et al., 1994). Alternatively, the high total fibre intake in treatment groups may have masked any specific effects of resistant starch.

Meal acceptability and gastrointestinal tolerance were not significantly different between the two treatments, with no major gastrointestinal side effects reported. Developing meal options that aligned with nutrition recommendations, were high in RS and palatable to individuals with T2D proved challenging. Less processed foods or those that are high in RS, while beneficial, are often less appealing. This challenge reflects the broader difficulty of encouraging the general population to increase their fibre or lower processed food intake. Furthermore, a threshold of 8 g RS per meal was selected, based on recommendations for a minimum daily intake of 24g resistant starch to modulate postprandial glycaemic response (Penn-Marshall et al., 2010). However, previous studies, using single doses of 25g RS 2, have reported heterogenous effects. One reporting a reduction in postprandial glucose response and another observing no significant effect (Behall & Scholfield, 2005; MacNeil et al., 2013), potentially suggesting that the resistant starch dose in the present study was insufficient to generate an effect on glycaemia.

8.7.4 **Individual variability in response**

Assessment of individual responses to the meals in Treatments A and B revealed some variability in postprandial responses to the same intervention. Such variability is well-established in healthy individuals (Berry et al., 2020), reflecting inherent physiological and metabolic differences that determine postprandial response (Zeevi et al., 2015). While it is not feasible to fully control for these factors, participants were instructed to abstain from alcohol and physical activity the day before and fast for 12-hours before their study visit to minimise variability. Nevertheless, other factors, such as evening meal, metformin dosage and baseline blood glucose control may have influenced outcomes. This increased variability could have weakened the consistency and directionality of the result, reducing the likelihood of detecting a significant effect.

To identify differences in the characteristics of responders and non-responders to the high-RS meals, mean HOMA-IR, HOMA-%B, HOMA-%S and HbA1c, amongst other metrics, were compared between the two groups. However, no significant differences were identified between these characteristics in responders versus non-responders. This analysis was conducted as some evidence suggests that the glycaemic benefits of fermentable carbohydrates, like RS, occur via enhanced insulin secretion, a mechanism reliant on reasonable β-cell function (Bodinham et al., 2012; Malin et al., 2019). Therefore, fibre and

resistant starch may function via other mechanisms to improve glycaemia in individuals with poor β-cell function. An avenue for personalised nutrition for individuals with T2D could involve to tailoring dietary fibre recommendations based on HOMA indices to improve glucose control.

8.7.5 **Limitations**

This study is one of the few that investigates the effects of RS 1, found within intact plant cell walls and RS 3, retrograded starch, on postprandial glucose and insulin in individuals with T2D. However, this study has several limitations.

Firstly, the smaller sample size in treatment B may have the skewed results. The reduced sample size was due to issues with the CGMs on the day of the visit that were not reported to researchers beforehand. Limited available funding for the CGMs and the lack provision for a potential extra study visit for participants made it unethical to request that participants return for an additional visit. Further, plasma glucose samples were collected but have not yet been analysed due to time and budget constraints. These samples may elucidate whether any true effects were missed by relying on the CGM data. One major reason for CGM failure was that participants were allowed to implement CGMs at home with remote assistance of researchers over the phone. Unfortunately, in some cases, the lack of familiarly with CGM technology meant that CGMs were occasionally implemented incorrectly and failures were not detected. Future studies should have follow-up phone calls with participants in the evening before the study visit to avoid this if self-implementation of CGMs is an option.

Another study limitation was that the use of online databases to calculate resistant starch content and glycaemic index may have introduced some inaccuracies. Particularly in the few instances where ingredients were not listed on the databases and data for the closest available substitute was used. Given this, estimations of resistant starch content could be inaccurate and the suggested minimal effective dose of 8g may not have been reached. Advanced techniques, such as microscopy should have been used to confirm the presence and preservation of RS 1 in Treatment A meals. This is a potential route for further investigation to understand whether this factor may have influenced the results of the primary outcome.

Lastly, several important outcomes are currently unanalysed, such as plasma glucose, circulating SCFA and GLP-1 or PYY concentrations. These outcomes could provide further insight into the observed lack of effect.

Lastly, the high fibre content in Treatment B meals complicates the interpretation of the results. Fibre intake can improve glycaemic response making it difficult to discern whether the lack of significant differences in results seen were because resistant starch does not affect glycaemia or whether total fibre content playing a larger role in modulating glycaemic responses in individuals with T2D. This is a potential avenue for further investigation.

8.8 Summary

The aim of the PPI panels and study was to understand public knowledge of resistant starch and investigate the effect of RS on postprandial glucose and insulin responses in individuals with T2D. Although no significant differences were found in the primary outcome of glucose iAUC 0 – 480 minutes, this study may provide some insight into the difference in effect of resistant starch in metabolically healthy individuals versus those with T2D. The glycaemic responses, from the breakfast meal, could suggest that factors such as viscosity, phenolic compounds, food processing methods and dietary fibre content modulate postprandial glycaemia in individuals with T2D. Furthermore, evidence on the effects of resistant starch in healthy individuals may not be translatable to those with T2D or may depend on T2D phenotype. Future work should explore whether RS in larger doses or over longer periods have a benefit on participants with T2D.

Chapter 9: General Discussion

9.1.1 **Summary of findings**

- Findings from the meta-analysis indicate that RS lowered postprandial and fasting glucose and insulin responses in individuals with T2D and pre-diabetes. RS 1 and 2 were found to have the greatest impact on glycaemic response, as determined by sub-analyses. Significant heterogeneity was detected within these meta-analyses that may have been driven by differences in resistant starch sources and dose size. It is apparent from this review that the metabolic effects of RS on glycaemic control in subsections of the population, such as those with T2D, remains underexplored.

- PPI panels provided insights into the dietary habits of individuals with T2D and the challenges they experience in regulating glucose control. Participants in the PPI panels offered feedback on the study materials and suggested meals they deemed to be acceptable and appropriate. Their unique contribution highlighted the importance of ensuring the intervention was adaptable and practical to promote adherence.

- An intervention using RS 1 and 3, the most readily available forms of resistant starch within the current food environment, had no effect on postprandial glucose and insulin in individuals with T2D. However, amounts of resistant starch within the study meals were estimated. Accurate analysis of resistant starch content and a better understanding of how the meals moved through the GI tract could provide insight into the observed results.

9.2 Introduction

T2D is characterised by chronic hyperglycaemia, stemming from hepatic and whole-body insulin resistance and a degeneration of pancreatic β-cell mass and function. Global prevalence of T2D is projected to reach 578 million by 2030 and T2D is becoming particularly pervasive in low- and middle- income countries (Saeedi et al., 2019). Current strategies for T2D management include lifestyle changes (diet, exercise), weight loss and pharmacological interventions. More extreme or invasive interventions like bariatric surgery or VLCDs that cause weight loss to cause T2D remission are not suitable for all. Dietary interventions to improve glycaemia include the Mediterranean, low-carbohydrate and DASH diets. With a focus on increasing wholegrains, fruit and vegetables and lean protein intake, their common characteristics are that they are higher in fibre and lower in processed carbohydrates than the normal, modern Western diet. This lowers glycaemic index, which is associated with improved metabolic outcomes (D. J. A. Jenkins et al., 2024).

RS, a form of dietary carbohydrate that remains mostly undigested in the upper gastrointestinal tract, has been associated with improvements in glycaemic control in healthy humans and rodent models. However, its role in glucose homeostasis in individuals with dysregulated glucose control remains poorly understood and under-researched. As resistant starch can be sourced from or incorporated into many commonly consumed foods, it is an accessible intervention for improved glucose control in populations with T2D. When designing a diet for a specific condition, it is challenging to ensure it is acceptable and adaptable to those living with the condition. Highlighting the importance of co-designing interventions in collaboration with the target population.

The work presented in this part of the thesis evaluates the effects of different types of resistant starch on glycaemia in individuals with disturbed glucose regulation and tests a co-designed intervention of meals made with readily available, high-RS foods compared with a low-RS control.

9.3 Discussion

In **Chapter 7,** the systematic review and meta-analysis indicated that acute intake of resistant starch lowered postprandial glucose and insulin responses, with sub-group analyses indicating that resistant starch types 1 and 2 show the greatest effect. However, due to the smaller sample (n=3) of studies using other resistant starch types, there is the potential for type two error. Furthermore, the current available evidence is based on interventions using RS 2 (high-amylose flours) which can easily be incorporated into traditional flour-based products such as biscuits, cakes and crackers. However, these are not readily available and therefore are not a current, realistic population-level intervention. The study in **Chapter 8** was designed to incorporate accessible foods with preserved cell wall structures (RS 1) or that can undergo retrogradation (RS 3) into meals and explore their effects on glucose control in individuals with type 2 diabetes. To ensure these foods would be acceptable to the target population, PPI panels with individuals with T2D were conducted so they could provide their insights on the study protocol design and meals.

Although findings from **Chapter 7** indicated that RS 1 had a significant effect on postprandial glucose response, this was not observed in iAUC responses in **Chapter 8**. It is difficult to draw conclusions about any effect of RS in the present study because RS content was not directly measured. In some studies using RS 1, encapsulated starch presence has been demonstrated via microscopy on food that have undergone thermal treatment (Edwards et al., 2020) or in *in vivo* samples taken from the human gastrointestinal tract (Edwards et al., 2015; Petropoulou et al., 2020). As microscopy was not conducted on food samples in the present study, the presence of encapsulated starch in the intervention cannot be confirmed. As a result, no definitive conclusions about the effect of RS 1 on glycaemia can be drawn from this study. Furthermore, Concentrations of RS 3 were enhanced in cooked and cooled potato and pasta products (Mishra et al., 2008; Riva et al., 2000). However, this cannot be confirmed in the present study, as the resistant starch content was only estimated.

Acute RS intake may still affect glycaemic response, as seen in **Chapter 7,** possibly through energy dilution, where available carbohydrates are substituted for non-digestible carbohydrates, attenuating glycaemic response and potentially reducing body weight over time (Keenan et al., 2006). However, after available carbohydrate matching in several studies included in the systematic review and meta-analysis, a reduced glucose response was still preserved (Behall & Scholfield, 2005; H. Larsen et al., 2000; Panlasigui & Thompson, 2006; Reynolds, Mann, et al., 2020)(Behall & Scholfield, 2005; H. Larsen et al., 2000; Panlasigui & Thompson, 2006; Reynolds, Mann, et al., 2020). This suggests there

may be other mechanisms at play, like improved glucose uptake into peripheral tissues or enhanced insulin secretion, however, they are yet to be established.

Although no acute significant differences were detected in the study in **Chapter 8**, this does not detract from the potential of resistant starch to have a beneficial effect in the long term. Findings from the meta-analysis in **Chapter 7** indicate that chronic resistant starch intake lowered fasting glucose and insulin. Unfortunately, this was only explored RS 1 and 2 as no studies of RS types 3 to 5 fit the review criteria. There is the potential that the prebiotic effect of resistant starch, functioning to diversify the gut microbiota and enhance release SCFAs is what elicits positive effects on insulin sensitivity and glucose homeostasis (Bodinham et al., 2014; Sandberg et al., 2018). SCFA may increase secretion of GLP-1, potentially enhancing insulin secretion or preserving β-cell mass, as discussed in **Chapter 2**. However, as mentioned in section one, the evidence for SCFA affecting glycaemic response is limited (Cherta-Murillo et al., 2022). Chronic resistant starch intake is also associated with reduced markers of low grade inflammation and reductions in insulin resistance (Bodinham et al., 2014; K. L. Johnston et al., 2010; Karimi et al., 2016; Kwak et al., 2012; Peterson et al., 2018; Sandberg et al., 2018).

The lack of effect seen in the current study (**Chapter 8**) is likely attributable to limitations in the study design, which may have prevented detection of significant results, rather than indicating that RS is an ineffective intervention for glucose homeostasis. However, this cannot be definitively confirmed.

Understanding the role of resistant starch in glycaemic control is challenging as RS interventions vary in type used, dose, duration and glycaemic assessment techniques. This results in significant heterogeneity being detected when they are pooled for meta-analysis. RS types appear to have a heterogenous effect on metabolic responses and further studies should be conducted, particularly on RS 3 to 5, to better understand these differences.

9.4 Future work

Proposed future work should:

1. Continue to involve stakeholders in the co-design of future research
2. Use available technologies, like Infogest and microscopy, to understand how the meals from **Chapter 8** where digested and whether resistant starch was preserved within plant cells
3. Investigate the mechanism driving physiological responses to different resistant starch types

9.5 Conclusions

Part two of this thesis has several novel findings. The systematic review is the first to suggest differential effects of RS types on glycaemia in individuals with T2D or pre-diabetes. Furthermore, the review and meta-analysis highlights the limited number of studies investigating the metabolic effects of RS in individuals with impaired glucose homeostasis. In the study in **Chapter 8,** RS intervention meals were co-designed with individuals with T2D through PPI groups to ensure acceptability and compliance. While the importance of public involvement in study design is gaining recognition, this remains one of the few studies to incorporate PPI in this manner. Furthermore, previous studies tend to use RS as an isolated intervention whereas this study investigated the effects of combining RS 1 and 3 in meals to provide an accessible method of glycaemic control for individuals with T2D. In conclusion, although there were no significant effects on postprandial glucose iAUC in the study in **Chapter 8**, RS interventions may still modulate glycaemic responses (**Chapter 7**). The use of RS as a complementary tool for managing blood glucose alongside other T2D treatments shows promise and warrants further investigation.

References

Adam, C. L., Gratz, S. W., Peinado, D. I., Thomson, L. M., Garden, K. E., Williams, P. A., Richardson, A. J., & Ross, A. W. (2016). Effects of Dietary Fibre (Pectin) and/or Increased Protein (Casein or Pea) on Satiety, Body Weight, Adiposity and Caecal Fermentation in High Fat Diet-Induced Obese Rats. *PLOS ONE*, *11*(5), e0155871. https://doi.org/10.1371/journal.pone.0155871

Adam, C. L., Thomson, L. M., Williams, P. A., & Ross, A. W. (2015). Soluble Fermentable Dietary Fibre (Pectin) Decreases Caloric Intake, Adiposity and Lipidaemia in High-Fat Diet-Induced Obese Rats. *PLOS ONE*, *10*(10), e0140392. https://doi.org/10.1371/journal.pone.0140392

Adler, A. I., Stratton, I. M., Neil, H. A. W., Yudkin, J. S., Matthews, D. R., Cull, C. A., Wright, A. D., Turner, R. C., & Holman, R. R. (2000). Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): Prospective observational study. *BMJ*, *321*(7258), 412–419. https://doi.org/10.1136/bmj.321.7258.412

Adler, G. K., Hornik, E. S., Murray, G., Bhandari, S., Yadav, Y., Heydarpour, M., Basu, R., Garg, R., & Tirosh, A. (2021). Acute effects of the food preservative propionic acid on glucose metabolism in humans. *BMJ Open Diabetes Research & Care*, *9*(1), e002336. https://doi.org/10.1136/bmjdrc-2021-002336

Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology*, *89*(5), 1070–1077. https://doi.org/10.1016/0016-5085(85)90211-2

Akanji, A. O., & Hockaday, T. D. (1990). Acetate tolerance and the kinetics of acetate utilization in diabetic and nondiabetic subjects. *The American Journal of Clinical Nutrition*, *51*(1), 112–118. https://doi.org/10.1093/ajcn/51.1.112

Alamowitch, C., Boillot, J., Boussairi, A., Ruskone-Fourmestraux, A., Chevalier, A., Rizkalla, S. W., Guyon, F., Bornet, F. R., & Slama, G. (1996). Lack of effect of an acute ileal perfusion of short-chain fatty acids on glucose metabolism in healthy men. *American Journal of Physiology-Endocrinology and Metabolism*, *271*(1), E199–E204. https://doi.org/10.1152/ajpendo.1996.271.1.E199

Alcazar, O., & Buchwald, P. (2019). Concentration-Dependency and Time Profile of Insulin Secretion: Dynamic Perifusion Studies With Human and Murine Islets. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00680

Alexander, S., Fabbro, D., Kelly, E., Mathie, A., Peters, J., Veale, E., Armstrong, J., Faccenda, E., Harding, S., & Davies, J. (2023). *The Concise Guide to PHARMACOLOGY 2023/24: Transporters. 180*, Suppl 2:S374-469.

Alfa, M. J., Strang, D., Tappia, P. S., Graham, M., Van Domselaar, G., Forbes, J. D., Laminman, V., Olson, N., DeGagne, P., Bray, D., Murray, B.-L., Dufault, B., & Lix, L. M. (2018). A randomized trial to determine the impact of a digestion resistant starch composition on the gut microbiome in older and mid-age adults. *Clinical Nutrition (Edinburgh, Scotland)*, *37*(3), 797–807. https://doi.org/10.1016/j.clnu.2017.03.025

Alhabeeb, H., Chambers, E. S., Frost, G., Morrison, D. J., & Preston, T. (2014). Inulin propionate ester increases satiety and decreases appetite but does not affect gastric emptying in healthy humans. *Proceedings of the Nutrition Society*, *73*(OCE1). https://doi.org/10.1017/S0029665114000354

Ali, Z., Ma, H., Wali, A., Ayim, I., Rashid, M. T., & Younas, S. (2019). A double-blinded, randomized, placebo-controlled study evaluating the impact of dates vinegar consumption on blood biochemical and hematological parameters in patients with

type 2 diabetes. *Tropical Journal of Pharmaceutical Research*, *17*(12), 2463. https://doi.org/10.4314/tjpr.v17i12.23

Al-Lahham, S. H., Peppelenbosch, M. P., Roelofsen, H., Vonk, R. J., & Venema, K. (2010). Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. *Biochimica Et Biophysica Acta*, *1801*(11), 1175–1183. https://doi.org/10.1016/j.bbalip.2010.07.007

Aller, E. E. J. G., Abete, I., Astrup, A., Martinez, J. A., & Baak, M. A. van. (2011). Starches, Sugars and Obesity. *Nutrients*, *3*(3), Article 3. https://doi.org/10.3390/nu3030341

Allman-Farinelli, M., Partridge, S. R., McGeechan, K., Balestracci, K., Hebden, L., Wong, A., Phongsavan, P., Denney-Wilson, E., Harris, M. F., & Bauman, A. (2016). A Mobile Health Lifestyle Program for Prevention of Weight Gain in Young Adults (TXT2BFiT): Nine-Month Outcomes of a Randomized Controlled Trial. *JMIR mHealth and uHealth*, *4*(2), e78. https://doi.org/10.2196/mhealth.5768

Al-Mana, N. M., & Robertson, M. D. (2018). Acute Effect of Resistant Starch on Food Intake, Appetite and Satiety in Overweight/Obese Males. *Nutrients*, *10*(12), Article 12. https://doi.org/10.3390/nu10121993

Almoraie, N. M., Saqaan, R., Alharthi, R., Alamoudi, A., Badh, L., & Shatwan, I. M. (2021). Snacking patterns throughout the life span: Potential implications on health. *Nutrition Research*, *91*, 81–94. https://doi.org/10.1016/j.nutres.2021.05.001

Alsaffar, A. A. (2011). Effect of food processing on the resistant starch content of cereals and cereal products – a review. *International Journal of Food Science & Technology*, *46*(3), 455–462. https://doi.org/10.1111/j.1365-2621.2010.02529.x

Al-Tamimi, E. K., Seib, P. A., Snyder, B. S., & Haub, M. D. (2010). Consumption of Cross-Linked Resistant Starch (RS4(XL)) on Glucose and Insulin Responses in Humans. *Journal of Nutrition and Metabolism*, *2010*, 651063. https://doi.org/10.1155/2010/651063

Amini, S., Mansoori, A., & Maghsumi-Norouzabad, L. (2021). The effect of acute consumption of resistant starch on appetite in healthy adults; a systematic review and meta-analysis of the controlled clinical trials. *Clinical Nutrition ESPEN*, *41*, 42–48. https://doi.org/10.1016/j.clnesp.2020.12.006

Aminian, A., Vidal, J., Salminen, P., Still, C. D., Nor Hanipah, Z., Sharma, G., Tu, C., Wood, G. C., Ibarzabal, A., Jimenez, A., Brethauer, S. A., Schauer, P. R., & Mahawar, K. (2020). Late Relapse of Diabetes After Bariatric Surgery: Not Rare, but Not a Failure. *Diabetes Care*, *43*(3), 534–540. https://doi.org/10.2337/dc19-1057

Ammar, A., Brach, M., Trabelsi, K., Chtourou, H., Boukhris, O., Masmoudi, L., Bouaziz, B., Bentlage, E., How, D., Ahmed, M., Müller, P., Müller, N., Aloui, A., Hammouda, O., Paineiras-Domingos, L. L., Braakman-Jansen, A., Wrede, C., Bastoni, S., Pernambuco, C. S., … On Behalf of the ECLB-COVID19 Consortium. (2020). Effects of COVID-19 Home Confinement on Eating Behaviour and Physical Activity: Results of the ECLB-COVID19 International Online Survey. *Nutrients*, *12*(6), Article 6. https://doi.org/10.3390/nu12061583

Anastasovska, J., Arora, T., Sanchez Canon, G. J., Parkinson, J. R. C., Touhy, K., Gibson, G. R., Nadkarni, N. A., So, P.-W., Goldstone, A. P., Thomas, E. L., Hankir, M. K., Van Loo, J., Modi, N., Bell, J. D., & Frost, G. (2012). Fermentable carbohydrate alters hypothalamic neuronal activity and protects against the obesogenic environment. *Obesity (Silver Spring, Md.)*, *20*(5), 1016–1023. https://doi.org/10.1038/oby.2012.6

Ang, K., Bourgy, C., Fenton, H., Regina, A., Newberry, M., Diepeveen, D., Lafiandra, D., Grafenauer, S., Hunt, W., & Solah, V. (2020). Noodles Made from High Amylose Wheat Flour Attenuate Postprandial Glycaemia in Healthy Adults. *Nutrients*, *12*(8), Article 8. https://doi.org/10.3390/nu12082171

Ang, Z., & Ding, J. L. (2016). GPR41 and GPR43 in Obesity and Inflammation—Protective or Causative? *Frontiers in Immunology*, *7*, 28. https://doi.org/10.3389/fimmu.2016.00028

Anika, S. M., Houpt, T. R., & Houpt, K. A. (1980). Insulin as a satiety hormone. *Physiology & Behavior*, *25*(1), 21–23. https://doi.org/10.1016/0031-9384(80)90175-4

Appel, L. J., Clark, J. M., Yeh, H.-C., Wang, N.-Y., Coughlin, J. W., Daumit, G., Miller, E. R., Dalcin, A., Jerome, G. J., Geller, S., Noronha, G., Pozefsky, T., Charleston, J., Reynolds, J. B., Durkin, N., Rubin, R. R., Louis, T. A., & Brancati, F. L. (2011). Comparative effectiveness of weight-loss interventions in clinical practice. *The New England Journal of Medicine*, *365*(21), 1959–1968. https://doi.org/10.1056/NEJMoa1108660

Arias-Córdova, Y., Ble-Castillo, J. L., García-Vázquez, C., Olvera-Hernández, V., Ramos-García, M., Navarrete-Cortes, A., Jiménez-Domínguez, G., Juárez-Rojop, I. E., Tovilla-Zárate, C. A., Martínez-López, M. C., & Méndez, J. D. (2021). Resistant Starch Consumption Effects on Glycemic Control and Glycemic Variability in Patients with Type 2 Diabetes: A Randomized Crossover Study. *Nutrients*, *13*(11), Article 11. https://doi.org/10.3390/nu13114052

Arnolds, S., Kuglin, B., Kapitza, C., & Heise, T. (2010). How pharmacokinetic and pharmacodynamic principles pave the way for optimal basal insulin therapy in type 2 diabetes. *International Journal of Clinical Practice*, *64*(10), 1415–1424. https://doi.org/10.1111/j.1742-1241.2010.02470.x

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum*, *17*(3), 183–190. https://doi.org/10.2337/diaspect.17.3.183

Arora, T., Loo, R. L., Anastasovska, J., Gibson, G. R., Tuohy, K. M., Sharma, R. K., Swann, J. R., Deaville, E. R., Sleeth, M. L., Thomas, E. L., Holmes, E., Bell, J. D., & Frost, G.

(2012). Differential Effects of Two Fermentable Carbohydrates on Central Appetite Regulation and Body Composition. *PLOS ONE*, *7*(8), e43263. https://doi.org/10.1371/journal.pone.0043263

Bach Knudsen, K. E., Lærke, H. N., Hedemann, M. S., Nielsen, T. S., Ingerslev, A. K., Gundelund Nielsen, D. S., Theil, P. K., Purup, S., Hald, S., Schioldan, A. G., Marco, M. L., Gregersen, S., & Hermansen, K. (2018). Impact of Diet-Modulated Butyrate Production on Intestinal Barrier Function and Inflammation. *Nutrients*, *10*(10), Article 10. https://doi.org/10.3390/nu10101499

Bacharach, S. Z., Tordoff, M. G., & Alhadeff, A. L. (2023). Glucose Sensing in the Hepatic Portal Vein and Its Role in Food Intake and Reward. *Cellular and Molecular Gastroenterology and Hepatology*, *16*(2), 189–199. https://doi.org/10.1016/j.jcmgh.2023.03.012

Baer, D. J., Rumpler, W. V., Miles, C. W., & Fahey, G. C. (1997). Dietary Fiber Decreases the Metabolizable Energy Content and Nutrient Digestibility of Mixed Diets Fed to Humans1. *The Journal of Nutrition*, *127*(4), 579–586. https://doi.org/10.1093/jn/127.4.579

Baggio, L. L., & Drucker, D. J. (2007). Biology of Incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), 2131–2157. https://doi.org/10.1053/j.gastro.2007.03.054

Bakaloudi, D. R., Barazzoni, R., Bischoff, S. C., Breda, J., Wickramasinghe, K., & Chourdakis, M. (2022). Impact of the first COVID-19 lockdown on body weight: A combined systematic review and a meta-analysis. *Clinical Nutrition*, *41*(12), 3046–3054. https://doi.org/10.1016/j.clnu.2021.04.015

Barrera, J. G., Sandoval, D. A., D'Alessio, D. A., & Seeley, R. J. (2011). GLP-1 and energy balance: An integrated model of short-term and long-term control. *Nature Reviews. Endocrinology*, *7*(9), 507. https://doi.org/10.1038/nrendo.2011.77

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J., Ghatei, M. A., Cone, R. D., & Bloom, S. R. (2002). Gut hormone PYY3-36 physiologically inhibits food intake. *Nature*, *418*(6898), 650–654. https://doi.org/10.1038/nature00887

Beek, C. M. van der, Canfora, E. E., Kip, A. M., Gorissen, S., Damink, S. O. O., Eijk, H. van, Holst, J., Blaak, E., Dejong, C., & Lenaerts, K. (2018). The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism: Clinical and Experimental*, *87*, 25–35. https://doi.org/10.1016/j.metabol.2018.06.009

Beek, C. M. van der, Canfora, E. E., Lenaerts, K., Troost, F., Damink, S. O., Holst, J., Masclee, A., Dejong, C., & Blaak, E. (2016). Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clinical Science*, *130 22*, 2073–2082. https://doi.org/10.1042/CS20160263

Behall, K. M., & Scholfield, D. J. (2005). Food Amylose Content Affects Postprandial Glucose and Insulin Responses. *Cereal Chemistry Journal*, *82*(6), 654–659. https://doi.org/10.1094/CC-82-0654

Behall, K. M., Scholfield, D. J., Yuhaniak, I., & Canary, J. (1989). Diets containing high amylose vs amylopectin starch: Effects on metabolic variables in human subjects. *The American Journal of Clinical Nutrition*, *49*(2), 337–344. https://doi.org/10.1093/ajcn/49.2.337

Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., Serrano, M., Fernø, J., Salvador, J., Escalada, J., Dieguez, C., Lopez, M., Frühbeck, G., & Nogueiras, R. (2014). GLP-1 Agonism Stimulates Brown Adipose Tissue Thermogenesis and Browning Through Hypothalamic AMPK. *Diabetes*, *63*(10), 3346–3358. https://doi.org/10.2337/db14-0302

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews*, *70*(2), 567–590. https://doi.org/10.1152/physrev.1990.70.2.567

Berry, S. E., Valdes, A. M., Drew, D. A., Asnicar, F., Mazidi, M., Wolf, J., Capdevila, J., Hadjigeorgiou, G., Davies, R., Al Khatib, H., Bonnett, C., Ganesh, S., Bakker, E., Hart, D., Mangino, M., Merino, J., Linenberg, I., Wyatt, P., Ordovas, J. M., … Spector, T. D. (2020). Human postprandial responses to food and potential for precision nutrition. *Nature Medicine*, *26*(6), 964–973. https://doi.org/10.1038/s41591-020-0934-0

Bes-Rastrollo, M., Sánchez-Villegas, A., Gómez-Gracia, E., Martínez, J. A., Pajares, R. M., & Martínez-González, M. A. (2006). Predictors of weight gain in a Mediterranean cohort: The Seguimiento Universidad de Navarra Study2. *The American Journal of Clinical Nutrition*, *83*(2), 362–370. https://doi.org/10.1093/ajcn/83.2.362

Bindels, L. B., Segura Munoz, R. R., Gomes-Neto, J. C., Mutemberezi, V., Martínez, I., Salazar, N., Cody, E. A., Quintero-Villegas, M. I., Kittana, H., de los Reyes-Gavilán, C. G., Schmaltz, R. J., Muccioli, G. G., Walter, J., & Ramer-Tait, A. E. (2017). Resistant starch can improve insulin sensitivity independently of the gut microbiota. *Microbiome*, *5*(1), 12. https://doi.org/10.1186/s40168-017-0230-5

Birt, D. F., Boylston, T., Hendrich, S., Jane, J.-L., Hollis, J., Li, L., McClelland, J., Moore, S., Phillips, G. J., Rowling, M., Schalinske, K., Scott, M. P., & Whitley, E. M. (2013). Resistant Starch: Promise for Improving Human Health. *Advances in Nutrition*, *4*(6), 587–601. https://doi.org/10.3945/an.113.004325

Bitar, K., Greenwood-Van Meerveld, B., Saad, R., & Wiley, J. (2011). Aging and Gastrointestinal Neuromuscular Function: Insights from Within and Outside the Gut. *Neurogastroenterology and Motility : The Official Journal of the European*

Gastrointestinal Motility Society, *23*(6), 490–501. https://doi.org/10.1111/j.1365-2982.2011.01678.x

Blaak, E. E., Antoine, J.-M., Benton, D., Björck, I., Bozzetto, L., Brouns, F., Diamant, M., Dye, L., Hulshof, T., Holst, J. J., Lamport, D. J., Laville, M., Lawton, C. L., Meheust, A., Nilson, A., Normand, S., Rivellese, A. A., Theis, S., Torekov, S. S., & Vinoy, S. (2012). Impact of postprandial glycaemia on health and prevention of disease. *Obesity Reviews*, *13*(10), 923–984. https://doi.org/10.1111/j.1467-789X.2012.01011.x

Ble-Castillo, J. L., Aparicio-Trápala, M. A., Francisco-Luria, M. U., Córdova-Uscanga, R., Rodríguez-Hernández, A., Méndez, J. D., & Díaz-Zagoya, J. C. (2010). Effects of Native Banana Starch Supplementation on Body Weight and Insulin Sensitivity in Obese Type 2 Diabetics. *International Journal of Environmental Research and Public Health*, *7*(5), Article 5. https://doi.org/10.3390/ijerph7051953

Bloemen, J. G., Venema, K., van de Poll, M. C., Olde Damink, S. W., Buurman, W. A., & Dejong, C. H. (2009). Short chain fatty acids exchange across the gut and liver in humans measured at surgery. *Clinical Nutrition (Edinburgh, Scotland)*, *28*(6), 657–661. https://doi.org/10.1016/j.clnu.2009.05.011

Blomain, E. S., Dirhan, D. A., Valentino, M. A., Kim, G. W., & Waldman, S. A. (2013). Mechanisms of Weight Regain following Weight Loss. *ISRN Obesity*, *2013*, 210524. https://doi.org/10.1155/2013/210524

Bodicoat, D. H., Gray, L. J., Henson, J., Webb, D., Guru, A., Misra, A., Gupta, R., Vikram, N., Sattar, N., Davies, M. J., & Khunti, K. (2014). Body Mass Index and Waist Circumference Cut-Points in Multi-Ethnic Populations from the UK and India: The ADDITION-Leicester, Jaipur Heart Watch and New Delhi Cross-Sectional Studies. *PLoS ONE*, *9*(3), e90813. https://doi.org/10.1371/journal.pone.0090813

Bodinham, C. L., Al-Mana, N. M., Smith, L., & Robertson, M. D. (2013). Endogenous plasma glucagon-like peptide-1 following acute dietary fibre consumption. *British Journal of Nutrition*, *110*(8), 1429–1433. https://doi.org/10.1017/S0007114513000731

Bodinham, C. L., Smith, L., Thomas, E. L., Bell, J. D., Swann, J. R., Costabile, A., Russell-Jones, D., Umpleby, A. M., & Robertson, M. D. (2014). Efficacy of increased resistant starch consumption in human type 2 diabetes. *Endocrine Connections*, *3*(2), 75–84. https://doi.org/10.1530/EC-14-0036

Bodinham, C. L., Smith, L., Wright, J., Frost, G. S., & Robertson, M. D. (2012). Dietary Fibre Improves First-phase Insulin Secretion in Overweight Individuals. *PLoS ONE*, *7*(7), e40834. https://doi.org/10.1371/journal.pone.0040834

Boets, E., Gomand, S. V., Deroover, L., Preston, T., Vermeulen, K., De Preter, V., Hamer, H. M., Van den Mooter, G., De Vuyst, L., Courtin, C. M., Annaert, P., Delcour, J. A., & Verbeke, K. A. (2017). Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: A stable isotope study. *Journal of Physiology*, *595*(2), 541–555. Scopus. https://doi.org/10.1113/JP272613

Boillot, J., Alamowitch, C., Berger, A. M., Luo, J., Bruzzo, F., Bornet, F. R., & Slama, G. (1995). Effects of dietary propionate on hepatic glucose production, whole-body glucose utilization, carbohydrate and lipid metabolism in normal rats. *The British Journal of Nutrition*, *73*(2), 241–251. https://doi.org/10.1079/bjn19950026

Bond, D. S., Phelan, S., Leahey, T. M., Hill, J. O., & Wing, R. R. (2009). Weight-loss maintenance in successful weight losers: Surgical vs non-surgical methods. *International Journal of Obesity*, *33*(1), Article 1. https://doi.org/10.1038/ijo.2008.256

Brighenti, F., Casiraghi, M. C., & Baggio, C. (1998). Resistant starch in the Italian diet. *British Journal of Nutrition*, *80*(4), 333–341. https://doi.org/10.1017/S0007114598001391

Brighenti, F., Castellani, G., Benini, L., Casiraghi, M. C., Leopardi, E., Crovetti, R., & Testolin, G. (1995). Effect of neutralized and native vinegar on blood glucose and

acetate responses to a mixed meal in healthy subjects. *European Journal of Clinical Nutrition*, *49*(4), 242–247.

Brites, C. M., Trigo, M. J., Carrapiço, B., Alviña, M., & Bessa, R. J. (2011). Maize and resistant starch enriched breads reduce postprandial glycemic responses in rats. *Nutrition Research*, *31*(4), 302–308. https://doi.org/10.1016/j.nutres.2011.02.001

British Nutrition Foundation. (n.d.). *Type 2 Diabetes Treatment Summaries*. Type 2 Diabetes. https://bnf.nice.org.uk/treatment-summaries/type-2-diabetes/

Broberger, C., L, D. L., Jg, S., & T, H. (1998). Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: Relationship to the neuropeptide Y and agouti gene-related protein systems. *The Journal of Comparative Neurology*, *402*(4). https://pubmed.ncbi.nlm.nih.gov/9862321/

Brooks, L., Viardot, A., Tsakmaki, A., Stolarczyk, E., Howard, J. K., Cani, P. D., Everard, A., Sleeth, M. L., Psichas, A., Anastasovskaj, J., Bell, J. D., Bell-Anderson, K., Mackay, C. R., Ghatei, M. A., Bloom, S. R., Frost, G., & Bewick, G. A. (2017). Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Molecular Metabolism*, *6*(1), 48–60. https://doi.org/10.1016/j.molmet.2016.10.011

Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., Muir, A. I., Wigglesworth, M. J., Kinghorn, I., Fraser, N. J., Pike, N. B., Strum, J. C., Steplewski, K. M., Murdock, P. R., Holder, J. C., Marshall, F. H., Szekeres, P. G., Wilson, S., Ignar, D. M., … Dowell, S. J. (2003). The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *The Journal of Biological Chemistry*, *278*(13), 11312–11319. https://doi.org/10.1074/jbc.M211609200

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: A meta-analysis*. *The American Journal of Clinical Nutrition*, *69*(1), 30–42. https://doi.org/10.1093/ajcn/69.1.30

Brown, T., Avenell, A., Edmunds, L. D., Moore, H., Whittaker, V., Avery, L., & Summerbell, C. (2009). Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, *10*(6), 627–638. https://doi.org/10.1111/j.1467-789X.2009.00641.x

Burton, P. R., Gurrin, L. C., & Campbell, M. J. (1998). Clinical significance not statistical significance: A simple Bayesian alternative to p values. *Journal of Epidemiology & Community Health*, *52*(5), 318–323. https://doi.org/10.1136/jech.52.5.318

Byrne, C. S., Chambers, E. S., Alhabeeb, H., Chhina, N., Morrison, D. J., Preston, T., Tedford, C., Fitzpatrick, J., Irani, C., Busza, A., Garcia-Perez, I., Fountana, S., Holmes, E., Goldstone, A. P., & Frost, G. S. (2016). Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *The American Journal of Clinical Nutrition*, *104*(1), 5–14. https://doi.org/10.3945/ajcn.115.126706

Byrne, C. S., Chambers, E. S., Morrison, D. J., & Frost, G. (2015). The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity*, *39*(9), Article 9. https://doi.org/10.1038/ijo.2015.84

Byrne, C. S., Chambers, E. S., Preston, T., Tedford, C., Brignardello, J., Garcia-Perez, I., Holmes, E., Wallis, G. A., Morrison, D. J., & Frost, G. S. (2019). Effects of Inulin Propionate Ester Incorporated into Palatable Food Products on Appetite and Resting Energy Expenditure: A Randomised Crossover Study. *Nutrients*, *11*(4), E861. https://doi.org/10.3390/nu11040861

Cai, M., Dou, B., Pugh, J. E., Lett, A. M., & Frost, G. S. (2021). The impact of starchy food structure on postprandial glycemic response and appetite: A systematic review with meta-analysis of randomized crossover trials. *The American Journal of Clinical Nutrition*, *114*(2), 472–487. https://doi.org/10.1093/ajcn/nqab098

Caleyachetty, R., Barber, T. M., Mohammed, N. I., Cappuccio, F. P., Hardy, R., Mathur, R., Banerjee, A., & Gill, P. (2021). Ethnicity-specific BMI cutoffs for obesity based on type 2 diabetes risk in England: A population-based cohort study. *The Lancet. Diabetes & Endocrinology*, *9*(7), 419–426. https://doi.org/10.1016/S2213-8587(21)00088-7

Campbell, J. E., & Drucker, D. J. (2013). Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. *Cell Metabolism*, *17*(6), 819–837. https://doi.org/10.1016/j.cmet.2013.04.008

Canfora, E. E., Hermes, G. D. A., Müller, M., Bastings, J., Vaughan, E. E., van Den Berg, M. A., Holst, J. J., Venema, K., Zoetendal, E. G., & Blaak, E. E. (2021). Fiber mixture-specific effect on distal colonic fermentation and metabolic health in lean but not in prediabetic men. *Gut Microbes*, *14*(1), 2009297. https://doi.org/10.1080/19490976.2021.2009297

Canfora, E. E., Jocken, J. W., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews. Endocrinology*, *11*(10), 577–591. https://doi.org/10.1038/nrendo.2015.128

Canfora, E. E., van der Beek, C. M., Jocken, J. W. E., Goossens, G. H., Holst, J. J., Olde Damink, S. W. M., Lenaerts, K., Dejong, C. H. C., & Blaak, E. E. (2017). Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: A randomized crossover trial. *Scientific Reports*, *7*(1), 2360. https://doi.org/10.1038/s41598-017-02546-x

Cani, P. D., Dewever, C., & Delzenne, N. M. (2004). Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and

ghrelin) in rats. *British Journal of Nutrition*, *92*(3), 521–526. https://doi.org/10.1079/BJN20041225

Cani, P. D., Neyrinck, A. M., Maton, N., & Delzenne, N. M. (2005). Oligofructose Promotes Satiety in Rats Fed a High-Fat Diet: Involvement of Glucagon-Like Peptide-1. *Obesity Research*, *13*(6), 1000–1007. https://doi.org/10.1038/oby.2005.117

Capurso, G., & Lahner, E. (2017). The interaction between smoking, alcohol and the gut microbiome. *Best Practice & Research Clinical Gastroenterology*, *31*(5), 579–588. https://doi.org/10.1016/j.bpg.2017.10.006

Carmody, R. N., Gerber, G. K., Luevano, J. M., Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J. (2015). Diet Dominates Host Genotype in Shaping the Murine Gut Microbiota. *Cell Host & Microbe*, *17*(1), 72–84. https://doi.org/10.1016/j.chom.2014.11.010

Cassidy, Y. M., McSorley, E. M., & Allsopp, P. J. (2018). Effect of soluble dietary fibre on postprandial blood glucose response and its potential as a functional food ingredient. *Journal of Functional Foods*, *46*, 423–439. https://doi.org/10.1016/j.jff.2018.05.019

Cavalot, F., Pagliarino, A., Valle, M., Martino, L. D., Bonomo, K., Massucco, P., Anfossi, G., & Trovati, M. (2011). Postprandial Blood Glucose Predicts Cardiovascular Events and All-Cause Mortality in Type 2 Diabetes in a 14-Year Follow-Up. *DIABETES CARE*, *34*.

Cerf, M. E. (2013). Beta Cell Dysfunction and Insulin Resistance. *Frontiers in Endocrinology*, *4*, 37. https://doi.org/10.3389/fendo.2013.00037

Ceriello, A. (2005). Postprandial Hyperglycemia and Diabetes Complications: Is It Time to Treat? *Diabetes*, *54*(1), 1–7. https://doi.org/10.2337/diabetes.54.1.1

Cersosimo, E., Triplitt, C., Solis-Herrera, C., Mandarino, L. J., & DeFronzo, R. A. (2000). Pathogenesis of Type 2 Diabetes Mellitus. In K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, W. W. de Herder, K. Dungan, A. Grossman, J. M. Hershman, J.

Hofland, G. Kaltsas, C. Koch, P. Kopp, M. Korbonits, R. McLachlan, J. E. Morley, M. New, J. Purnell, F. Singer, C. A. Stratakis, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK279115/

Cesur, F., Seçkiner, S., Kucukerdonmez, O., Cagindi, O., Saygili, L. F., & Meseri, R. (2023). Can Enzyme-Resistant Starch Snack Prevent Blood Glucose Fluctuations? A Pilot Trial in Patients with Insulin-Treated Type 2 Diabetes. *Starch - Stärke*, *75*(9–10), 2200279. https://doi.org/10.1002/star.202200279

Chambers, E. S., Byrne, C. S., Aspey, K., Chen, Y., Khan, S., Morrison, D. J., & Frost, G. (2018). Acute oral sodium propionate supplementation raises resting energy expenditure and lipid oxidation in fasted humans. *Diabetes, Obesity & Metabolism*, *20*(4), 1034–1039. https://doi.org/10.1111/dom.13159

Chambers, E. S., Byrne, C. S., Morrison, D. J., Murphy, K. G., Preston, T., Tedford, C., Garcia-Perez, I., Fountana, S., Serrano-Contreras, J. I., Holmes, E., Reynolds, C. J., Roberts, J. F., Boyton, R. J., Altmann, D. M., McDonald, J. A. K., Marchesi, J. R., Akbar, A. N., Riddell, N. E., Wallis, G. A., & Frost, G. S. (2019). Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: A randomised cross-over trial. *Gut*, *68*(8), 1430–1438. https://doi.org/10.1136/gutjnl-2019-318424

Chambers, E. S., Byrne, C. S., Rugyendo, A., Morrison, D. J., Preston, T., Tedford, C., Bell, J. D., Thomas, L., Akbar, A. N., Riddell, N. E., Sharma, R., Thursz, M. R., Manousou, P., & Frost, G. (2019). The effects of dietary supplementation with inulin and inulin-propionate ester on hepatic steatosis in adults with non-alcoholic fatty liver disease. *Diabetes, Obesity & Metabolism*, *21*(2), 372–376. https://doi.org/10.1111/dom.13500

Chambers, E. S., Morrison, D. J., & Frost, G. (2015). Control of appetite and energy intake by SCFA: What are the potential underlying mechanisms? *Proceedings of the Nutrition Society*, *74*(3), 328–336. https://doi.org/10.1017/S0029665114001657

Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-Varghese, S. E. K., MacDougall, K., Preston, T., Tedford, C., Finlayson, G. S., Blundell, J. E., Bell, J. D., Thomas, E. L., Mt-Isa, S., Ashby, D., Gibson, G. R., Kolida, S., Dhillo, W. S., Bloom, S. R., … Frost, G. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut*, *64*(11), 1744–1754. https://doi.org/10.1136/gutjnl-2014-307913

Chandarana, K., Drew, M. E., Emmanuel, J., Karra, E., Gelegen, C., Chan, P., Cron, N. J., & Batterham, R. L. (2009). Subject standardization, acclimatization, and sample processing affect gut hormone levels and appetite in humans. *Gastroenterology*, *136*(7), 2115–2126. https://doi.org/10.1053/j.gastro.2009.02.047

Chang, S.-H., Stoll, C. R. T., Song, J., Varela, J. E., Eagon, C. J., & Colditz, G. A. (2014). The Effectiveness and Risks of Bariatric Surgery: An Updated Systematic Review and Meta-analysis, 2003-2012. *JAMA Surgery*, *149*(3), 275–287. https://doi.org/10.1001/jamasurg.2013.3654

Chaudhri, O., Small, C., & Bloom, S. (2006). Gastrointestinal hormones regulating appetite. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1471), 1187–1209. https://doi.org/10.1098/rstb.2006.1856

Chen, L., Liu, R., Qin, C., Meng, Y., Zhang, J., Wang, Y., & Xu, G. (2010). Sources and intake of resistant starch in the Chinese diet. *Asia Pacific Journal of Clinical Nutrition*, *19*(2), 274–282.

Cherbut, C., Ferrier, L., Rozé, C., Anini, Y., Blottière, H., Lecannu, G., & Galmiche, J.-P. (1998). Short-chain fatty acids modify colonic motility through nerves and polypeptide

YY release in the rat. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *275*(6), G1415–G1422. https://doi.org/10.1152/ajpgi.1998.275.6.G1415

Cherta-Murillo, A., Pugh, J. E., Alaraj-Alshehhi, S., Hajjar, D., Chambers, E. S., & Frost, G. S. (2022). The effects of SCFAs on glycemic control in humans: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, *116*(2), 335–361. https://doi.org/10.1093/ajcn/nqac085

Chong, B., Jayabaskaran, J., Kong, G., Chan, Y. H., Chin, Y. H., Goh, R., Kannan, S., Ng, C. H., Loong, S., Kueh, M. T. W., Lin, C., Anand, V. V., Lee, E. C. Z., Chew, H. S. J., Tan, D. J. H., Chan, K. E., Wang, J.-W., Muthiah, M., Dimitriadis, G. K., … Chew, N. W. S. (2023). Trends and predictions of malnutrition and obesity in 204 countries and territories: An analysis of the Global Burden of Disease Study 2019. *eClinicalMedicine*, *57*. https://doi.org/10.1016/j.eclinm.2023.101850

Christiansen, C. B., Gabe, M. B. N., Svendsen, B., Dragsted, L. O., Rosenkilde, M. M., & Holst, J. J. (2018). The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *315*(1), G53–G65. https://doi.org/10.1152/ajpgi.00346.2017

Chu, J., Igbetar, B. D., & Orfila, C. (2017). Fibrous Cellular Structures are Found in a Commercial Fruit Smoothie and Remain Intact during Simulated Digestion. *Journal of Nutrition & Food Sciences*, *07*(01). https://doi.org/10.4172/2155-9600.1000576

Church, T. S., Thomas, D. M., Tudor-Locke, C., Katzmarzyk, P. T., Earnest, C. P., Rodarte, R. Q., Martin, C. K., Blair, S. N., & Bouchard, C. (2011). Trends over 5 Decades in U.S. Occupation-Related Physical Activity and Their Associations with Obesity. *PLoS ONE*, *6*(5), e19657. https://doi.org/10.1371/journal.pone.0019657

Clifton, P., & Keogh, J. (2016). Starch. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (pp. 146–151). Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00661-9

Cone, R. D., Cowley, M. A., Butler, A. A., Fan, W., Marks, D. L., & Low, M. J. (2001). The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *International Journal of Obesity*, *25*(5), S63–S67. https://doi.org/10.1038/sj.ijo.0801913

Cook, T. M., Gavini, C. K., Jesse, J., Aubert, G., Gornick, E., Bonomo, R., Gautron, L., Layden, B. T., & Mansuy-Aubert, V. (2021). Vagal neuron expression of the microbiota-derived metabolite receptor, free fatty acid receptor (FFAR3), is necessary for normal feeding behavior. *Molecular Metabolism*, *54*, 101350. https://doi.org/10.1016/j.molmet.2021.101350

Cornu, M., Yang, J.-Y., Jaccard, E., Poussin, C., Widmann, C., & Thorens, B. (2009). Glucagon-Like Peptide-1 Protects β-Cells Against Apoptosis by Increasing the Activity of an Igf-2/Igf-1 Receptor Autocrine Loop. *Diabetes*, *58*(8), 1816–1825. https://doi.org/10.2337/db09-0063

Corrado, M., Cherta-Murillo, A., Chambers, E. S., Wood, A. J., Plummer, A., Lovegrove, A., Edwards, C. H., Frost, G. S., & Hazard, B. A. (2020). Effect of semolina pudding prepared from starch branching enzyme IIa and b mutant wheat on glycaemic response in vitro and in vivo: A randomised controlled pilot study. *Food & Function*, *11*(1), 617–627. https://doi.org/10.1039/C9FO02460C

Crummett, L. T., & Grosso, R. J. (2022). Postprandial Glycemic Response to Whole Fruit versus Blended Fruit in Healthy, Young Adults. *Nutrients*, *14*(21), 4565. https://doi.org/10.3390/nu14214565

Cummings, J. H. (1981). Short chain fatty acids in the human colon. *Gut*, *22*(9), 763–779. https://doi.org/10.1136/gut.22.9.763

Cummings, J. H., & Macfarlane, G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology*, *70*(6), 443–459. Scopus. https://doi.org/10.1111/j.1365-2672.1991.tb02739.x

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, *28*(10), 1221–1227. https://doi.org/10.1136/gut.28.10.1221

Cummings, J. H., & Stephen, A. M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition*, *61 Suppl 1*, S5-18. https://doi.org/10.1038/sj.ejcn.1602936

Curtis, R. G., Olds, T., Ferguson, T., Fraysse, F., Dumuid, D., Esterman, A., Hendrie, G. A., Brown, W. J., Lagiseti, R., & Maher, C. A. (2021). Changes in diet, activity, weight, and wellbeing of parents during COVID-19 lockdown. *PLOS ONE*, *16*(3), e0248008. https://doi.org/10.1371/journal.pone.0248008

Custódio, T. F., Paulsen, P. A., Frain, K. M., & Pedersen, B. P. (2021). Structural comparison of GLUT1 to GLUT3 reveal transport regulation mechanism in sugar porter family. *Life Science Alliance*, *4*(4). https://doi.org/10.26508/lsa.202000858

Dainty, S. A., Klingel, S. L., Pilkey, S. E., McDonald, E., McKeown, B., Emes, M. J., & Duncan, A. M. (2016). Resistant Starch Bagels Reduce Fasting and Postprandial Insulin in Adults at Risk of Type 2 Diabetes. *The Journal of Nutrition*, *146*(11), 2252–2259. https://doi.org/10.3945/jn.116.239418

D'Alessio, D. A., Kahn, S. E., Leusner, C. R., & Ensinck, J. W. (1994, May 1). *Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal.* American Society for Clinical Investigation. https://doi.org/10.1172/JCI117225

Darwiche, G., Östman, E. M., Liljeberg, H. G., Kallinen, N., Björgell, O., Björck, I. M., & Almér, L.-O. (2001). Measurements of the gastric emptying rate by use of ultrasonography: Studies in humans using bread with added sodium propionate. *The American Journal of Clinical Nutrition*, *74*(2), 254–258. https://doi.org/10.1093/ajcn/74.2.254

Darzi, J., Frost, G. S., Montaser, R., Yap, J., & Robertson, M. D. (2014). Influence of the tolerability of vinegar as an oral source of short-chain fatty acids on appetite control and food intake. *International Journal of Obesity*, *38*(5), 675–681. https://doi.org/10.1038/ijo.2013.157

Darzi, J., Frost, G. S., & Robertson, M. D. (2012). Effects of a novel propionate-rich sourdough bread on appetite and food intake. *European Journal of Clinical Nutrition*, *66*(7), 789–794. https://doi.org/10.1038/ejcn.2012.1

Das, S. K., Gilhooly, C. H., Golden, J. K., Pittas, A. G., Fuss, P. J., Cheatham, R. A., Tyler, S., Tsay, M., McCrory, M. A., Lichtenstein, A. H., Dallal, G. E., Dutta, C., Bhapkar, M. V., DeLany, J. P., Saltzman, E., & Roberts, S. B. (2007). Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: A 1-y randomized controlled trial2. *The American Journal of Clinical Nutrition*, *85*(4), 1023–1030. https://doi.org/10.1093/ajcn/85.4.1023

Daud, N. M., Ismail, N. A., Thomas, E. L., Fitzpatrick, J. A., Bell, J. D., Swann, J. R., Costabile, A., Childs, C. E., Pedersen, C., Goldstone, A. P., & Frost, G. S. (2014). The impact of oligofructose on stimulation of gut hormones, appetite regulation and adiposity. *Obesity*, *22*(6), 1430–1438. https://doi.org/10.1002/oby.20754

Davidson, G. I., EneObong, H. N., & Nnawuihe, O. O. (2019). Variations in nutrient composition and glycemic index of standardized traditional cocoyam and corn-based dishes consumed in Nsukka Local Government Area of Enugu State, Nigeria. *Progress in Nutrition*, *21*(3), 641–651. Scopus. https://doi.org/10.23751/pn.v21i3.7319

de Roos, N., Heijnen, M. L., de Graaf, C., Woestenenk, G., & Hobbel, E. (1995). Resistant starch has little effect on appetite, food intake and insulin secretion of healthy young men. *European Journal of Clinical Nutrition*, *49*(7), 532–541.

De Silva, A., & Bloom, S. R. (2012). Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. *Gut and Liver*, *6*(1), 10–20. https://doi.org/10.5009/gnl.2012.6.1.10

Deehan, E. C., Yang, C., Perez-Muñoz, M. E., Nguyen, N. K., Cheng, C. C., Triador, L., Zhang, Z., Bakal, J. A., & Walter, J. (2020). Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host & Microbe*, *27*(3), 389-404.e6. https://doi.org/10.1016/j.chom.2020.01.006

Deeks, J. J., Higgins, J. P., & Altman, D. G. (n.d.). *Chapter 10: Analysing data and undertaking meta-analyses*. Retrieved 4 August 2021, from https://training.cochrane.org/handbook/current/chapter-10

Defronzo, R. A. (2004). Dysfunctional fat cells, lipotoxicity and type 2 diabetes. *International Journal of Clinical Practice*, *58*(s143), 9–21. https://doi.org/10.1111/j.1368-504X.2004.00389.x

DeFronzo, R. A., Ferrannini, E., Hendler, R., Wahren, J., & Felig, P. (1978). Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. *Proceedings of the National Academy of Sciences*, *75*(10), 5173–5177. https://doi.org/10.1073/pnas.75.10.5173

DeFronzo, R. A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., & Felber, J. P. (1981). The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and Hepatic and Femoral Venous Catheterization. *Diabetes*, *30*(12), 1000–1007. https://doi.org/10.2337/diab.30.12.1000

Delmée, E., Cani, P. D., Gual, G., Knauf, C., Burcelin, R., Maton, N., & Delzenne, N. M. (2006). Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. *Life Sciences*, *79*(10), 1007–1013. https://doi.org/10.1016/j.lfs.2006.05.013

Delzenne, N. M., Cani, P. D., Daubioul, C., & Neyrinck, A. M. (2005). Impact of inulin and oligofructose on gastrointestinal peptides. *British Journal of Nutrition*, *93*(S1), S157–S161. https://doi.org/10.1079/BJN20041342

den Besten, G., Bleeker, A., Gerding, A., Eunen, K. van, Havinga, R., Dijk, T. H. van, Oosterveer, M. H., Jonker, J. W., Groen, A. K., Reijngoud, D.-J., & Bakker, B. M. (2015). Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes*, *64*(7), 2398–2408. https://doi.org/10.2337/db14-1213

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Derakhshandeh-Rishehri, S., Heidari-Beni, M., Feizi, A., Askari, G.-R., & Entezari, M. (2014). Effect of Honey Vinegar Syrup on Blood Sugar and Lipid Profile in Healthy. *International Journal of Preventive Medicine*, *5*(12), 9.

De Silva, A., Salem, V., Long, C. J., Makwana, A., Newbould, R. D., Rabiner, E. A., Ghatei, M. A., Bloom, S. R., Matthews, P. M., Beaver, J. D., & Dhillo, W. S. (2011). The Gut Hormones PYY3-36 and GLP-17-36 amide Reduce Food Intake and Modulate Brain Activity in Appetite Centers in Humans. *Cell Metabolism*, *14*(5), 700–706. https://doi.org/10.1016/j.cmet.2011.09.010

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., Bäckhed, F., & Mithieux, G. (2014). Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. *Cell*, *156*(1), 84–96. https://doi.org/10.1016/j.cell.2013.12.016

Dhital, S., Gidley, M. J., & Warren, F. J. (2015). Inhibition of α-amylase activity by cellulose: Kinetic analysis and nutritional implications. *Carbohydrate Polymers*, *123*, 305–312. https://doi.org/10.1016/j.carbpol.2015.01.039

Diabetes UK. (2024). *How many people in the UK have diabetes?* Diabetes UK. https://www.diabetes.org.uk/about-us/about-the-charity/our-strategy/statistics

Dicken, S. J., Mitchell, J. J., Vay, J. N. L., Beard, E., Kale, D., Herbec, A., & Shahab, L. (2021). Impact of COVID-19 Pandemic on Weight and BMI among UK Adults: A Longitudinal Analysis of Data from the HEBECO Study. *Nutrients*, *13*(9), 2911. https://doi.org/10.3390/nu13092911

Dikeman, C. L., & Fahey Jr., G. C. (2006). Viscosity as Related to Dietary Fiber: A Review. *Critical Reviews in Food Science and Nutrition*, *46*(8), 649–663. https://doi.org/10.1080/10408390500511862

Dimitriadis, G. K., Randeva, M. S., & Miras, A. D. (2017). Potential Hormone Mechanisms of Bariatric Surgery. *Current Obesity Reports*, *6*(3), 253–265. https://doi.org/10.1007/s13679-017-0276-5

Dodd, H., Williams, S., Brown, R., & Venn, B. (2011). Calculating meal glycemic index by using measured and published food values compared with directly measured meal glycemic index123. *The American Journal of Clinical Nutrition*, *94*(4), 992–996. https://doi.org/10.3945/ajcn.111.012138

Dodevska, M. S., Sobajic, S. S., Djordjevic, P. B., Dimitrijevic-Sreckovic, V. S., Spasojevic-Kalimanovska, V. V., & Djordjevic, B. I. (2016). Effects of total fibre or resistant starch-rich diets within lifestyle intervention in obese prediabetic adults. *European Journal of Nutrition*, *55*(1), 127–137. https://doi.org/10.1007/s00394-015-0831-3

Donald, A. M. (2004). 5—Understanding starch structure and functionality. In A.-C. Eliasson (Ed.), *Starch in Food* (pp. 156–184). Woodhead Publishing. https://doi.org/10.1533/9781855739093.1.156

Drake, H. L. (1994). Acetogenesis, Acetogenic Bacteria, and the Acetyl-CoA "Wood/Ljungdahl" Pathway: Past and Current Perspectives. In H. L. Drake (Ed.), *Acetogenesis* (pp. 3–60). Springer US. https://doi.org/10.1007/978-1-4615-1777-1_1

Drewnowski, A. (1998). Energy Density, Palatability, and Satiety: Implications for Weight Control. *Nutrition Reviews*, *56*(12), 347–353. https://doi.org/10.1111/j.1753-4887.1998.tb01677.x

Drucker, D. J. (2006). The biology of incretin hormones. *Cell Metabolism*, *3*(3), 153–165. https://doi.org/10.1016/j.cmet.2006.01.004

Du, H., van der A, D. L., Boshuizen, H. C., Forouhi, N. G., Wareham, N. J., Halkjær, J., Tjønneland, A., Overvad, K., Jakobsen, M. U., Boeing, H., Buijsse, B., Masala, G., Palli, D., Sørensen, T. I., Saris, W. H., & Feskens, E. J. (2010). Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *The American Journal of Clinical Nutrition*, *91*(2), 329–336. https://doi.org/10.3945/ajcn.2009.28191

Dumoulin, V., Moro, F., Barcelo, A., Dakka, T., & Cuber, J.-C. (1998). Peptide YY, Glucagon-Like Peptide-1, and Neurotensin Responses to Luminal Factors in the Isolated Vascularly Perfused Rat Ileum. *Endocrinology*, *139*(9), 3780–3786. https://doi.org/10.1210/endo.139.9.6202

Dupre, J., Ross, S. A., Watson, D., & Brown, J. C. (1973). STIMULATION OF INSULIN SECRETION BY GASTRIC INHIBITORY POLYPEPTIDE IN MAN.1. *The Journal of Clinical Endocrinology & Metabolism*, *37*(5), 826–828. https://doi.org/10.1210/jcem-37-5-826

EASO Secretariat. (2025, January 24). *EASO response to the Lancet Commission Report on Obesity Diagnosis and Management*. EASO. https://easo.org/easo-response-to-the-lancet-commission-report-on-obesity-diagnosis-and-management/

Eaton, S. B. (2006). The ancestral human diet: What was it and should it be a paradigm for contemporary nutrition? *Proceedings of the Nutrition Society*, *65*(1), 1–6. https://doi.org/10.1079/PNS2005471

Edwards, C. H., Grundy, M. M., Grassby, T., Vasilopoulou, D., Frost, G. S., Butterworth, P. J., Berry, S. E., Sanderson, J., & Ellis, P. R. (2015). Manipulation of starch bioaccessibility in wheat endosperm to regulate starch digestion, postprandial glycemia, insulinemia, and gut hormone responses: A randomized controlled trial in healthy ileostomy participants12. *The American Journal of Clinical Nutrition*, *102*(4), 791–800. https://doi.org/10.3945/ajcn.114.106203

Edwards, C. H., Ryden, P., Mandalari, G., Butterworth, P. J., & Ellis, P. R. (2021). Structure-function studies of chickpea and durum wheat uncover mechanisms by which cell wall properties influence starch bioaccessibility. *Nature Food*, *2*(2), 118–126. https://doi.org/10.1038/s43016-021-00230-y

Edwards, C. H., Ryden, P., Pinto, A. M., van der Schoot, A., Stocchi, C., Perez-Moral, N., Butterworth, P. J., Bajka, B., Berry, S. E., Hill, S. E., & Ellis, P. R. (2020). Chemical, physical and glycaemic characterisation of PulseON®: A novel legume cell-powder ingredient for use in the design of functional foods. *Journal of Functional Foods*, *68*, 103918. https://doi.org/10.1016/j.jff.2020.103918

Eelderink, C., Noort, M. W. J., Sozer, N., Koehorst, M., Holst, J. J., Deacon, C. F., Rehfeld, J. F., Poutanen, K., Vonk, R. J., Oudhuis, L., & Priebe, M. G. (2017). Difference in postprandial GLP-1 response despite similar glucose kinetics after consumption of wheat breads with different particle size in healthy men. *European Journal of Nutrition*, *56*(3), 1063–1076. https://doi.org/10.1007/s00394-016-1156-6

El-Salhy, M., Grimelius, L., Wilander, E., Ryberg, B., Terenius, L., Lundberg, J. M., & Tatemoto, K. (1983). Immunocytochemical identification of polypeptide YY (PYY)

cells in the human gastrointestinal tract. *Histochemistry*, *77*(1), 15–23. https://doi.org/10.1007/BF00496632

Englyst, H. N., & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *The American Journal of Clinical Nutrition*, *42*(5), 778–787. https://doi.org/10.1093/ajcn/42.5.778

Englyst, H. N., & Hudson, G. J. (1996). The classification and measurement of dietary carbohydrates. *Food Chemistry*, *57*(1), 15–21. https://doi.org/10.1016/0308-8146(96)00056-8

Eshghi, F., Bakhshimoghaddam, F., Rasmi, Y., & Alizadeh, M. (2019). Effects of Resistant Starch Supplementation on Glucose Metabolism, Lipid Profile, Lipid Peroxidation Marker, and Oxidative Stress in Overweight and Obese Adults: Randomized, Double-Blind, Crossover Trial. *Clinical Nutrition Research*, *8*(4), 318–328. https://doi.org/10.7762/cnr.2019.8.4.318

Etchison, W. C., Bloodgood, E. A., Minton, C. P., Thompson, N. J., Collins, M. A., Hunter, S. C., & Dai, H. (2011). Body Mass Index and Percentage of Body Fat as Indicators for Obesity in an Adolescent Athletic Population. *Sports Health*, *3*(3), 249–252. https://doi.org/10.1177/1941738111404655

Farup, P. G., & Valeur, J. (2020). Changes in faecal short-chain fatty acids after weight-loss interventions in subjects with morbid obesity. *Nutrients*, *12*(3). Scopus. https://doi.org/10.3390/nu12030802

Feise, N. K., & Johnston, C. S. (2020). Commercial Vinegar Tablets Do Not Display the Same Physiological Benefits for Managing Postprandial Glucose Concentrations as Liquid Vinegar. *Journal of Nutrition and Metabolism*, *2020*, 1–5. https://doi.org/10.1155/2020/9098739

Fildes, A., Charlton, J., Rudisill, C., Littlejohns, P., Prevost, A. T., & Gulliford, M. C. (2015). Probability of an Obese Person Attaining Normal Body Weight: Cohort Study Using

Electronic Health Records. *American Journal of Public Health*, *105*(9), e54-59. https://doi.org/10.2105/AJPH.2015.302773

Filippo, C. D., Cavalieri, D., Paola, M. D., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences*, *107*(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107

Flint, A., Raben, A., Astrup, A., & Holst, J. J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *Journal of Clinical Investigation*, *101*(3), 515. https://doi.org/10.1172/JCI990

Ford, E. S., & Dietz, W. H. (2013). Trends in energy intake among adults in the United States: Findings from NHANES123. *The American Journal of Clinical Nutrition*, *97*(4), 848–853. https://doi.org/10.3945/ajcn.112.052662

Frampton, J. (2022). *Dietary carbohydrate, exercise, and human health*.

Frampton, J., Murphy, K. G., Frost, G., & Chambers, E. S. (2020). Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nature Metabolism*, *2*(9), Article 9. https://doi.org/10.1038/s42255-020-0188-7

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *55*(2), 628–634. https://doi.org/10.1152/jappl.1983.55.2.628

Frayn, K. N. (2013). *Metabolic Regulation: A Human Perspective*. John Wiley & Sons.

Freeland, K. R., & Wolever, T. M. S. (2010). Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-α. *British Journal of Nutrition*, *103*(3), 460–466. https://doi.org/10.1017/S0007114509991863

Frontier Economics. (2023). *Unhealthy Numbers: The Rising Cost of Obesity in the UK*. https://www.institute.global/insights/public-services/unhealthy-numbers-the-rising-cost-of-obesity-in-the-uk

Frost, G. S., Walton, G. E., Swann, J. R., Psichas, A., Costabile, A., Johnson, L. P., Sponheimer, M., Gibson, G. R., & Barraclough, T. G. (2014). Impacts of plant-based foods in ancestral hominin diets on the metabolism and function of gut microbiota in vitro. *mBio*, *5*(3), e00853-00814. https://doi.org/10.1128/mBio.00853-14

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J. R., Gibson, G., Viardot, A., Morrison, D., Louise Thomas, E., & Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, *5*(1), 3611. https://doi.org/10.1038/ncomms4611

Fu, L., Zhang, G., Qian, S., Zhang, Q., & Tan, M. (2022). Associations between dietary fiber intake and cardiovascular risk factors: An umbrella review of meta-analyses of randomized controlled trials. *Frontiers in Nutrition*, *9*, 972399. https://doi.org/10.3389/fnut.2022.972399

Fushimi, T., Tayama, K., Fukaya, M., Kitakoshi, K., Nakai, N., Tsukamoto, Y., & Sato, Y. (2001). Acetic Acid Feeding Enhances Glycogen Repletion in Liver and Skeletal Muscle of Rats. *The Journal of Nutrition*, *131*(7), 1973–1977. https://doi.org/10.1093/jn/131.7.1973

Fushimi, T., Tayama, K., Fukaya, M., Kitakoshi, K., Nakai, N., Tsukamoto, Y., & Sato, Y. (2002). The Efficacy of Acetic Acid for Glycogen Repletion in Rat Skeletal Muscle after Exercise. *International Journal of Sports Medicine*, *23*(3), 218–222. https://doi.org/10.1055/s-2002-23172

Ganz, M. L., Wintfeld, N., Li, Q., Alas, V., Langer, J., & Hammer, M. (2014). The association of body mass index with the risk of type 2 diabetes: A case–control study nested in

an electronic health records system in the United States. *Diabetology & Metabolic Syndrome*, *6*(1), 50. https://doi.org/10.1186/1758-5996-6-50

Gao, C., Rao, M., Huang, W., Wan, Q., Yan, P., Long, Y., Guo, M., Xu, Y., & Xu, Y. (2019). Resistant starch ameliorated insulin resistant in patients of type 2 diabetes with obesity: A systematic review and meta-analysis. *Lipids in Health and Disease*, *18*(1), 205. https://doi.org/10.1186/s12944-019-1127-z

Gao, M., Piernas, C., Astbury, N. M., Hippisley-Cox, J., O'Rahilly, S., Aveyard, P., & Jebb, S. A. (2021). Associations between body-mass index and COVID-19 severity in 6·9 million people in England: A prospective, community-based, cohort study. *The Lancet. Diabetes & Endocrinology*, *9*(6), 350–359. https://doi.org/10.1016/S2213-8587(21)00089-9

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T., & Ye, J. (2009). Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes*, *58*(7), 1509–1517. https://doi.org/10.2337/db08-1637

Garcia-Gutierrez, E., & Sayavedra, L. (2022). 3.07—Diet, Microbiota and the Gut-Brain Axis. In M. Glibetic (Ed.), *Comprehensive Gut Microbiota* (pp. 69–83). Elsevier. https://doi.org/10.1016/B978-0-12-819265-8.00089-9

García-Vázquez, C., Ble-Castillo, J., Arias-Córdova, Y., Córdova-Uscanga, R., Tovilla-Zárate, C., Juárez-Rojop, I., Olvera-Hernández, V., Alvarez-Villagomez, C., Nolasco-Coleman, A. M., & Díaz-Zagoya, J. C. (2019). Effects of Resistant Starch Ingestion on Postprandial Lipemia and Subjective Appetite in Overweight or Obese Subjects. *International Journal of Environmental Research and Public Health*, *16*, null. https://doi.org/10.3390/ijerph16203827

García-Vázquez, C., Ble-Castillo, J., Arias-Córdova, Y., Ramos-García, M., Olvera-Hernández, V., Guzmán-Priego, C., Martínez-López, M. C., Jiménez-Domínguez, G., & Hernández-Becerra, J. A. (2023). Effects of resistant starch on glycemic response,

postprandial lipemia and appetite in subjects with type 2 diabetes. *European Journal of Nutrition*, *62*, 2269–2278. https://doi.org/10.1007/s00394-023-03154-4

Gentilcore, D., Chaikomin, R., Jones, K. L., Russo, A., Feinle-Bisset, C., Wishart, J. M., Rayner, C. K., & Horowitz, M. (2006). Effects of Fat on Gastric Emptying of and the Glycemic, Insulin, and Incretin Responses to a Carbohydrate Meal in Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *91*(6), 2062–2067. https://doi.org/10.1210/jc.2005-2644

Gheflati, A., Bashiri, R., Ghadiri-Anari, A., Reza, J. Z., Kord, M. T., & Nadjarzadeh, A. (2019). The effect of apple vinegar consumption on glycemic indices, blood pressure, oxidative stress, and homocysteine in patients with type 2 diabetes and dyslipidemia: A randomized controlled clinical trial. *Clinical Nutrition ESPEN*, *33*, 132–138. https://doi.org/10.1016/j.clnesp.2019.06.006

Giacco, R., Clemente, G., Brighenti, F., Mancini, M., D'Avanzo, A., Coppola, S., Ruffa, G., Lasorella, G., Rivieccio, A. M., Rivellese, A., & Riccardi, G. (1998). Metabolic effects of resistant starch in patients with Type 2 diabetes. *Diabetes, Nutrition & Metabolism*, *11*, 330–335.

Gill, P. A., Bogatyrev, A., van Zelm, M. C., Gibson, P. R., & Muir, J. G. (2021). Delivery of Acetate to the Peripheral Blood after Consumption of Foods High in Short-Chain Fatty Acids. *Molecular Nutrition & Food Research*, *65*(4), 2000953. https://doi.org/10.1002/mnfr.202000953

Gill, T., King, L., & Caterson, I. (2005). Obesity prevention: Necessary and possible. A structured approach for effective planning. *Proceedings of the Nutrition Society*, *64*(2), 255–261. https://doi.org/10.1079/PNS2005425

Goldenberg, J. Z., Day, A., Brinkworth, G. D., Sato, J., Yamada, S., Jönsson, T., Beardsley, J., Johnson, J. A., Thabane, L., & Johnston, B. C. (2021). Efficacy and safety of low and very low carbohydrate diets for type 2 diabetes remission: Systematic review and

meta-analysis of published and unpublished randomized trial data. *BMJ*, *372*, m4743. https://doi.org/10.1136/bmj.m4743

Goletzke, J., Atkinson, F. S., Ek, K. L., Bell, K., Brand-Miller, J. C., & Buyken, A. E. (2016). Glycaemic and insulin index of four common German breads. *European Journal of Clinical Nutrition*, *70*(7), 808–811. Scopus. https://doi.org/10.1038/ejcn.2016.9

González Hernández, M. A., Canfora, E. E., Jocken, J. W. E., & Blaak, E. E. (2019). The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients*, *11*(8), Article 8. https://doi.org/10.3390/nu11081943

Gower, B. A., Bergman, R., Stefanovski, D., Darnell, B., Ovalle, F., Fisher, G., Sweatt, S. K., Resuehr, H. S., & Pelkman, C. (2016). Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: A randomized, controlled trial. *Nutrition & Metabolism*, *13*(1), 2. https://doi.org/10.1186/s12986-016-0062-5

Gray, G. M. (1975). Carbohydrate Digestion and Absorption. *New England Journal of Medicine*, *292*(23), 1225–1230. https://doi.org/10.1056/NEJM197506052922308

Gressier, M., & Frost, G. (2022). Minor changes in fibre intake in the UK population between 2008/2009 and 2016/2017. *European Journal of Clinical Nutrition*, *76*(2), 322–327. https://doi.org/10.1038/s41430-021-00933-2

Guh, D. P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C. L., & Anis, A. H. (2009). The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. *BMC Public Health*, *9*, 88. https://doi.org/10.1186/1471-2458-9-88

Guice, J., Bendiks, Z. A., Coulon, D., Raggio, A. M., Page, R. C., Carvajal-Aldaz, D. G., Lou, M., Welsh, D. A., Marx, B. D., Taylor, C. M., Husseneder, C., Marco, M. L., & Keenan, M. J. (2020). Differences in Capacity of High-Amylose Resistant Starch, Whole-Grain Flour, and a Combination of Both to Modify Intestinal Responses of Male Sprague

Dawley Rats Fed Moderate and High Fat Diets. *Journal of Agricultural and Food Chemistry*, *68*(51), 15176–15185. Scopus. https://doi.org/10.1021/acs.jafc.0c05285

Guo, X., Long, P., Meng, Q., Ho, C., & Zhang, L. (2018). An emerging strategy for evaluating the grades of Keemun black tea by combinatory liquid chromatography-Orbitrap mass spectrometry-based untargeted metabolomics and inhibition effects on α-glucosidase and α-amylase. *Food Chemistry*, *246*. https://doi.org/10.1016/j.foodchem.2017.10.148

Guraya, H. S., Kadan, R. S., & Champagne, E. T. (1997). Effect of Rice Starch-Lipid Complexes on In Vitro Digestibility, Complexing Index, and Viscosity. *Cereal Chemistry*, *74*(5), 561–565. https://doi.org/10.1094/CCHEM.1997.74.5.561

Hahn, T. M., Breininger, J. F., Baskin, D. G., & Schwartz, M. W. (1998). Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nature Neuroscience*, *1*(4), 271–272. https://doi.org/10.1038/1082

Hall, K. D. (2018). Did the food environment cause the obesity epidemic? *Obesity (Silver Spring, Md.)*, *26*(1), 11. https://doi.org/10.1002/oby.22073

Hall, K. D., Ayuketah, A., Brychta, R., Cai, H., Cassimatis, T., Chen, K. Y., Chung, S. T., Costa, E., Courville, A., Darcey, V., Fletcher, L. A., Forde, C. G., Gharib, A. M., Guo, J., Howard, R., Joseph, P. V., McGehee, S., Ouwerkerk, R., Raisinger, K., … Zhou, M. (2019). Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell Metabolism*, *30*(1), 67-77.e3. https://doi.org/10.1016/j.cmet.2019.05.008

Hallström, E., Sestili, F., Lafiandra, D., Björck, I., & Östman, E. (2011). A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. *Food & Nutrition Research*, *55*, 10.3402/fnr.v55i0.7074. https://doi.org/10.3402/fnr.v55i0.7074

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R.-J. (2008). Review article: The role of butyrate on colonic function. *Alimentary Pharmacology and Therapeutics*, *27*(2), 104–119. Scopus. https://doi.org/10.1111/j.1365-2036.2007.03562.x

Hammer, H. F., Fox, M. R., Keller, J., Salvatore, S., Basilisco, G., Hammer, J., Lopetuso, L., Benninga, M., Borrelli, O., Dumitrascu, D., Hauser, B., Herszenyi, L., Nakov, R., Pohl, D., Thapar, N., Sonyi, M., & European H2-CH4-breath test group. (2022). European guideline on indications, performance, and clinical impact of hydrogen and methane breath tests in adult and pediatric patients: European Association for Gastroenterology, Endoscopy and Nutrition, European Society of Neurogastroenterology and Motility, and European Society for Paediatric Gastroenterology Hepatology and Nutrition consensus. *United European Gastroenterology Journal*, *10*(1), 15–40. https://doi.org/10.1002/ueg2.12133

Hammer, H. F., Petritsch, W., Pristautz, H., & Krejs, G. J. (1996). *Assessment of the influence of hydrogen nonexcretion on the usefulness of the hydrogen breath test and lactose tolerance test*. Wiener Klinische Wochenschrift. https://pubmed.ncbi.nlm.nih.gov/8901127/

Hammoud, R., & Drucker, D. J. (2023). Beyond the pancreas: Contrasting cardiometabolic actions of GIP and GLP1. *Nature Reviews Endocrinology*, *19*(4), 201–216. https://doi.org/10.1038/s41574-022-00783-3

Han, J.-H., Kim, I.-S., Jung, S.-H., Lee, S.-G., Son, H.-Y., & Myung, C.-S. (2014). The Effects of Propionate and Valerate on Insulin Responsiveness for Glucose Uptake in 3T3-L1 Adipocytes and C2C12 Myotubes via G Protein-Coupled Receptor 41. *PLOS ONE*, *9*(4), e95268. https://doi.org/10.1371/journal.pone.0095268

Hankosky, E. R., Schapiro, D., Gunn, K. B., Lubelczyk, E. B., Mitroi, J., & Nelson, D. R. (2023). Gaps Remain for Achieving HbA1c Targets for People with Type 1 or Type 2

Diabetes Using Insulin: Results from NHANES 2009–2020. *Diabetes Therapy*, *14*(6), 967–975. https://doi.org/10.1007/s13300-023-01399-0

Haralampu, S. G. (2000). Resistant starch—A review of the physical properties and biological impact of RS3. *Carbohydrate Polymers*, *41*(3), 285–292. https://doi.org/10.1016/S0144-8617(99)00147-2

Hartvigsen, M. L., Gregersen, S., Lærke, H. N., Holst, J. J., Bach Knudsen, K. E., & Hermansen, K. (2014). Effects of concentrated arabinoxylan and β-glucan compared with refined wheat and whole grain rye on glucose and appetite in subjects with the metabolic syndrome: A randomized study. *European Journal of Clinical Nutrition*, *68*(1), 84–90. https://doi.org/10.1038/ejcn.2013.236

Hattori, M., Kondo, T., Kishi, M., & Yamagami, K. (2010). A Single Oral Administration of Acetic Acid Increased Energy Expenditure in C57BL/6J Mice. *Bioscience, Biotechnology, and Biochemistry*, *74*(10), 2158–2159. https://doi.org/10.1271/bbb.100486

Haub, M. D., Hubach, K. L., Al-tamimi, E. K., Ornelas, S., & Seib, P. A. (2010). Different Types of Resistant Starch Elicit Different Glucose Reponses in Humans. *Journal of Nutrition and Metabolism*, *2010*, 230501. https://doi.org/10.1155/2010/230501

Hayashi, Y. (2021). Glucagon regulates lipolysis and fatty acid oxidation through inositol triphosphate receptor 1 in the liver. *Journal of Diabetes Investigation*, *12*(1), 32–34. https://doi.org/10.1111/jdi.13315

Henderson, J. R., Daniel, P. M., & Fraser, P. A. (1981). The pancreas as a single organ: The influence of the endocrine upon the exocrine part of the gland. *Gut*, *22*(2), 158–167. https://doi.org/10.1136/gut.22.2.158

Herrmann, E., Young, W., Reichert-Grimm, V., Weis, S., Riedel, C. U., Rosendale, D., Stoklosinski, H., Hunt, M., & Egert, M. (2018). In Vivo Assessment of Resistant Starch Degradation by the Caecal Microbiota of Mice Using RNA-Based Stable Isotope

Probing—A Proof-of-Principle Study. *Nutrients*, *10*(2), Article 2. https://doi.org/10.3390/nu10020179

Hex, N., MacDonald, R., Pocock, J., Uzdzinska, B., Taylor, M., Atkin, M., Wild, S. H., Beba, H., & Jones, R. (2024). Estimation of the direct health and indirect societal costs of diabetes in the UK using a cost of illness model. *Diabetic Medicine*, *41*(9), e15326. https://doi.org/10.1111/dme.15326

Heymsfield, S. B., & Wadden, T. A. (2017). Mechanisms, Pathophysiology, and Management of Obesity. *New England Journal of Medicine*, *376*(3), 254–266. https://doi.org/10.1056/NEJMra1514009

Higgins, J. P. T., Savović, J., Page, M., Elbers, R., & Sterne, J. (2021). *Chapter 8: Assessing risk of bias in a randomized trial. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021).* Cochrane. www.training.cochrane.org/handbook

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ*, *327*(7414), 557–560. https://doi.org/10.1136/bmj.327.7414.557

Hill, J. O., Wyatt, H. R., Reed, G. W., & Peters, J. C. (2003). Obesity and the Environment: Where Do We Go from Here? *Science*, *299*(5608), 853–855. https://doi.org/10.1126/science.1079857

Hill, Wyatt Holly R., & Peters John C. (2012). Energy Balance and Obesity. *Circulation*, *126*(1), 126–132. https://doi.org/10.1161/CIRCULATIONAHA.111.087213

Hlebowicz, J., Darwiche, G., Björgell, O., & Almér, L.-O. (2007). Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: A pilot study. *BMC Gastroenterology*, *7*(1), 46. https://doi.org/10.1186/1471-230X-7-46

Hodgkinson, K., El Abbar, F., Dobranowski, P., Manoogian, J., Butcher, J., Figeys, D., Mack, D., & Stintzi, A. (2023). Butyrate's role in human health and the current progress towards its clinical application to treat gastrointestinal disease. *Clinical Nutrition*, *42*(2), 61–75. https://doi.org/10.1016/j.clnu.2022.10.024

Holland, C., Ryden, P., Edwards, C. H., & Grundy, M. M.-L. (2020). Plant Cell Walls: Impact on Nutrient Bioaccessibility and Digestibility. *Foods*, *9*(2), 201. https://doi.org/10.3390/foods9020201

Holm, J., Björck, I., Ostrowska, S., Eliasson, A.-C., Asp, N.-G., Larsson, K., & Lundquist, I. (1983). Digestibility of Amylose-Lipid Complexes in-vitro and in-vivo. *Starch - Stärke*, *35*(9), 294–297. https://doi.org/10.1002/star.19830350902

Holt, S. H. A., & Miller, J. B. (1995). Increased insulin responses to ingested foods are associated with lessened satiety. *Appetite*, *24*(1), 43–54. https://doi.org/10.1016/S0195-6663(95)80005-0

Hong, Y.-H., Nishimura, Y., Hishikawa, D., Tsuzuki, H., Miyahara, H., Gotoh, C., Choi, K.-C., Feng, D. D., Chen, C., Lee, H.-G., Katoh, K., Roh, S.-G., & Sasaki, S. (2005). Acetate and Propionate Short Chain Fatty Acids Stimulate Adipogenesis via GPCR43. *Endocrinology*, *146*(12), 5092–5099. https://doi.org/10.1210/en.2005-0545

Hooper, L. V. (2015). Chapter 3—Epithelial Cell Contributions to Intestinal Immunity. In F. W. Alt (Ed.), *Advances in Immunology* (Vol. 126, pp. 129–172). Academic Press. https://doi.org/10.1016/bs.ai.2014.11.003

Hosseini, Z. S. M., Hosseini, J., Nabati, S., Hasanshahi, G., & Mahmoodi, M. (2011). Survey on the anti-diabetic effects of vinegar on some biochemical factors in type 2 diabetic patients. *Clinical Biochemistry*, *44*(13), S226. https://doi.org/10.1016/j.clinbiochem.2011.08.543

Hu, J., Kyrou, I., Tan, B. K., Dimitriadis, G. K., Ramanjaneya, M., Tripathi, G., Patel, V., James, S., Kawan, M., Chen, J., & Randeva, H. S. (2016). Short-Chain Fatty Acid

Acetate Stimulates Adipogenesis and Mitochondrial Biogenesis via GPR43 in Brown Adipocytes. *Endocrinology*, *157*(5), 1881–1894. https://doi.org/10.1210/en.2015-1944

Hu, J., Wang, J., Li, Y., Xue, K., & Kan, J. (2023). Use of Dietary Fibers in Reducing the Risk of Several Cancer Types: An Umbrella Review. *Nutrients*, *15*(11), 2545. https://doi.org/10.3390/nu15112545

Huang, S., & Czech, M. P. (2007). The GLUT4 glucose transporter. *Cell Metabolism*, *5*(4), 237–252. https://doi.org/10.1016/j.cmet.2007.03.006

Huang, X.-F., Yu, Y., Beck, E. J., South, T., Li, Y., Batterham, M. J., Tapsell, L. C., & Chen, J. (2011). Diet high in oat β-glucan activates the gut-hypothalamic (PYY3–36-NPY) axis and increases satiety in diet-induced obesity in mice. *Molecular Nutrition & Food Research*, *55*(7), 1118–1121. https://doi.org/10.1002/mnfr.201100095

Hughes, R. L., Horn, W. H., Finnegan, P., Newman, J. W., Marco, M. L., Keim, N. L., & Kable, M. E. (2021). Resistant Starch Type 2 from Wheat Reduces Postprandial Glycemic Response with Concurrent Alterations in Gut Microbiota Composition. *Nutrients*, *13*(2), Article 2. https://doi.org/10.3390/nu13020645

Huwiler, V. V., Schönenberger, K. A., Segesser von Brunegg, A., Reber, E., Mühlebach, S., Stanga, Z., & Balmer, M. L. (2022). Prolonged Isolated Soluble Dietary Fibre Supplementation in Overweight and Obese Patients: A Systematic Review with Meta-Analysis of Randomised Controlled Trials. *Nutrients*, *14*(13), Article 13. https://doi.org/10.3390/nu14132627

International Diabetes Foundation. (2021). *IDF Diabetes Atlas | Tenth Edition*. https://diabetesatlas.org/

Jakubowicz, D., Froy, O., Ahrén, B., Boaz, M., Landau, Z., Bar-Dayan, Y., Ganz, T., Barnea, M., & Wainstein, J. (2014). Incretin, insulinotropic and glucose-lowering effects of

whey protein pre-load in type 2 diabetes: A randomised clinical trial. *Diabetologia*, *57*(9), 1807–1811. https://doi.org/10.1007/s00125-014-3305-x

Jannasch, F., Kröger, J., & Schulze, M. B. (2017). Dietary patterns and Type 2 diabetes: A systematic literature review and meta- analysis of prospective studies. *Journal of Nutrition*, *147*(6), 1174–1182. Scopus. https://doi.org/10.3945/jn.116.242552

Janssen, P., Vanden Berghe, P., Verschueren, S., Lehmann, A., Depoortere, I., & Tack, J. (2011). Review article: The role of gastric motility in the control of food intake. *Alimentary Pharmacology & Therapeutics*, *33*(8), 880–894. https://doi.org/10.1111/j.1365-2036.2011.04609.x

Järvi, A. E., Karlström, B. E., Granfeldt, Y. E., Björck, I. M., Vessby, B. O., & Asp, N. G. (1995). The influence of food structure on postprandial metabolism in patients with non-insulin-dependent diabetes mellitus. *The American Journal of Clinical Nutrition*, *61*(4), 837–842. https://doi.org/10.1093/ajcn/61.4.837

Jarvi, A., Karlström, B., Granfeldt, Y., Björck, I., Asp, N., & Vessby, B. (1999). Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycaemic index diet in type 2 diabetes. *Diabetes Care*, *22*, 10–18. https://doi.org/10.2337/diacare.22.1.10

Jasbi, P., Baker, O., Shi, X., Gonzalez, L. A., Wang, S., Anderson, S., Xi, B., Gu, H., & Johnston, C. S. (2019). Daily red wine vinegar ingestion for eight weeks improves glucose homeostasis and affects the metabolome but does not reduce adiposity in adults. *Food & Function*, *10*(11), 7343–7355. https://doi.org/10.1039/C9FO01082C

Jenkins, D. J. ., Kendall, C. W., & Ransom, T. P. P. (1998). Dietary fiber, the evolution of the human diet and coronary heart disease. *Nutrition Research*, *18*(4), 633–652. https://doi.org/10.1016/S0271-5317(98)00050-5

Jenkins, D. J. A., Willett, W. C., Yusuf, S., Hu, F. B., Glenn, A. J., Liu, S., Mente, A., Miller, V., Bangdiwala, S. I., Gerstein, H. C., Sieri, S., Ferrari, P., Patel, A. V., McCullough,

M. L., Marchand, L. L., Freedman, N. D., Loftfield, E., Sinha, R., Shu, X.-O., … Yang, W. (2024). Association of glycaemic index and glycaemic load with type 2 diabetes, cardiovascular disease, cancer, and all-cause mortality: A meta-analysis of mega cohorts of more than 100 000 participants. *The Lancet Diabetes & Endocrinology*, *12*(2), 107–118. https://doi.org/10.1016/S2213-8587(23)00344-3

Jenkins, D. J. A., Wolever, T. M. S., Jenkins, A. L., Thorne, M. J., Lee, R., Kalmusky, J., Reichert, R., & Wong, G. S. (1983). The glycaemic index of foods tested in diabetic patients: A new basis for carbohydrate exchange favouring the use of legumes. *Diabetologia*, *24*(4), 257–264. https://doi.org/10.1007/BF00282710

Jenkins, D. J., Vuksan, V., Kendall, C. W. C., Würsch, P., Jeffcoat, R., Waring, S., Mehling, C. C., Vidgen, E., Augustin, L. S. A., & Wong, E. (1998). Physiological Effects of Resistant Starches on Fecal Bulk, Short Chain Fatty Acids, Blood Lipids and Glycemic Index. *Journal of the American College of Nutrition*, *17*(6), 609–616. https://doi.org/10.1080/07315724.1998.10718810

Jenkins, D. J., Wolever, T. M., Leeds, A. R., Gassull, M. A., Haisman, P., Dilawari, J., Goff, D. V., Metz, G. L., & Alberti, K. G. (1978). Dietary fibres, fibre analogues, and glucose tolerance: Importance of viscosity. *Br Med J*, *1*(6124), 1392–1394. https://doi.org/10.1136/bmj.1.6124.1392

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., Jenkins, A. L., & Goff, D. V. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, *34*(3), 362–366. https://doi.org/10.1093/ajcn/34.3.362

Jeong, S.-M., Lee, D. H., Rezende, L. F. M., & Giovannucci, E. L. (2023). Different correlation of body mass index with body fatness and obesity-related biomarker according to age, sex and race-ethnicity. *Scientific Reports*, *13*(1), 3472. https://doi.org/10.1038/s41598-023-30527-w

Jiang, G., & Zhang, B. B. (2003). Glucagon and regulation of glucose metabolism. *American Journal of Physiology-Endocrinology and Metabolism*, *284*(4), E671–E678. https://doi.org/10.1152/ajpendo.00492.2002

Jiao, A., Yu, B., He, J., Yu, J., Zheng, P., Luo, Y., Luo, J., Yan, H., Wang, Q., Wang, H., Mao, X., & Chen, D. (2021). Sodium acetate, propionate, and butyrate reduce fat accumulation in mice via modulating appetite and relevant genes. *Nutrition*, *87–88*, 111198. https://doi.org/10.1016/j.nut.2021.111198

Jiménez-Domínguez, G., Ble-Castillo, J. L., Aparicio-Trápala, M. A., Juárez-Rojop, I. E., Tovilla-Zárate, C. A., Ble-Castillo, D. J., García-Vázquez, C., Olvera-Hernández, V., Pérez-Pimienta, B., Diaz-Zagoya, J. C., & Mendez, J. D. (2015). Effects of Acute Ingestion of Native Banana Starch on Glycemic Response Evaluated by Continuous Glucose Monitoring in Obese and Lean Subjects. *International Journal of Environmental Research and Public Health*, *12*(7), Article 7. https://doi.org/10.3390/ijerph120707491

Johnson, K. O., Shannon, O. M., Matu, J., Holliday, A., Ispoglou, T., & Deighton, K. (2020). Differences in circulating appetite-related hormone concentrations between younger and older adults: A systematic review and meta-analysis. *Aging Clinical and Experimental Research*, *32*(7), 1233–1244. https://doi.org/10.1007/s40520-019-01292-6

Johnston, C. S., & Buller, A. J. (2005). Vinegar and Peanut Products as Complementary Foods to Reduce Postprandial Glycemia. *Journal of the American Dietetic Association*, *105*(12), 1939–1942. https://doi.org/10.1016/j.jada.2005.07.012

Johnston, C. S., & Gaas, C. A. (2006). Vinegar: Medicinal Uses and Antiglycemic Effect. *Medscape General Medicine*, *8*(2), 61.

Johnston, C. S., Kim, C. M., & Buller, A. J. (2004). Vinegar Improves Insulin Sensitivity to a High-Carbohydrate Meal in Subjects With Insulin Resistance or Type 2 Diabetes. *Diabetes Care*, *27*(1), 281–282. https://doi.org/10.2337/diacare.27.1.281

Johnston, C. S., Steplewska, I., Long, C. A., Harris, L. N., & Ryals, R. H. (2010). Examination of the Antiglycemic Properties of Vinegar in Healthy Adults. *Annals of Nutrition and Metabolism*, *56*(1), 74–79. https://doi.org/10.1159/000272133

Johnston, K. L., Thomas, E. L., Bell, J. D., Frost, G. S., & Robertson, M. D. (2010). Resistant starch improves insulin sensitivity in metabolic syndrome. *Diabetic Medicine*, *27*(4), 391–397. https://doi.org/10.1111/j.1464-5491.2010.02923.x

Johnstone, A. M., Murison, S. D., Duncan, J. S., Rance, K. A., & Speakman, J. R. (2005). Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine2. *The American Journal of Clinical Nutrition*, *82*(5), 941–948. https://doi.org/10.1093/ajcn/82.5.941

Jovanovic, A., Gerrard, J., & Taylor, R. (2009). The Second-Meal Phenomenon in Type 2 Diabetes. *Diabetes Care*, *32*(7), 1199–1201. https://doi.org/10.2337/dc08-2196

Jurado-Fasoli, L., Sanchez-Delgado, G., Alcantara, J. M. A., Acosta, F. M., Sanchez-Sanchez, R., Labayen, I., Ortega, F. B., Martinez-Tellez, B., & Ruiz, J. R. (2024). Adults with metabolically healthy overweight or obesity present more brown adipose tissue and higher thermogenesis than their metabolically unhealthy counterparts. *eBioMedicine*, *100*. https://doi.org/10.1016/j.ebiom.2023.104948

Kabisch, S., Meyer, N. M. T., Honsek, C., Gerbracht, C., Dambeck, U., Kemper, M., Osterhoff, M. A., Birkenfeld, A. L., Arafat, A. M., Weickert, M. O., & Pfeiffer, A. F. H. (2019). Obesity does not modulate the glycometabolic benefit of insoluble cereal fibre in subjects with prediabetes—A stratified post hoc analysis of the optimal fibre trial (Optifit). *Nutrients*, *11*(11). Scopus. https://doi.org/10.3390/nu11112726

Kadyan, S., Park, G., Wang, B., Singh, P., Arjmandi, B., & Nagpal, R. (2023). Resistant starches from dietary pulses modulate the gut metabolome in association with microbiome in a humanized murine model of ageing. *Scientific Reports*, *13*(1), Article 1. https://doi.org/10.1038/s41598-023-37036-w

Kaji, I., Karaki, S., & Kuwahara, A. (2014). Short-Chain Fatty Acid Receptor and Its Contribution to Glucagon-Like Peptide-1 Release. *Digestion*, *89*(1), 31–36. https://doi.org/10.1159/000356211

Kant, A. K., & Graubard, B. I. (2015). 40-Year Trends in Meal and Snack Eating Behaviors of American Adults. *Journal of the Academy of Nutrition and Dietetics*, *115*(1), 50–63. https://doi.org/10.1016/j.jand.2014.06.354

Karimi, P., Farhangi, M. A., Sarmadi, B., Gargari, B. P., Javid, A. Z., Pouraghaei, M., & Dehghan, P. (2016). The Therapeutic Potential of Resistant Starch in Modulation of Insulin Resistance, Endotoxemia, Oxidative Stress and Antioxidant Biomarkers in Women with Type 2 Diabetes: A Randomized Controlled Clinical Trial. *Annals of Nutrition and Metabolism*, *68*(2), 85–93. https://doi.org/10.1159/000441683

Kassir, R., Debs, T., Blanc, P., Gugenheim, J., Ben Amor, I., Boutet, C., & Tiffet, O. (2016). Complications of bariatric surgery: Presentation and emergency management. *International Journal of Surgery*, *27*, 77–81. https://doi.org/10.1016/j.ijsu.2016.01.067

Katsoulis, M., Lai, A. G., Diaz-Ordaz, K., Gomes, M., Pasea, L., Banerjee, A., Denaxas, S., Tsilidis, K., Lagiou, P., Misirli, G., Bhaskaran, K., Wannamethee, G., Dobson, R., Batterham, R. L., Kipourou, D.-K., Lumbers, R. T., Wen, L., Wareham, N., Langenberg, C., & Hemingway, H. (2021). Identifying adults at high-risk for change in weight and BMI in England: A longitudinal, large-scale, population-based cohort study using electronic health records. *The Lancet Diabetes & Endocrinology*, *9*(10), 681–694. https://doi.org/10.1016/S2213-8587(21)00207-2

Keenan, M. J., Zhou, J., McCutcheon, K. L., Raggio, A. M., Bateman, H. G., Todd, E., Jones, C. K., Tulley, R. T., Melton, S., Martin, R. J., & Hegsted, M. (2006). Effects of Resistant Starch, A Non-digestible Fermentable Fiber, on Reducing Body Fat. *Obesity*, *14*(9), 1523–1534. https://doi.org/10.1038/oby.2006.176

Kemp, D. M., & Habener, J. F. (2004). Glucagon and Glucagon-like Peptides. In L. Martini (Ed.), *Encyclopedia of Endocrine Diseases* (pp. 220–224). Elsevier. https://doi.org/10.1016/B0-12-475570-4/01450-5

Khalid, A., Khalid, A. S., Ashiq, E., & Ahtisham. (2022). Effect of the Apple Cider Vinegar on Weight Management, Blood Glucose Levels and Lipid Profile among Obese/Overweight Adults: A Randomised Control Trial. *Pakistan Journal of Medical & Health Sciences*, *16*(07), Article 07. https://doi.org/10.53350/pjmhs22167286

Khosravi, Z., Hadi, A., Tutunchi, H., Asghari-Jafarabadi, M., Naeinie, F., Roshanravan, N., Ostadrahimi, A., & Fadel, A. (2022). The effects of butyrate supplementation on glycemic control, lipid profile, blood pressure, nitric oxide level and glutathione peroxidase activity in type 2 diabetic patients: A randomized triple -blind, placebo-controlled trial. *Clinical Nutrition ESPEN*, *49*, 79–85. https://doi.org/10.1016/j.clnesp.2022.03.008

Kianmehr, H., Zhang, P., Luo, J., Guo, J., Pavkov, M. E., Bullard, K. M., Gregg, E. W., Ospina, N. S., Fonseca, V., Shi, L., & Shao, H. (2022). Potential Gains in Life Expectancy Associated With Achieving Treatment Goals in US Adults With Type 2 Diabetes. *JAMA Network Open*, *5*(4), e227705. https://doi.org/10.1001/jamanetworkopen.2022.7705

Kim, K. N., Yao, Y., & Ju, S. Y. (2019). Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. *Nutrients*, *11*(10), Article 10. https://doi.org/10.3390/nu11102512

Kim, S. J., de Souza, R. J., Choo, V. L., Ha, V., Cozma, A. I., Chiavaroli, L., Mirrahimi, A., Mejia, S. B., di Buono, M., Bernstein, A. M., Leiter, L. A., Kris-Etherton, P. M., Vuksan, V., Beyene, J., Kendall, C. W., Jenkins, D. J., & Sievenpiper, J. L. (2015). The Effects of Dietary Pulse Consumption on Body Weight: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Canadian Journal of Diabetes*, *39*, S58. https://doi.org/10.1016/j.jcjd.2015.01.219

Kim, S.-J., Winter, K., Nian, C., Tsuneoka, M., Koda, Y., & McIntosh, C. H. S. (2005). Glucose-dependent Insulinotropic Polypeptide (GIP) Stimulation of Pancreatic β-Cell Survival Is Dependent upon Phosphatidylinositol 3-Kinase (PI3K)/Protein Kinase B (PKB) Signaling, Inactivation of the Forkhead Transcription Factor Foxo1, and Down-regulation of bax Expression *. *Journal of Biological Chemistry*, *280*(23), 22297–22307. https://doi.org/10.1074/jbc.M500540200

Kim, Y., Keogh, J. B., & Clifton, P. M. (2016). Polyphenols and Glycemic Control. *Nutrients*, *8*(1), 17. https://doi.org/10.3390/nu8010017

Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., Kobayashi, M., Hirasawa, A., & Tsujimoto, G. (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proceedings of the National Academy of Sciences*, *108*(19), 8030–8035. https://doi.org/10.1073/pnas.1016088108

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., Terasawa, K., Kashihara, D., Hirano, K., Tani, T., Takahashi, T., Miyauchi, S., Shioi, G., Inoue, H., & Tsujimoto, G. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nature Communications*, *4*, 1829. https://doi.org/10.1038/ncomms2852

Kirkpatrick, C. F., Bolick, J. P., Kris-Etherton, P. M., Sikand, G., Aspry, K. E., Soffer, D. E., Willard, K.-E., & Maki, K. C. (2019). Review of current evidence and clinical

recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: A scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. *Journal of Clinical Lipidology*, *13*(5), 689-711.e1. https://doi.org/10.1016/j.jacl.2019.08.003

Kistorp, C. N., Toubro, S., Astrup, A., & Svendsen, O. L. (2000). Measurements of Body Composition by Dual-Energy X-ray Absorptiometry Improve Prediction of Energy Expenditure. *Annals of the New York Academy of Sciences*, *904*(1), 79–84. https://doi.org/10.1111/j.1749-6632.2000.tb06424.x

Kjems, L. L., Holst, J. J., Vølund, A., & Madsbad, S. (2003). The Influence of GLP-1 on Glucose-Stimulated Insulin Secretion: Effects on β-Cell Sensitivity in Type 2 and Nondiabetic Subjects. *Diabetes*, *52*(2), 380–386. https://doi.org/10.2337/diabetes.52.2.380

Koh, A., Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell*, *165*, 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Kokrashvili, Z., Mosinger, B., & Margolskee, R. F. (2009). Taste signaling elements expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of gut hormones123. *The American Journal of Clinical Nutrition*, *90*(3), 822S-825S. https://doi.org/10.3945/ajcn.2009.27462T

Kozak, L. P. (2010). Brown Fat and the Myth of Diet-Induced Thermogenesis. *Cell Metabolism*, *11*(4), 263–267. https://doi.org/10.1016/j.cmet.2010.03.009

Kreymann, B., Ghatei, M. A., Williams, G., & Bloom, S. R. (1987). Glucagon-like Peptide-1 17-36: A Physiological Incretin in Man. *The Lancet*, *330*(8571), 1300–1304. https://doi.org/10.1016/S0140-6736(87)91194-9

Krezowski, P. A., Nuttall, F. Q., Gannon, M. C., Billington, C. J., & Parker, S. (1987). Insulin and Glucose Responses to Various Starch-Containing Foods in Type II Diabetic Subjects. *Diabetes Care*, *10*(2), 205–212. https://doi.org/10.2337/diacare.10.2.205

Kwak, J. H., Paik, J. K., Kim, H. I., Kim, O. Y., Shin, D. Y., Kim, H.-J., Lee, J. H., & Lee, J. H. (2012). Dietary treatment with rice containing resistant starch improves markers of endothelial function with reduction of postprandial blood glucose and oxidative stress in patients with prediabetes or newly diagnosed type 2 diabetes. *Atherosclerosis*, *224*(2), 457–464. https://doi.org/10.1016/j.atherosclerosis.2012.08.003

Lachat, C., Nago, E., Verstraeten, R., Roberfroid, D., Van Camp, J., & Kolsteren, P. (2012). Eating out of home and its association with dietary intake: A systematic review of the evidence. *Obesity Reviews*, *13*(4), 329–346. https://doi.org/10.1111/j.1467-789X.2011.00953.x

Lambert, J. E., Parnell, J. A., Tunnicliffe, J. M., Han, J., Sturzenegger, T., & Reimer, R. A. (2017). Consuming yellow pea fiber reduces voluntary energy intake and body fat in overweight/obese adults in a 12-week randomized controlled trial. *Clinical Nutrition (Edinburgh, Scotland)*, *36*(1), 126–133. https://doi.org/10.1016/j.clnu.2015.12.016

Langkilde, A. M., Champ, M., & Andersson, H. (2002). Effects of high-resistant-starch banana flour (RS(2)) on in vitro fermentation and the small-bowel excretion of energy, nutrients, and sterols: An ileostomy study. *The American Journal of Clinical Nutrition*, *75*(1), 104–111. https://doi.org/10.1093/ajcn/75.1.104

Larraufie, P., Martin-Gallausiaux, C., Lapaque, N., Dore, J., Gribble, F. M., Reimann, F., & Blottiere, H. M. (2018). SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Scientific Reports*, *8*(1), 74. https://doi.org/10.1038/s41598-017-18259-0

Larsen, H., Rasmussen, O., Rasmussen, P., Alstrup, K., Biswas, S., Tetens, I., Thilsted, S., & Hermansen, K. (2000). Glycaemic index of parboiled rice depends on the severity of processing: Study in type 2 diabetic subjects. *European Journal of Clinical Nutrition*, *54*(5), 380–385. https://doi.org/10.1038/sj.ejcn.1600969

Larsen, P. J., Tang-Christensen, M., Holst, J. J., & Ørskov, C. (1997). Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience*, *77*(1), 257–270. https://doi.org/10.1016/S0306-4522(96)00434-4

Laurent, C., Simoneau, C., Marks, L., Braschi, S., Champ, M., Charbonnel, B., & Krempf, M. (1995). Effect of acetate and propionate on fasting hepatic glucose production in humans. *European Journal of Clinical Nutrition*, *49*(7), 484–491.

Le Blay, G., Michel, C., Blottière, H. M., & Cherbut, C. (1999). Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. *British Journal of Nutrition*, *82*(5), 419–426. Scopus. https://doi.org/10.1017/s000711459900166x

Le Poul, E., Loison, C., Struyf, S., Springael, J.-Y., Lannoy, V., Decobecq, M.-E., Brezillon, S., Dupriez, V., Vassart, G., Damme, J. V., Parmentier, M., & Detheux, M. (2003). Functional Characterization of Human Receptors for Short Chain Fatty Acids and Their Role in Polymorphonuclear Cell Activation *. *Journal of Biological Chemistry*, *278*(28), 25481–25489. https://doi.org/10.1074/jbc.M301403200

Lean, M. E., Leslie, W. S., Barnes, A. C., Brosnahan, N., Thom, G., McCombie, L., Kelly, T., Irvine, K., Peters, C., Zhyzhneuskaya, S., Hollingsworth, K. G., Adamson, A. J., Sniehotta, F. F., Mathers, J. C., McIlvenna, Y., Welsh, P., McConnachie, A., McIntosh, A., Sattar, N., & Taylor, R. (2024). 5-year follow-up of the randomised Diabetes Remission Clinical Trial (DiRECT) of continued support for weight loss

maintenance in the UK: An extension study. *The Lancet Diabetes & Endocrinology*, *12*(4), 233–246. https://doi.org/10.1016/S2213-8587(23)00385-6

Leeman, M., Östman, E., & Björck, I. (2005). Vinegar dressing and cold storage of potatoes lowers postprandial glycaemic and insulinaemic responses in healthy subjects. *European Journal of Clinical Nutrition*, *59*(11), 1266–1271. https://doi.org/10.1038/sj.ejcn.1602238

Leitner, B. P., Huang, S., Brychta, R. J., Duckworth, C. J., Baskin, A. S., McGehee, S., Tal, I., Dieckmann, W., Gupta, G., Kolodny, G. M., Pacak, K., Herscovitch, P., Cypess, A. M., & Chen, K. Y. (2017). Mapping of human brown adipose tissue in lean and obese young men. *Proceedings of the National Academy of Sciences*, *114*(32), 8649–8654. https://doi.org/10.1073/pnas.1705287114

Levy, R. B., Rauber, F., Chang, K., Louzada, M. L. da C., Monteiro, C. A., Millett, C., & Vamos, E. P. (2021). Ultra-processed food consumption and type 2 diabetes incidence: A prospective cohort study. *Clinical Nutrition*, *40*(5), 3608–3614. https://doi.org/10.1016/j.clnu.2020.12.018

Lewis, C. E., Jacobs, D. R., McCreath, H., Kiefe, C. I., Schreiner, P. J., Smith, D. E., & Williams, O. D. (2000). Weight gain continues in the 1990s: 10-year trends in weight and overweight from the CARDIA study. Coronary Artery Risk Development in Young Adults. *American Journal of Epidemiology*, *151*(12), 1172–1181. https://doi.org/10.1093/oxfordjournals.aje.a010167

Lhuissier, A., Tichit, C., Caillavet, F., Cardon, P., Masullo, A., Martin-Fernandez, J., Parizot, I., & Chauvin, P. (2013). Who still eats three meals a day? Findings from a quantitative survey in the Paris area. *Appetite*, *63*, 59–69. https://doi.org/10.1016/j.appet.2012.12.012

Li, G., Su, H., Zhou, Z., & Yao, W. (2014). Identification of the Porcine G Protein-Coupled Receptor 41 and 43 Genes and Their Expression Pattern in Different Tissues and

Development Stages. *PLOS ONE*, *9*(5), e97342. https://doi.org/10.1371/journal.pone.0097342

Li, Z., Yi, C.-X., Katiraei, S., Kooijman, S., Zhou, E., Chung, C. K., Gao, Y., van den Heuvel, J. K., Meijer, O. C., Berbée, J. F. P., Heijink, M., Giera, M., Willems van Dijk, K., Groen, A. K., Rensen, P. C. N., & Wang, Y. (2018). Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*, *67*(7), 1269–1279. https://doi.org/10.1136/gutjnl-2017-314050

Liatis, S., Grammatikou, S., Poulia, K.-A., Perrea, D., Makrilakis, K., Diakoumopoulou, E., & Katsilambros, N. (2010). Vinegar reduces postprandial hyperglycaemia in patients with type II diabetes when added to a high, but not to a low, glycaemic index meal. *European Journal of Clinical Nutrition*, *64*(7), 727–732. https://doi.org/10.1038/ejcn.2010.89

Lichtash, C. T., Cui, J., Guo, X., Chen, Y.-D. I., Hsueh, W. A., Rotter, J. I., & Goodarzi, M. O. (2013). Body Adiposity Index versus Body Mass Index and Other Anthropometric Traits as Correlates of Cardiometabolic Risk Factors. *PLOS ONE*, *8*(6), e65954. https://doi.org/10.1371/journal.pone.0065954

Liljeberg, H., & Björck, I. (1998). Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. *European Journal of Clinical Nutrition*, *52*(5), 368–371. https://doi.org/10.1038/sj.ejcn.1600572

Lim, J. J., Liu, Y., Lu, L. W., Sequeira, I. R., & Poppitt, S. D. (2023). No Evidence That Circulating GLP-1 or PYY Are Associated with Increased Satiety during Low Energy Diet-Induced Weight Loss: Modelling Biomarkers of Appetite. *Nutrients*, *15*(10), Article 10. https://doi.org/10.3390/nu15102399

Lin, C.-H., Chang, D.-M., Wu, D.-J., Peng, H.-Y., & Chuang, L.-M. (2015). Assessment of Blood Glucose Regulation and Safety of Resistant Starch Formula-Based Diet in

Healthy Normal and Subjects With Type 2 Diabetes. *Medicine*, *94*(33), e1332. https://doi.org/10.1097/MD.0000000000001332

Lin, H. V., Frassetto, A., Kowalik, E. J., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., Hubert, J. A., Szeto, D., Yao, X., Forrest, G., & Marsh, D. J. (2012). Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PloS One*, *7*(4), e35240. https://doi.org/10.1371/journal.pone.0035240

Lin, N., Li, Y., Tang, L., Shi, J., & Chen, Y. (2013). In vivo effect of oat cereal β-glucan on metabolic indexes and satiety-related hormones in diet-induced obesity C57-Bl mice. *Molecular Nutrition & Food Research*, *57*(7), 1291–1294. https://doi.org/10.1002/mnfr.201200695

Lin, Y., Huybrechts, I., Vandevijvere, S., Bolca, S., De Keyzer, W., De Vriese, S., Polet, A., De Neve, M., Van Oyen, H., Van Camp, J., De Backer, G., & De Henauw, S. (2011). Fibre intake among the Belgian population by sex-age and sex-education groups and its association with BMI and waist circumference. *The British Journal of Nutrition*, *105*(11), 1692–1703. https://doi.org/10.1017/S0007114510005088

Liou, A. P., Paziuk, M., Luevano, J.-M., Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Science Translational Medicine*, *5*(178), 178ra41. https://doi.org/10.1126/scitranslmed.3005687

Lobley, G. E., Holtrop, G., Bremner, D. M., Calder, A. G., Milne, E., & Johnstone, A. M. (2013). Impact of short term consumption of diets high in either non-starch polysaccharides or resistant starch in comparison with moderate weight loss on indices of insulin sensitivity in subjects with metabolic syndrome. *Nutrients*, *5*(6), 2144–2172. https://doi.org/10.3390/nu5062144

Lovegrove, A., Edwards, C. H., De Noni, I., Patel, H., El, S. N., Grassby, T., Zielke, C., Ulmius, M., Nilsson, L., Butterworth, P. J., Ellis, P. R., & Shewry, P. R. (2017). Role of polysaccharides in food, digestion, and health. *Critical Reviews in Food Science and Nutrition*, *57*(2), 237–253. Scopus. https://doi.org/10.1080/10408398.2014.939263

Ludwig, D. S., Pereira, M. A., Kroenke, C. H., Hilner, J. E., Van Horn, L., Slattery, M. L., & Jacobs, J., David R. (1999). Dietary Fiber, Weight Gain, and Cardiovascular Disease Risk Factors in Young Adults. *JAMA*, *282*(16), 1539–1546. https://doi.org/10.1001/jama.282.16.1539

Luitse, M. J., Biessels, G. J., Rutten, G. E., & Kappelle, L. J. (2012). Diabetes, hyperglycaemia, and acute ischaemic stroke. *The Lancet Neurology*, *11*(3), 261–271. https://doi.org/10.1016/S1474-4422(12)70005-4

Lundberg, J. M., Tatemoto, K., Terenius, L., Hellström, P. M., Mutt, V., Hökfelt, T., & Hamberger, B. (1982). Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. *Proceedings of the National Academy of Sciences*, *79*(14), 4471–4475. https://doi.org/10.1073/pnas.79.14.4471

Macfarlane, G. T., Gibson, G. R., & Cummings, J. H. (1992). Comparison of fermentation reactions in different regions of the human colon. *Journal of Applied Bacteriology*, *72*(1), 57–64. Scopus. https://doi.org/10.1111/j.1365-2672.1992.tb04882.x

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*, *62*(1), 67–72. https://doi.org/10.1079/PNS2002207

Mackie, A. R., Bajka, B. H., Rigby, N. M., Wilde, P. J., Alves-Pereira, F., Mosleth, E. F., Rieder, A., Kirkhus, B., & Salt, L. J. (2017). Oatmeal particle size alters glycemic index but not as a function of gastric emptying rate. *American Journal of Physiology-*

Gastrointestinal and Liver Physiology, *313*(3), G239–G246. https://doi.org/10.1152/ajpgi.00005.2017

MacNeil, S., Rebry, R. M., Tetlow, I. J., Emes, M. J., McKeown, B., & Graham, T. E. (2013). Resistant starch intake at breakfast affects postprandial responses in type 2 diabetics and enhances the glucose-dependent insulinotropic polypeptide – insulin relationship following a second meal. *Applied Physiology, Nutrition, and Metabolism*, *38*(12), 1187–1195. https://doi.org/10.1139/apnm-2013-0023

Maki, K. C., Pelkman, C. L., Finocchiaro, E. T., Kelley, K. M., Lawless, A. L., Schild, A. L., & Rains, T. M. (2012). Resistant Starch from High-Amylose Maize Increases Insulin Sensitivity in Overweight and Obese Men123. *The Journal of Nutrition*, *142*(4), 717–723. https://doi.org/10.3945/jn.111.152975

Malam, S., Prior, G., Phillips, R., & O'Driscoll, C. (2014). *The 2014 Food and You Survey. England Bulletin 3. Eating outside the home.* Food Standards Agency. https://www.food.gov.uk/sites/default/files/media/document/england-bulletin-3-food-and-you-2014_0.pdf

Malin, S. K., Kullman, E. L., Scelsi, A. R., Godin, J., Ross, A. B., & Kirwan, J. P. (2019). A Whole-Grain Diet Increases Glucose-Stimulated Insulin Secretion Independent of Gut Hormones in Adults at Risk for Type 2 Diabetes. *Molecular Nutrition & Food Research*, *63*(7), 1800967. https://doi.org/10.1002/mnfr.201800967

Malkova, D., Polyviou, T., Rizou, E., Gerasimidis, K., Chambers, E. S., Preston, T., Tedford, M. C., Frost, G., & Morrison, D. J. (2020). Moderate intensity exercise training combined with inulin-propionate ester supplementation increases whole body resting fat oxidation in overweight women. *Metabolism*, *104*, 154043. https://doi.org/10.1016/j.metabol.2019.154043

Marchetti, P., Lupi, R., Bugliani, M., Kirkpatrick, C. L., Sebastiani, G., Grieco, F. A., Del Guerra, S., D'Aleo, V., Piro, S., Marselli, L., Boggi, U., Filipponi, F., Tinti, L., Salvini,

L., Wollheim, C. B., Purrello, F., & Dotta, F. (2012). A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia*, *55*(12), 3262–3272. https://doi.org/10.1007/s00125-012-2716-9

Marchini, J. S., Faisant, N., Champ, M., Ranganathan, S., Azoulay, C., Kergueris, M., Piloquet, H., & Krempf, M. (1998). Effects of an acute raw resistant potato starch supplement on postprandial glycemia, insulinemia, lipemia in healthy adults. *Nutrition Research*, *18*(7), 1135–1145. https://doi.org/10.1016/S0271-5317(98)00095-5

Margolskee, R. F. (2002). Molecular Mechanisms of Bitter and Sweet Taste Transduction *. *Journal of Biological Chemistry*, *277*(1), 1–4. https://doi.org/10.1074/jbc.R100054200

Mårin, P., Rebuffé-Scrive, M., Smith, U., & Björntorp, P. (1987). Glucose uptake in human adipose tissue. *Metabolism*, *36*(12), 1154–1160. https://doi.org/10.1016/0026-0495(87)90242-3

Marlett, J. A., & Cheung, T.-F. (1997). Database and Quick Methods of Assessing Typical Dietary Fiber Intakes using data for 228 Commonly Consumed Foods. *Journal of the American Dietetic Association*, *97*(10), 1139–1151. https://doi.org/10.1016/S0002-8223(97)00275-7

Martín, M. Á., & Ramos, S. (2021). Dietary Flavonoids and Insulin Signaling in Diabetes and Obesity. *Cells*, *10*(6), 1474. https://doi.org/10.3390/cells10061474

Martínez, I., Kim, J., Duffy, P. R., Schlegel, V. L., & Walter, J. (2010). Resistant Starches Types 2 and 4 Have Differential Effects on the Composition of the Fecal Microbiota in Human Subjects. *PLOS ONE*, *5*(11), e15046. https://doi.org/10.1371/journal.pone.0015046

Martínez-Sánchez, M. A., Balaguer-Román, A., Fernández-Ruiz, V. E., Almansa-Saura, S., García-Zafra, V., Ferrer-Gómez, M., Frutos, M. D., Queipo-Ortuño, M. I., Ruiz-Alcaraz, A. J., Núñez-Sánchez, M. Á., & Ramos-Molina, B. (2023). Plasma short-chain fatty acid changes after bariatric surgery in patients with severe obesity.

Surgery for Obesity and Related Diseases, *19*(7), 727–734. https://doi.org/10.1016/j.soard.2022.12.041

Martín-Peláez, S., Fito, M., & Castaner, O. (2020). Mediterranean Diet Effects on Type 2 Diabetes Prevention, Disease Progression, and Related Mechanisms. A Review. *Nutrients*, *12*(8). https://doi.org/10.3390/nu12082236

Maruta, H., Yoshimura, Y., Araki, A., Kimoto, M., Takahashi, Y., & Yamashita, H. (2016). Activation of AMP-Activated Protein Kinase and Stimulation of Energy Metabolism by Acetic Acid in L6 Myotube Cells. *PLOS ONE*, *11*(6), e0158055. https://doi.org/10.1371/journal.pone.0158055

Mas, A., Torija, M. J., García-Parrilla, M. del C., & Troncoso, A. M. (2014). Acetic Acid Bacteria and the Production and Quality of Wine Vinegar. *The Scientific World Journal*, *2014*, 1–6. https://doi.org/10.1155/2014/394671

Mather, A., & Pollock, C. (2011). Glucose handling by the kidney. *Kidney International*, *79*, S1–S6. https://doi.org/10.1038/ki.2010.509

Maziarz, M. P., Preisendanz, S., Juma, S., Imrhan, V., Prasad, C., & Vijayagopal, P. (2017). Resistant starch lowers postprandial glucose and leptin in overweight adults consuming a moderate-to-high-fat diet: A randomized-controlled trial. *Nutrition Journal*, *16*(1), 14. https://doi.org/10.1186/s12937-017-0235-8

McNeil, N. (1984). The contribution of the large intestine to energy supplies in man. *The American Journal of Clinical Nutrition*, *39*(2), 338–342. https://doi.org/10.1093/ajcn/39.2.338

McNelis, J. C., Lee, Y. S., Mayoral, R., van der Kant, R., Johnson, A. M. F., Wollam, J., & Olefsky, J. M. (2015). GPR43 Potentiates β-Cell Function in Obesity. *Diabetes*, *64*(9), 3203–3217. https://doi.org/10.2337/db14-1938

Meijer, J. L., Roderka, M. N., Chinburg, E. L., Renier, T. J., McClure, A. C., Rothstein, R. I., Barry, E. L., Billmeier, S., & Gilbert-Diamond, D. (2022). Alterations in Fecal Short-

Chain Fatty Acids after Bariatric Surgery: Relationship with Dietary Intake and Weight Loss. *Nutrients*, *14*(20), Article 20. https://doi.org/10.3390/nu14204243

Meng, Y., & Chen, L. (2015). *Effects of resistant staple food on patients with type 2 diabetes mellitus*. https://doi.org/10.13325/j.cnki.acta.nutr.sin.2015.03.008

Mete, E., Haszard, J., Perry, T., Oey, I., Mann, J., & Te Morenga, L. (2021). Effect of Wholegrain Flour Particle Size in Bread on Glycaemic and Insulinaemic Response among People with Risk Factors for Type 2 Diabetes: A Randomised Crossover Trial. *Nutrients*, *13*(8), Article 8. https://doi.org/10.3390/nu13082579

Mettler, S., Schwarz, I., & Colombani, P. C. (2009). Additive postprandial blood glucose-attenuating and satiety-enhancing effect of cinnamon and acetic acid. *Nutrition Research (New York, N.Y.)*, *29*(10), 723–727. https://doi.org/10.1016/j.nutres.2009.10.002

Mishra, S., Hardacre, A., Monro, J., Mishra, S., Hardacre, A., & Monro, J. (2012). Food Structure and Carbohydrate Digestibility. In *Carbohydrates—Comprehensive Studies on Glycobiology and Glycotechnology*. IntechOpen. https://doi.org/10.5772/51969

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. https://doi.org/10.1002/star.200800209

Mithieux, G., Andreelli, F., & Magnan, C. (2009). Intestinal gluconeogenesis: Key signal of central control of energy and glucose homeostasis. *Current Opinion in Clinical Nutrition and Metabolic Care*, *12*(4), 419–423. https://doi.org/10.1097/MCO.0b013e32832c4d6a

Mithieux, G., & Gautier-Stein, A. (2014). Intestinal glucose metabolism revisited. *Diabetes Research and Clinical Practice*, *105*(3), 295–301. https://doi.org/10.1016/j.diabres.2014.04.008

Mitrou, P., Petsiou, E., Papakonstantinou, E., Maratou, E., Lambadiari, V., Dimitriadis, P., Spanoudi, F., Raptis, S. A., & Dimitriadis, G. (2015a). The role of acetic acid on glucose uptake and blood flow rates in the skeletal muscle in humans with impaired glucose tolerance. *European Journal of Clinical Nutrition*, *69*(6), 734–739. https://doi.org/10.1038/ejcn.2014.289

Mitrou, P., Petsiou, E., Papakonstantinou, E., Maratou, E., Lambadiari, V., Dimitriadis, P., Spanoudi, F., Raptis, S. A., & Dimitriadis, G. (2015b). Vinegar Consumption Increases Insulin-Stimulated Glucose Uptake by the Forearm Muscle in Humans with Type 2 Diabetes. *Journal of Diabetes Research*, *2015*, 1–7. https://doi.org/10.1155/2015/175204

Moll, H., Frey, E., Gerber, P., Geidl, B., Kaufmann, M., Braun, J., Beuschlein, F., Puhan, M. A., & Yebyo, H. G. (2024). GLP-1 receptor agonists for weight reduction in people living with obesity but without diabetes: A living benefit–harm modelling study. *eClinicalMedicine*, *73*. https://doi.org/10.1016/j.eclinm.2024.102661

Moody, A. (2020). *Health Survey for England, 2019. Overweight and Obesity in Adults and Children*. NatCen Social Research, Health and Social Care Information Centre: London, UK. https://files.digital.nhs.uk/9D/4195D5/HSE19-Overweight-obesity-rep.pdf

Moongngarm, A. (2013). Chemical Compositions and Resistant Starch Content in Starchy Foods. *American Journal of Agricultural and Biological Sciences*, *8*(2), 107–113. https://doi.org/10.3844/ajabssp.2013.107.113

Moore, M. C., Coate, K. C., Winnick, J. J., An, Z., & Cherrington, A. D. (2012). Regulation of Hepatic Glucose Uptake and Storage In Vivo. *Advances in Nutrition*, *3*(3), 286–294. https://doi.org/10.3945/an.112.002089

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*, *7*(3), 189–200. https://doi.org/10.1080/19490976.2015.1134082

Morton, G. J., Cummings, D. E., Baskin, D. G., Barsh, G. S., & Schwartz, M. W. (2006). Central nervous system control of food intake and body weight. *Nature*, *443*(7109), 289–295. https://doi.org/10.1038/nature05026

Muir, J. G., Lu, Z. X., Young, G. P., Cameron-Smith, D., Collier, G. R., & O'Dea, K. (1995). Resistant starch in the diet increases breath hydrogen and serum acetate in human subjects. *American Journal of Clinical Nutrition*, *61*(4), 792–799. Scopus. https://doi.org/10.1093/ajcn/61.4.792

Murakami, Y., Ojima-Kato, T., Saburi, W., Mori, H., Matsui, H., Tanabe, S., & Suzuki, T. (2015). Supplemental epilactose prevents metabolic disorders through uncoupling protein-1 induction in the skeletal muscle of mice fed high-fat diets. *The British Journal of Nutrition*, *114*(11), 1774–1783. https://doi.org/10.1017/S0007114515003505

Murphy, K. G., & Bloom, S. R. (2006). Gut hormones and the regulation of energy homeostasis. *Nature*, *444*(7121), 854–859. https://doi.org/10.1038/nature05484

Murphy, M. M., Douglass, J. S., & Birkett, A. (2008). Resistant starch intakes in the United States. *Journal of the American Dietetic Association*, *108*(1), 67–78. https://doi.org/10.1016/j.jada.2007.10.012

Musa-Veloso, K., Noori, D., Venditti, C., Poon, T., Johnson, J., Harkness, L. S., O'Shea, M., & Chu, Y. (2021). A Systematic Review and Meta-Analysis of Randomized Controlled Trials on the Effects of Oats and Oat Processing on Postprandial Blood Glucose and Insulin Responses. *The Journal of Nutrition*, *151*(2), 341–351. https://doi.org/10.1093/jn/nxaa349

Myhre, J. B., Løken, E. B., Wandel, M., & Andersen, L. F. (2015). The contribution of snacks to dietary intake and their association with eating location among Norwegian adults – results from a cross-sectional dietary survey. *BMC Public Health*, *15*(1), 369. https://doi.org/10.1186/s12889-015-1712-7

Mytton, O. T., Nnoaham, K., Eyles, H., Scarborough, P., & Ni Mhurchu, C. (2014). Systematic review and meta-analysis of the effect of increased vegetable and fruit consumption on body weight and energy intake. *BMC Public Health*, *14*(1), 886. https://doi.org/10.1186/1471-2458-14-886

Nagashima, H., Morio, Y., Meshitsuka, S., Yamane, K., Nanjo, Y., & Teshima, R. (2010). High-resolution nuclear magnetic resonance spectroscopic study of metabolites in the cerebrospinal fluid of patients with cervical myelopathy and lumbar radiculopathy. *European Spine Journal*, *19*(8), 1363. https://doi.org/10.1007/s00586-010-1453-3

Narayan, K. M. V., Boyle, J. P., Thompson, T. J., Gregg, E. W., & Williamson, D. F. (2007). Effect of BMI on Lifetime Risk for Diabetes in the U.S. *Diabetes Care*, *30*(6), 1562–1566. https://doi.org/10.2337/dc06-2544

Navarro, M., De Fonseca, F. R., Alvarez, E., Chowen, J. A., Zueco, J. A., Gomez, R., Eng, J., & Blázquez, E. (1996). Colocalization of Glucagon-Like Peptide-1 (GLP-1) Receptors, Glucose Transporter GLUT-2, and Glucokinase mRNAs in Rat Hypothalamic Cells: Evidence for a Role of GLP-1 Receptor Agonists as an Inhibitory Signal for Food and Water Intake. *Journal of Neurochemistry*, *67*(5), 1982–1991. https://doi.org/10.1046/j.1471-4159.1996.67051982.x

Neis, E. P., Eijk, H. M. van, Lenaerts, K., Damink, S. W. O., Blaak, E. E., Dejong, C. H., & Rensen, S. S. (2019). Distal versus proximal intestinal short-chain fatty acid release in man. *Gut*, *68*(4), 764–765. https://doi.org/10.1136/gutjnl-2018-316161

Nelson, M. C., Story, M., Larson, N. I., Neumark-Sztainer, D., & Lytle, L. A. (2008). Emerging Adulthood and College-aged Youth: An Overlooked Age for Weight-related Behavior Change. *Obesity*, *16*(10), 2205–2211. https://doi.org/10.1038/oby.2008.365

Neuenschwander, M., Ballon, A., Weber, K. S., Norat, T., Aune, D., Schwingshackl, L., & Schlesinger, S. (2019). Role of diet in type 2 diabetes incidence: Umbrella review of meta-analyses of prospective observational studies. *BMJ*, l2368. https://doi.org/10.1136/bmj.l2368

Newcombe, R. G. (1998). Interval estimation for the difference between independent proportions: Comparison of eleven methods. *Statistics in Medicine*, *17*(8), 873–890. https://doi.org/10.1002/(SICI)1097-0258(19980430)17:8<873::AID-SIM779>3.0.CO;2-I

Ng, S. W., & Popkin, B. M. (2012). Time use and physical activity: A shift away from movement across the globe. *Obesity Reviews*, *13*(8), 659–680. https://doi.org/10.1111/j.1467-789X.2011.00982.x

NHS. (2020). *Statistics on Obesity, Physical Activity and Diet, England, 2020*. NHS. https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-obesity-physical-activity-and-diet/england-2020

NICE. (2015, December 2). *Recommendations | Type 2 diabetes in adults: Management | Guidance | NICE*. NICE. https://www.nice.org.uk/guidance/ng28/chapter/recommendations

Nichenametla, S. N., Weidauer, L. A., Wey, H. E., Beare, T. M., Specker, B. L., & Dey, M. (2014). Resistant starch type 4-enriched diet lowered blood cholesterols and improved body composition in a double blind controlled cross-over intervention. *Molecular Nutrition & Food Research*, *58*(6), 1365–1369. https://doi.org/10.1002/mnfr.201300829

Nicolai, J. P., Lupiani, J. H., & Wolf, A. J. (2018). Chapter 37—An Integrative Approach to Obesity. In D. Rakel (Ed.), *Integrative Medicine (Fourth Edition)* (pp. 382-394.e2). Elsevier. https://doi.org/10.1016/B978-0-323-35868-2.00037-2

Nilsson, N., Kotarsky, K., Owman, C., & Olde, B. (2003). Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochemical and Biophysical Research Communications*, *303*(4). https://doi.org/10.1016/s0006-291x(03)00488-1

Nishida, A., Miyamoto, J., Shimizu, H., & Kimura, I. (2021). Gut microbial short-chain fatty acids-mediated olfactory receptor 78 stimulation promotes anorexigenic gut hormone peptide YY secretion in mice. *Biochemical and Biophysical Research Communications*, *557*, 48–54. https://doi.org/10.1016/j.bbrc.2021.03.167

Noah, L., Guillon, F., Bouchet, B., Buléon, A., Molis, C., Gratas, M., & Champ, M. (1998). Digestion of Carbohydrate from White Beans (*Phaseolus vulgaris* L.) in Healthy Humans1. *The Journal of Nutrition*, *128*(6), 977–985. https://doi.org/10.1093/jn/128.6.977

Noakes, M., Clifton, P., Nestel, P., Le Leu, R., & McIntosh, G. (1996). Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. *The American Journal of Clinical Nutrition*, *64*(6). https://doi.org/10.1093/ajcn/64.6.944

Nøhr, M. K., Pedersen, M. H., Gille, A., Egerod, K. L., Engelstoft, M. S., Husted, A. S., Sichlau, R. M., Grunddal, K. V., Seier Poulsen, S., Han, S., Jones, R. M., Offermanns, S., & Schwartz, T. W. (2013). GPR41/FFAR3 and GPR43/FFAR2 as Cosensors for Short-Chain Fatty Acids in Enteroendocrine Cells vs FFAR3 in Enteric Neurons and FFAR2 in Enteric Leukocytes. *Endocrinology*, *154*(10), 3552–3564. https://doi.org/10.1210/en.2013-1142

Nordgaard, I., Mortensen, P. B., & Langkilde, A. M. (1995). Small intestinal malabsorption and colonic fermentation of resistant starch and resistant peptides to short-chain fatty acids. *Nutrition (Burbank, Los Angeles County, Calif.)*, *11*(2), 129–137.

Norman, J. E., Bild, D., Lewis, C. E., Liu, K., & West, D. S. (2003). The impact of weight change on cardiovascular disease risk factors in young black and white adults: The CARDIA study. *International Journal of Obesity*, *27*(3), Article 3. https://doi.org/10.1038/sj.ijo.0802243

Nowakowska, M., Zghebi, S. S., Ashcroft, D. M., Buchan, I., Chew-Graham, C., Holt, T., Mallen, C., Van Marwijk, H., Peek, N., Perera-Salazar, R., Reeves, D., Rutter, M. K., Weng, S. F., Qureshi, N., Mamas, M. A., & Kontopantelis, E. (2019). The comorbidity burden of type 2 diabetes mellitus: Patterns, clusters and predictions from a large English primary care cohort. *BMC Medicine*, *17*(1), Article 1. https://doi.org/10.1186/s12916-019-1373-y

Nowotny, K., Jung, T., Höhn, A., Weber, D., & Grune, T. (2015). Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. *Biomolecules*, *5*(1), Article 1. https://doi.org/10.3390/biom5010194

Office for Health Improvement and Disparities. (2021). *National Diet and Nutrition Survey: Diet, nutrition and physical activity in 2020 A follow up study during COVID-19*.

Ogawa, N., Satsu, H., Watanabe, H., Fukaya, M., Tsukamoto, Y., Miyamoto, Y., & Shimizu, M. (2000). Acetic Acid Suppresses the Increase in Disaccharidase Activity That Occurs during Culture of Caco-2 Cells. *The Journal of Nutrition*, *130*(3), 507–513. https://doi.org/10.1093/jn/130.3.507

Ohlsson, C., Bygdell, M., Sondén, A., Rosengren, A., & Kindblom, J. M. (2016). Association between excessive BMI increase during puberty and risk of cardiovascular mortality in adult men: A population-based cohort study. *The Lancet Diabetes & Endocrinology*, *4*(12), 1017–1024. https://doi.org/10.1016/S2213-8587(16)30273-X

O'Keefe, S. J. (2019). The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *The Lancet Gastroenterology & Hepatology*, *4*(12), 984–996. https://doi.org/10.1016/S2468-1253(19)30257-2

Olesen, M., Rumessen, J. J., & Gudmand-Høyer, E. (1994). Intestinal transport and fermentation of resistant starch evaluated by the hydrogen breath test. *European Journal of Clinical Nutrition*, *48*(10), 692–701.

Östman, E., Granfeldt, Y., Persson, L., & Björck, I. (2005). Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. *European Journal of Clinical Nutrition*, *59*(9), 983–988. https://doi.org/10.1038/sj.ejcn.1602197

Panlasigui, L. N., & Thompson, L. U. (2006). Blood glucose lowering effects of brown rice in normal and diabetic subjects. *International Journal of Food Sciences and Nutrition*, *57*(3–4), 151–158. https://doi.org/10.1080/09637480500410879

Pantalone, K. M., Hobbs, T. M., Chagin, K. M., Kong, S. X., Wells, B. J., Kattan, M. W., Bouchard, J., Sakurada, B., Milinovich, A., Weng, W., Bauman, J., Misra-Hebert, A. D., Zimmerman, R. S., & Burguera, B. (2017). Prevalence and recognition of obesity and its associated comorbidities: Cross-sectional analysis of electronic health record data from a large US integrated health system. *BMJ Open*, *7*(11), e017583. https://doi.org/10.1136/bmjopen-2017-017583

Parada, J., & Aguilera, J. M. (2012). Effect of native crystalline structure of isolated potato starch on gelatinization behavior and consequently on glycemic response. *Food Research International*, *45*(1), 238–243. https://doi.org/10.1016/j.foodres.2011.10.042

Pascale, A., Marchesi, N., Marelli, C., Coppola, A., Luzi, L., Govoni, S., Giustina, A., & Gazzaruso, C. (2018). Microbiota and metabolic diseases. *Endocrine*, *61*(3), 357–371. https://doi.org/10.1007/s12020-018-1605-5

Patterson, M. A., Maiya, M., & Stewart, M. L. (2020). Resistant Starch Content in Foods Commonly Consumed in the United States: A Narrative Review. *Journal of the Academy of Nutrition and Dietetics*, *120*(2), 230–244. https://doi.org/10.1016/j.jand.2019.10.019

Payne, A. N., Zihler, A., Chassard, C., & Lacroix, C. (2012). Advances and perspectives in in vitro human gut fermentation modeling. *Trends in Biotechnology*, *30*(1), 17–25. https://doi.org/10.1016/j.tibtech.2011.06.011

Pedersen-Bjergaard, U., Høt, U., Kelbæk, H., Schifter, S., Rehfeld, J. F., Faber, J., & Christensen, N. J. (1996). Influence of meal composition on postprandial peripheral plasma concentrations of vasoactive peptides in man. *Scandinavian Journal of Clinical and Laboratory Investigation*, *56*(6), 497–503. https://doi.org/10.3109/00365519609088805

Penn-Marshall, M., Holtzman, G. I., & Barbeau, W. E. (2010). African americans may have to consume more than 12 grams a day of resistant starch to lower their risk for type 2 diabetes. *Journal of Medicinal Food*, *13*(4), 999–1004. https://doi.org/10.1089/jmf.2009.0195

Peters, S. G., Pomare, E. W., & Fisher, C. A. (1992). Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. *Gut*, *33*(9), 1249–1252. https://doi.org/10.1136/gut.33.9.1249

Petersen, P. S., & Moran, T. H. (2013). Regulation | Anorexigenic and Orexigenic Gut Peptides. In J. Jez (Ed.), *Encyclopedia of Biological Chemistry III (Third Edition)* (pp. 524–529). Elsevier. https://doi.org/10.1016/B978-0-12-819460-7.00508-9

Peterson, C. M., Beyl, R. A., Marlatt, K. L., Martin, C. K., Aryana, K. J., Marco, M. L., Martin, R. J., Keenan, M. J., & Ravussin, E. (2018). Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: A randomized controlled trial. *The American Journal of Clinical Nutrition*, *108*(3), 492–501. https://doi.org/10.1093/ajcn/nqy121

Petropoulou, K., Salt, L. J., Edwards, C. H., Warren, F. J., Garcia-Perez, I., Chambers, E. S., Alshaalan, R., Khatib, M., Perez-Moral, N., Cross, K. L., Kellingray, L., Stanley, R., Koev, T., Khimyak, Y. Z., Narbad, A., Penney, N., Serrano-Contreras, J. I., Charalambides, M. N., Miguens Blanco, J., … Frost, G. S. (2020). A natural mutation in Pisum sativum L. (pea) alters starch assembly and improves glucose homeostasis in humans. *Nature Food*, *1*(11), Article 11. https://doi.org/10.1038/s43016-020-00159-8

Pietiläinen, K. H., Kaprio, J., Borg, P., Plasqui, G., Yki-Järvinen, H., Kujala, U. M., Rose, R. J., Westerterp, K. R., & Rissanen, A. (2008). Physical inactivity and obesity: A vicious circle. *Obesity (Silver Spring, Md.)*, *16*(2), 409. https://doi.org/10.1038/oby.2007.72

Pingitore, A., Chambers, E. S., Hill, T., Maldonado, I. R., Liu, B., Bewick, G., Morrison, D. J., Preston, T., Wallis, G. A., Tedford, C., Castañera González, R., Huang, G. C., Choudhary, P., Frost, G., & Persaud, S. J. (2017). The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. *Diabetes, Obesity and Metabolism*, *19*(2), 257–265. https://doi.org/10.1111/dom.12811

Pingitore, A., Gonzalez-Abuin, N., Ruz-Maldonado, I., Huang, G. C., Frost, G., & Persaud, S. J. (2019). Short chain fatty acids stimulate insulin secretion and reduce apoptosis in mouse and human islets in vitro: Role of free fatty acid receptor 2. *Diabetes, Obesity and Metabolism*, *21*(2), 330–339. https://doi.org/10.1111/dom.13529

Poitout, V., Amyot, J., Semache, M., Zarrouki, B., Hagman, D., & Fontés, G. (2010). Glucolipotoxicity of the pancreatic beta cell. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1801*(3), 289–298. https://doi.org/10.1016/j.bbalip.2009.08.006

Polyviou, T., MacDougall, K., Chambers, E. S., Viardot, A., Psichas, A., Jawaid, S., Harris, H. C., Edwards, C. A., Simpson, L., Murphy, K. G., Zac-Varghese, S. E. K., Blundell, J. E., Dhillo, W. S., Bloom, S. R., Frost, G. S., Preston, T., Tedford, M. C., & Morrison, D. J. (2016). Randomised clinical study: Inulin short-chain fatty acid esters for targeted delivery of short-chain fatty acids to the human colon. *Alimentary Pharmacology & Therapeutics*, *44*(7), 662–672. https://doi.org/10.1111/apt.13749

Popkin, B. M., Corvalan, C., & Grummer-Strawn, L. M. (2020). Dynamics of the double burden of malnutrition and the changing nutrition reality. *The Lancet*, *395*(10217), 65–74. https://doi.org/10.1016/S0140-6736(19)32497-3

Popovich, D. G., Jenkins, D. J., Kendall, C. W., Dierenfeld, E. S., Carroll, R. W., Tariq, N., & Vidgen, E. (1997). The western lowland gorilla diet has implications for the health of humans and other hominoids. *The Journal of Nutrition*, *127*(10), 2000–2005. https://doi.org/10.1093/jn/127.10.2000

Pouteau, E., Vahedi, K., Messing, B., Flourié, B., Nguyen, P., Darmaun, D., & Krempf, M. (1998). Production rate of acetate during colonic fermentation of lactulose: A stable-isotope study in humans. *The American Journal of Clinical Nutrition*, *68*(6), 1276–1283. https://doi.org/10.1093/ajcn/68.6.1276

Pramfalk, C., Pavlides, M., Banerjee, R., McNeil, C. A., Neubauer, S., Karpe, F., & Hodson, L. (2015). Sex-Specific Differences in Hepatic Fat Oxidation and Synthesis May Explain the Higher Propensity for NAFLD in Men. *The Journal of Clinical Endocrinology and Metabolism*, *100*(12), 4425–4433. https://doi.org/10.1210/jc.2015-2649

Priyadarshini, M., Villa, S. R., Fuller, M., Wicksteed, B., Mackay, C. R., Alquier, T., Poitout, V., Mancebo, H., Mirmira, R. G., Gilchrist, A., & Layden, B. T. (2015). An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. *Molecular Endocrinology*, *29*(7), 1055–1066. https://doi.org/10.1210/me.2015-1007

Prospective Studies Collaboration. (2009). Body-mass index and cause-specific mortality in 900 000 adults: Collaborative analyses of 57 prospective studies. *The Lancet*, *373*(9669), 1083–1096. https://doi.org/10.1016/S0140-6736(09)60318-4

Psichas, A., Reimann, F., & Gribble, F. (2015). Gut chemosensing mechanisms. *The Journal of Clinical Investigation*, *125*(3). https://doi.org/10.1172/JCI76309

Psichas, A., Sleeth, M. L., Murphy, K. G., Brooks, L., Bewick, G. A., Hanyaloglu, A. C., Ghatei, M. A., Bloom, S. R., & Frost, G. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *International Journal of Obesity (2005)*, *39*(3), 424–429. https://doi.org/10.1038/ijo.2014.153

Public Health England. (2020). *National Diet and Nutrition Survey*. GOV.UK. https://www.gov.uk/government/collections/national-diet-and-nutrition-survey

Public Health England, & Food Standards Agency. (2021, September). *National Diet and Nutrition Survey: Diet, nutrition and physical activity*. Public Health England. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1019663/Follow_up_stud_2020_main_report.pdf

Pugh, J. E., Cai, M., Altieri, N., & Frost, G. (2023). A comparison of the effects of resistant starch types on glycemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis. *Frontiers in Nutrition*, *10*. https://www.frontiersin.org/articles/10.3389/fnut.2023.1118229

Pyke, C., Heller, R. S., Kirk, R. K., Ørskov, C., Reedtz-Runge, S., Kaastrup, P., Hvelplund, A., Bardram, L., Calatayud, D., & Knudsen, L. B. (2014). GLP-1 Receptor Localization

in Monkey and Human Tissue: Novel Distribution Revealed With Extensively Validated Monoclonal Antibody. *Endocrinology*, *155*(4), 1280–1290. https://doi.org/10.1210/en.2013-1934

Qiu, M.-H., Liu, W., Qu, W.-M., Urade, Y., Lu, J., & Huang, Z.-L. (2012). The Role of Nucleus Accumbens Core/Shell in Sleep-Wake Regulation and their Involvement in Modafinil-Induced Arousal. *PLOS ONE*, *7*(9), e45471. https://doi.org/10.1371/journal.pone.0045471

Raben, A., Tagliabue, A., Christensen, N. J., Madsen, J., Holst, J. J., & Astrup, A. (1994). Resistant starch: The effect on postprandial glycemia, hormonal response, and satiety. *The American Journal of Clinical Nutrition*, *60*(4), 544–551. https://doi.org/10.1093/ajcn/60.4.544

Rahat-Rozenbloom, S., Fernandes, J., Cheng, J., Gloor, G. B., & Wolever, T. M. (2017). The acute effects of inulin and resistant-starch on postprandial serum short-chain fatty acids and second-meal glycaemic response in lean and overweight humans. *European Journal of Clinical Nutrition*, *71*(2), 227–233. https://doi.org/10.1038/ejcn.2016.248

Raigond, P., Dutt, S., & Singh, B. (2019). *Resistant Starch in Food* (pp. 815–846). https://doi.org/10.1007/978-3-319-78030-6_30

Ramirez, J., Guarner, F., Bustos Fernandez, L., Maruy, A., Sdepanian, V. L., & Cohen, H. (2020). Antibiotics as Major Disruptors of Gut Microbiota. *Frontiers in Cellular and Infection Microbiology*, *10*. https://doi.org/10.3389/fcimb.2020.572912

Ratnayake, W. S., & Jackson, D. S. (2008). Chapter 5 Starch Gelatinization. In *Advances in Food and Nutrition Research* (Vol. 55, pp. 221–268). Academic Press. https://doi.org/10.1016/S1043-4526(08)00405-1

Reader, D. M., O'connell, B. S., Johnson, M. L., & Franz, M. (2002). Glycemic and Insulinemic Response of Subjects with Type 2 Diabetes after Consumption of Three

Energy Bars. *Journal of the American Dietetic Association*, *102*(8), 1139–1142. https://doi.org/10.1016/S0002-8223(02)90253-1

Reas, D. L., Nygård, J. F., Svensson, E., Sørensen, T., & Sandanger, I. (2007). Changes in body mass index by age, gender, and socio-economic status among a cohort of Norwegian men and women (1990–2001). *BMC Public Health*, *7*(1), 269. https://doi.org/10.1186/1471-2458-7-269

Rechkemmer, G., Rönnau, K., & Engelhardt, W. V. (1988). Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. *Comparative Biochemistry and Physiology Part A: Physiology*, *90*(4), 563–568. https://doi.org/10.1016/0300-9629(88)90668-8

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., Flint, H. J., & Louis, P. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME Journal*, *8*(6), 1323–1335. Scopus. https://doi.org/10.1038/ismej.2014.14

Reimer, R. A., Maurer, A. D., Eller, L. K., Hallam, M. C., Shaykhutdinov, R., Vogel, H. J., & Weljie, A. M. (2012). Satiety Hormone and Metabolomic Response to an Intermittent High Energy Diet Differs in Rats Consuming Long-Term Diets High in Protein or Prebiotic Fiber. *Journal of Proteome Research*, *11*(8), 4065–4074. https://doi.org/10.1021/pr300487s

Reynolds, A. N., Akerman, A. P., & Mann, J. (2020). Dietary fibre and whole grains in diabetes management: Systematic review and meta-analyses. *PLoS Medicine*, *17*(3). https://doi.org/10.1371/journal.pmed.1003053

Reynolds, A. N., Mann, J., Cummings, J., Winter, N., Mete, E., & Morenga, L. T. (2019). Carbohydrate quality and human health: A series of systematic reviews and meta-analyses. *The Lancet*, *393*(10170), 434–445. https://doi.org/10.1016/S0140-6736(18)31809-9

Reynolds, A. N., Mann, J., Elbalshy, M., Mete, E., Robinson, C., Oey, I., Silcock, P., Downes, N., Perry, T., & Te Morenga, L. (2020). Wholegrain Particle Size Influences Postprandial Glycemia in Type 2 Diabetes: A Randomized Crossover Study Comparing Four Wholegrain Breads. *Diabetes Care*, *43*(2), 476–479. https://doi.org/10.2337/dc19-1466

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., … Gordon, J. I. (2013). Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice. *Science (New York, N.Y.)*, *341*(6150), 10.1126/science.1241214. https://doi.org/10.1126/science.1241214

Riva, M., Fessas, D., & Schiraldi, A. (2000). Starch Retrogradation in Cooked Pasta and Rice. *Cereal Chemistry*, *77*(4), 433–438. https://doi.org/10.1094/CCHEM.2000.77.4.433

Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H., & Frayn, K. N. (2005). Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *The American Journal of Clinical Nutrition*, *82*(3), 559–567. https://doi.org/10.1093/ajcn/82.3.559

Robertson, M. D., Currie, J. M., Morgan, L. M., Jewell, D. P., & Frayn, K. N. (2003). Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. *Diabetologia*, *46*(5), 659–665. https://doi.org/10.1007/s00125-003-1081-0

Robertson, M. D., Wright, J. W., Loizon, E., Debard, C., Vidal, H., Shojaee-Moradie, F., Russell-Jones, D., & Umpleby, A. M. (2012). Insulin-Sensitizing Effects on Muscle and Adipose Tissue after Dietary Fiber Intake in Men and Women with Metabolic

Syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *97*(9), 3326–3332. https://doi.org/10.1210/jc.2012-1513

Robertson, T. M., Brown, J. E., Fielding, B. A., & Robertson, M. D. (2021). The cumulative effects of chilling and reheating a carbohydrate-based pasta meal on the postprandial glycaemic response: A pilot study. *European Journal of Clinical Nutrition*, *75*(3), Article 3. https://doi.org/10.1038/s41430-020-00736-x

Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine*, *48*(3), e219. https://doi.org/10.1038/emm.2016.6

Roediger, W. E. W. (2008). Review article: Nitric oxide from dysbiotic bacterial respiration of nitrate in the pathogenesis and as a target for therapy of ulcerative colitis. *Alimentary Pharmacology and Therapeutics*, *27*(7), 531–541. Scopus. https://doi.org/10.1111/j.1365-2036.2008.03612.x

Rosas-Pérez, A. M., Honma, K., & Goda, T. (2020). Sustained effects of resistant starch on the expression of genes related to carbohydrate digestion/absorption in the small intestine. *International Journal of Food Sciences and Nutrition*, *71*(5), 572–580. https://doi.org/10.1080/09637486.2019.1711362

Rosenbaum, M., & Leibel, R. L. (2010). Adaptive thermogenesis in humans. *International Journal of Obesity (2005)*, *34*(0 1), S47. https://doi.org/10.1038/ijo.2010.184

Roshanravan, N., Mahdavi, R., Alizadeh, E., Jafarabadi, M. A., Hedayati, M., Ghavami, A., Alipour, S., Alamdari, N. M., Barati, M., & Ostadrahimi, A. (2017). Effect of Butyrate and Inulin Supplementation on Glycemic Status, Lipid Profile and Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes: A Randomized Double-Blind, Placebo-Controlled Trial. *Hormone and Metabolic Research*, *49*(11), 886–891. https://doi.org/10.1055/s-0043-119089

Roshanravan, N., Mahdavi, R., Jafarabadi, M. A., Alizadeh, E., Ghavami, A., Saadat, Y. R., Alamdari, N. M., Dastouri, M. R., Alipour, S., & Ostadrahimi, A. (2018). The effects of sodium butyrate and high-performance inulin supplementation on the promotion of gut bacterium Akkermansia muciniphila growth and alterations in miR-375 and KLF5 expression in type 2 diabetic patients: A randomized, double-blind, placebo-controlled trial. *European Journal of Integrative Medicine*, *18*, 1–7. https://doi.org/10.1016/j.eujim.2017.12.011

Rubino, F., Cummings, D. E., Eckel, R. H., Cohen, R. V., Wilding, J. P. H., Brown, W. A., Stanford, F. C., Batterham, R. L., Farooqi, I. S., Farpour-Lambert, N. J., Roux, C. W. le, Sattar, N., Baur, L. A., Morrison, K. M., Misra, A., Kadowaki, T., Tham, K. W., Sumithran, P., Garvey, W. T., … Mingrone, G. (2025). Definition and diagnostic criteria of clinical obesity. *The Lancet Diabetes & Endocrinology*, *13*(3), 221–262. https://doi.org/10.1016/S2213-8587(24)00316-4

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., & IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Sagili, V. S., Chakrabarti, P., Jayanty, S., Kardile, H., & Sathuvalli, V. (2022). The Glycemic Index and Human Health with an Emphasis on Potatoes. *Foods*, *11*(15), 2302. https://doi.org/10.3390/foods11152302

Sahuri-Arisoylu, M., Brody, L. P., Parkinson, J. R., Parkes, H., Navaratnam, N., Miller, A. D., Thomas, E. L., Frost, G., & Bell, J. D. (2016). Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *International Journal of Obesity*, *40*(6), Article 6. https://doi.org/10.1038/ijo.2016.23

Saini, R., & Badole, S. L. (2015). Chapter 28—Bioactive Compounds Increase Incretins with Beneficial Effects on Diabetes. In R. R. Watson & B. B. Dokken (Eds.), *Glucose Intake and Utilization in Pre-Diabetes and Diabetes* (pp. 349–353). Academic Press. https://doi.org/10.1016/B978-0-12-800093-9.00028-4

Salazar, N., Arboleya, S., Fernández-Navarro, T., de los Reyes-Gavilán, C. G., Gonzalez, S., & Gueimonde, M. (2019). Age-Associated Changes in Gut Microbiota and Dietary Components Related with the Immune System in Adulthood and Old Age: A Cross-Sectional Study. *Nutrients*, *11*(8), 1765. https://doi.org/10.3390/nu11081765

Salazar, N., Ponce-Alonso, M., Garriga, M., Sanchez-Carrillo, S., Hernández-Barranco, A. M., Redruello, B., Fernández, M., Botella-Carretero, J. I., Vega-Piñero, B., Galeano, J., Zamora, J., Ferrer, M., de Los Reyes-Gavilán, C. G., & Del Campo, R. (2022). Fecal Metabolome and Bacterial Composition in Severe Obesity: Impact of Diet and Bariatric Surgery. *Gut Microbes*, *14*(1), 2106102. https://doi.org/10.1080/19490976.2022.2106102

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, *414*(6865), 799–806. https://doi.org/10.1038/414799a

Samuel, B. S., Shaito, A., Motoike, T., Rey, F. E., Backhed, F., Manchester, J. K., Hammer, R. E., Williams, S. C., Crowley, J., Yanagisawa, M., & Gordon, J. I. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(43), 16767–16772. https://doi.org/10.1073/pnas.0808567105

Sánchez-Tapia, M., Hernández-Velázquez, I., Pichardo-Ontiveros, E., Granados-Portillo, O., Gálvez, A., R Tovar, A., & Torres, N. (2020). Consumption of Cooked Black Beans Stimulates a Cluster of Some Clostridia Class Bacteria Decreasing Inflammatory

Response and Improving Insulin Sensitivity. *Nutrients*, *12*(4), Article 4. https://doi.org/10.3390/nu12041182

Sandberg, J. C., Björck, I. M. E., & Nilsson, A. C. (2017). Effects of whole grain rye, with and without resistant starch type 2 supplementation, on glucose tolerance, gut hormones, inflammation and appetite regulation in an 11-14.5 hour perspective; a randomized controlled study in healthy subjects. *Nutrition Journal*, *16*(1). Scopus. https://doi.org/10.1186/s12937-017-0246-5

Sandberg, J. C., Björck, I. M. E., & Nilsson, A. C. (2018). Impact of rye-based evening meals on cognitive functions, mood and cardiometabolic risk factors: A randomized controlled study in healthy middle-aged subjects. *Nutrition Journal*, *17*(1), 102. https://doi.org/10.1186/s12937-018-0412-4

Sandoval, D. A., & Patti, M. E. (2023). Glucose metabolism after bariatric surgery: Implications for T2DM remission and hypoglycaemia. *Nature Reviews Endocrinology*, *19*(3), 164–176. https://doi.org/10.1038/s41574-022-00757-5

Schellenberg, E. S., Dryden, D. M., Vandermeer, B., Ha, C., & Korownyk, C. (2013). Lifestyle Interventions for Patients With and at Risk for Type 2 Diabetes. *Annals of Internal Medicine*, *159*(8), 543–551. https://doi.org/10.7326/0003-4819-159-8-201310150-00007

Scheppach, W., Cummings, J. H., Branch, W. J., & Schrezenmeir, J. (1988). Effect of gut-derived acetate on oral glucose tolerance in man. *Clinical Science (London, England: 1979)*, *75*(4), 355–361. https://doi.org/10.1042/cs0750355

Scheppach, W., Wiggins, H. S., Halliday, D., Self, R., Howard, J., Branch, W. J., Schrezenmeir, J., & Cummings, J. H. (1988). Effect of gut-derived acetate on glucose turnover in man. *Clinical Science*, *75*(4), 363–370. https://doi.org/10.1042/cs0750363

Schofield, C. J., & Sutherland, C. (2012). Disordered insulin secretion in the development of insulin resistance and Type 2 diabetes. *Diabetic Medicine*, *29*(8), 972–979. https://doi.org/10.1111/j.1464-5491.2012.03655.x

Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition Clinical Nutrition*, *39 Suppl 1*, 5–41.

Schwartz, S. E., Levine, R. A., Singh, A., Scheidecker, J. R., & Track, N. S. (1982). Sustained pectin ingestion delays gastric emptying. *Gastroenterology*, *83*(4), 812–817.

Scott, K. M., Bruffaerts, R., Simon, G. E., Alonso, J., Angermeyer, M., de Girolamo, G., Demyttenaere, K., Gasquet, I., Haro, J. M., Karam, E., Kessler, R. C., Levinson, D., Medina Mora, M. E., Oakley Browne, M. A., Ormel, J., Villa, J. P., Uda, H., & Von Korff, M. (2008). Obesity and mental disorders in the general population: Results from the world mental health surveys. *International Journal of Obesity*, *32*(1), 192–200. https://doi.org/10.1038/sj.ijo.0803701

Scott, K. P., Duncan, S. H., & Flint, H. J. (2008). Dietary fibre and the gut microbiota. *Nutrition Bulletin*, *33*(3), 201–211. https://doi.org/10.1111/j.1467-3010.2008.00706.x

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Sharma, R. B., & Alonso, L. C. (2014). Lipotoxicity in the Pancreatic Beta Cell: Not Just Survival and Function, but Proliferation as Well? *Current Diabetes Reports*, *14*(6), 492. https://doi.org/10.1007/s11892-014-0492-2

Shen, L., Keenan, M. J., Raggio, A., Williams, C., & Martin, R. J. (2011). Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. *Molecular Nutrition & Food Research*, *55*(10), 1499–1508. https://doi.org/10.1002/mnfr.201000605

Shi, Q., Nong, K., Vandvik, P. O., Guyatt, G. H., Schnell, O., Rydén, L., Marx, N., Brosius, F. C., Mustafa, R. A., Agarwal, A., Zou, X., Mao, Y., Asadollahifar, A., Chowdhury, S. R., Zhai, C., Gupta, S., Gao, Y., Lima, J. P., Numata, K., … Li, S. (2023). Benefits and harms of drug treatment for type 2 diabetes: Systematic review and network meta-analysis of randomised controlled trials. *BMJ*, *381*, e074068. https://doi.org/10.1136/bmj-2022-074068

Shishehbor, F., Mansoori, A., & Shirani, F. (2017). Vinegar consumption can attenuate postprandial glucose and insulin responses; a systematic review and meta-analysis of clinical trials. *Diabetes Research and Clinical Practice*, *127*, 1–9. https://doi.org/10.1016/j.diabres.2017.01.021

Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., & Shulman, R. G. (1990). Quantitation of Muscle Glycogen Synthesis in Normal Subjects and Subjects with Non-Insulin-Dependent Diabetes by 13C Nuclear Magnetic Resonance Spectroscopy. *New England Journal of Medicine*, *322*(4), 223–228. https://doi.org/10.1056/NEJM199001253220403

Siemieniuk, R., & Guyatt, G. (n.d.). *What is GRADE?* | *BMJ Best Practice*. Retrieved 8 August 2023, from https://bestpractice.bmj.com/info/toolkit/learn-ebm/what-is-grade/

Sieuwerts, S., Bok, F. A. M. de, Hugenholtz, J., & Vlieg, J. E. T. van H. (2008). Unraveling Microbial Interactions in Food Fermentations: From Classical to Genomics Approaches. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.00113-08

Singh, V., Park, Y.-J., Lee, G., Unno, T., & Shin, J.-H. (2022). Dietary regulations for microbiota dysbiosis among post-menopausal women with type 2 diabetes. *Critical Reviews in Food Science and Nutrition*, *0*(0), 1–16. https://doi.org/10.1080/10408398.2022.2076651

Slavin, J., & Green, H. (2007). Dietary fibre and satiety. *Nutrition Bulletin*, *32*(s1), 32–42. https://doi.org/10.1111/j.1467-3010.2007.00603.x

Slavin, J. L., & Lloyd, B. (2012). Health Benefits of Fruits and Vegetables1. *Advances in Nutrition*, *3*(4), 506–516. https://doi.org/10.3945/an.112.002154

Sloth, B., Holst, J. J., Flint, A., Gregersen, N. T., & Astrup, A. (2007). Effects of PYY1–36 and PYY3–36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. *American Journal of Physiology-Endocrinology and Metabolism*, *292*(4), E1062–E1068. https://doi.org/10.1152/ajpendo.00450.2006

Sonia, S., Witjaksono, F., & Ridwan, R. (2015). Effect of cooling of cooked white rice on resistant starch content and glycemic response. *Asia Pacific Journal of Clinical Nutrition*, *24*(4), 620–625. https://doi.org/10.6133/apjcn.2015.24.4.13

Sonnenburg, J., & Bäckhed, F. (2016). Diet–microbiota interactions as moderators of human metabolism. *Nature*, *535*, 56–64. https://doi.org/10.1038/nature18846

Spreckley, E., & Murphy, K. G. (2015). The L-Cell in Nutritional Sensing and the Regulation of Appetite. *Frontiers in Nutrition*, *2*, 23. https://doi.org/10.3389/fnut.2015.00023

Srour, B., Fezeu, L. K., Kesse-Guyot, E., Allès, B., Debras, C., Druesne-Pecollo, N., Chazelas, E., Deschasaux, M., Hercberg, S., Galan, P., Monteiro, C. A., Julia, C., & Touvier, M. (2020). Ultraprocessed Food Consumption and Risk of Type 2 Diabetes Among Participants of the NutriNet-Santé Prospective Cohort. *JAMA Internal Medicine*, *180*(2), 283–291. https://doi.org/10.1001/jamainternmed.2019.5942

Stedman, M., Lunt, M., Davies, M., Livingston, M., Duff, C., Fryer, A., Anderson, S. G., Gadsby, R., Gibson, M., Rayman, G., & Heald, A. (2020). Cost of hospital treatment of type 1 diabetes (T1DM) and type 2 diabetes (T2DM) compared to the non-diabetes population: A detailed economic evaluation. *BMJ Open*, *10*(5), e033231. https://doi.org/10.1136/bmjopen-2019-033231

Stephenson, J., Smith, C. M., Kearns, B., Haywood, A., & Bissell, P. (2021). The association between obesity and quality of life: A retrospective analysis of a large-scale population-based cohort study. *BMC Public Health*, *21*(1), 1990. https://doi.org/10.1186/s12889-021-12009-8

Stewart, M. L., Wilcox, M. L., Bell, M., Buggia, M. A., & Maki, K. C. (2018). Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. *Nutrients*, *10*(2). https://doi.org/10.3390/nu10020129

Stewart, M. L., & Zimmer, J. P. (2017). A High Fiber Cookie Made with Resistant Starch Type 4 Reduces Post-Prandial Glucose and Insulin Responses in Healthy Adults. *Nutrients*, *9*(3). https://doi.org/10.3390/nu9030237

Stewart, M. L., & Zimmer, J. P. (2018). Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: A double-blind, randomized, controlled trial. *Nutrition*, *53*, 59–63. https://doi.org/10.1016/j.nut.2018.01.002

Stöckli, J., Fazakerley, D. J., & James, D. E. (2011). GLUT4 exocytosis. *Journal of Cell Science*, *124*(24), 4147–4159. https://doi.org/10.1242/jcs.097063

Sukkar, A. (2021). *The impact of acute oral sodium propionate supplementation on energy metabolism*. https://doi.org/10.25560/91753

Sukkar, A. H., Lett, A. M., Frost, G., & Chambers, E. S. (2019). Regulation of energy expenditure and substrate oxidation by short-chain fatty acids. *Journal of Endocrinology*, *242*(2), R1–R8. https://doi.org/10.1530/JOE-19-0098

Sun, H., Ma, X., Zhang, S., Zhao, D., & Liu, X. (2018). Resistant starch produces antidiabetic effects by enhancing glucose metabolism and ameliorating pancreatic dysfunction in type 2 diabetic rats. *International Journal of Biological Macromolecules*, *110*, 276–284. Scopus. https://doi.org/10.1016/j.ijbiomac.2017.11.162

Surmely, J.-F., Schneiter, P., Henry, S., Paquot, N., Jequier, E., & Tappy, L. (1999). Effects of glucagon in the control of endogenous glucose production in man. *Nutrition*, *15*(4), 267–273. https://doi.org/10.1016/S0899-9007(99)00036-2

Suzuki, K., Iwasaki, K., Murata, Y., Harada, N., S, Y., Hamasaki, A., Shibue, K., Joo, E., Sankoda, A., Fujiwara, Y., Hayashi, Y., & Inagaki, N. (2018). Distribution and hormonal characterization of primary murine L cells throughout the gastrointestinal tract. *Journal of Diabetes Investigation*, *9*(1). https://doi.org/10.1111/jdi.12681

Szczerba, E., Barbaresko, J., Schiemann, T., Stahl-Pehe, A., Schwingshackl, L., & Schlesinger, S. (2023). Diet in the management of type 2 diabetes: Umbrella review of systematic reviews with meta-analyses of randomised controlled trials. *BMJ Medicine*, *2*(1). https://doi.org/10.1136/bmjmed-2023-000664

Tak, Y. J., & Lee, S. Y. (2021). Long-Term Efficacy and Safety of Anti-Obesity Treatment: Where Do We Stand? *Current Obesity Reports*, *10*(1), 14–30. https://doi.org/10.1007/s13679-020-00422-w

Tan, L., Duan, W., Chen, M., Mei, Y., Qi, X., & Zhang, Y. (2022). Naturally cultured high resistant starch rice improved postprandial glucose levels in patients with type 2 diabetes: A randomized, double-blinded, controlled trial. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1019868

Tang, C., Ahmed, K., Gille, A., Lu, S., Gröne, H.-J., Tunaru, S., & Offermanns, S. (2015). Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nature Medicine*, *21*(2), 173–177. https://doi.org/10.1038/nm.3779

Tang-Christensen, M., Larsen, P. J., Goke, R., Fink-Jensen, A., Jessop, D. S., Moller, M., & Sheikh, S. P. (1996). Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *271*(4), R848–R856. https://doi.org/10.1152/ajpregu.1996.271.4.R848

Taylor, R. (2013). Type 2 Diabetes: Etiology and reversibility. *Diabetes Care*, *36*(4), 1047–1055. https://doi.org/10.2337/dc12-1805

The Emerging Risk Factors Collaboration, T. E. R. F. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *The Lancet*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Thompson, G. N., Walter, J. H., Bresson, J.-L., Ford, G. C., Lyonnet, S. L., Chalmers, R. A., Saudubray, J.-M., Leonard, J. V., & Halliday, D. (1990). Sources of propionate in inborn errors of propionate metabolism. *Metabolism*, *39*(11), 1133–1137. https://doi.org/10.1016/0026-0495(90)90084-P

Thompson, S. V., Hannon, B. A., An, R., & Holscher, H. D. (2017). Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: A systematic review and meta-analysis of randomized controlled trials1. *The American Journal of Clinical Nutrition*, *106*(6), 1514–1528. https://doi.org/10.3945/ajcn.117.163246

Thorens, B. (2015). GLUT2, glucose sensing and glucose homeostasis. *Diabetologia*, *58*(2), 221–232. https://doi.org/10.1007/s00125-014-3451-1

Thorens, B., Sarkar, H. K., Kaback, H. R., & Lodish, H. F. (1988). Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and β-pancreatic islet cells. *Cell*, *55*(2), 281–290. https://doi.org/10.1016/0092-8674(88)90051-7

Thorsen, K., Drengstig, T., & Ruoff, P. (2014). Transepithelial glucose transport and Na+/K+ homeostasis in enterocytes: An integrative model. *American Journal of Physiology-Cell Physiology*, *307*(4), C320–C337. https://doi.org/10.1152/ajpcell.00068.2013

Tian, J., Ogawa, Y., Shi, J., Chen, S., Zhang, H., Liu, D., & Ye, X. (2019). The microstructure of starchy food modulates its digestibility. *Critical Reviews in Food Science and Nutrition*, *59*(19), 3117–3128. https://doi.org/10.1080/10408398.2018.1484341

Tirosh, A., Calay, E. S., Tuncman, G., Claiborn, K. C., Inouye, K. E., Eguchi, K., Alcala, M., Rathaus, M., Hollander, K. S., Ron, I., Livne, R., Heianza, Y., Qi, L., Shai, I., Garg, R., & Hotamisligil, G. S. (2019). The short-chain fatty acid propionate increases glucagon and FABP4 production, impairing insulin action in mice and humans. *Science Translational Medicine*, *11*(489), eaav0120. https://doi.org/10.1126/scitranslmed.aav0120

Tison, G. H., Avram, R., Kuhar, P., Abreau, S., Marcus, G. M., Pletcher, M. J., & Olgin, J. E. (2020). Worldwide Effect of COVID-19 on Physical Activity: A Descriptive Study. *Annals of Internal Medicine*, *173*(9), 767–770. https://doi.org/10.7326/M20-2665

Todd, J. F., & Bloom, S. R. (2007). Incretins and other peptides in the treatment of diabetes. *Diabetic Medicine*, *24*(3), 223–232. https://doi.org/10.1111/j.1464-5491.2006.02071.x

Todesco, T., Rao, A. V., Bosello, O., & Jenkins, D. J. (1991). Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. *The American Journal of Clinical Nutrition*, *54*(5), 860–865. https://doi.org/10.1093/ajcn/54.5.860

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F., & Gribble, F. M. (2012). Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein–Coupled Receptor FFAR2. *Diabetes*, *61*(2), 364–371. https://doi.org/10.2337/db11-1019

Topping, D. L., & Clifton, P. M. (2001). Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. *Physiological Reviews*, *81*(3), 1031–1064. https://doi.org/10.1152/physrev.2001.81.3.1031

Tovar, J., Sayago-Ayerdi, S., Peñalver, C., Paredes-Lopez, O., & Bello-Pérez, L. (2003). In Vitro Starch Hydrolysis Index and Predicted Glycemic Index of CornTortilla, Black Beans (Phaseolus vulgaris L.), and Mexican “taco”. *Cereal Chemistry*, *80*, 533–535. https://doi.org/10.1094/CCHEM.2003.80.5.533

Tran, L. T., Park, S., Kim, S. K., Lee, J. S., Kim, K. W., & Kwon, O. (2022). Hypothalamic control of energy expenditure and thermogenesis. *Experimental & Molecular Medicine*, *54*(4), 358–369. https://doi.org/10.1038/s12276-022-00741-z

Trowell, H. (1972). Crude fibre, dietary fibre and atherosclerosis. *Atherosclerosis*, *16*(1), 138–140. https://doi.org/10.1016/0021-9150(72)90017-2

Troy, S., Soty, M., Ribeiro, L., Laval, L., Migrenne, S., Fioramonti, X., Pillot, B., Fauveau, V., Aubert, R., Viollet, B., Foretz, M., Leclerc, J., Duchampt, A., Zitoun, C., Thorens, B., Magnan, C., Mithieux, G., & Andreelli, F. (2008). Intestinal Gluconeogenesis Is a Key Factor for Early Metabolic Changes after Gastric Bypass but Not after Gastric Lap-Band in Mice. *Cell Metabolism*, *8*(3), 201–211. https://doi.org/10.1016/j.cmet.2008.08.008

Truesdale, K. P., Stevens, J., Lewis, C. E., Schreiner, P. J., Loria, C. M., & Cai, J. (2006). Changes in risk factors for cardiovascular disease by baseline weight status in young adults who maintain or gain weight over 15 years: The CARDIA study. *International Journal of Obesity*, *30*(9), Article 9. https://doi.org/10.1038/sj.ijo.0803307

Tucker, A. J., Vandermey, J. S., Robinson, L. E., Graham, T. E., Bakovic, M., & Duncan, A. M. (2014). Effects of breads of varying carbohydrate quality on postprandial glycaemic, incretin and lipidaemic response after first and second meals in adults with diet-controlled type 2 diabetes. *Journal of Functional Foods*, *6*, 116–125. https://doi.org/10.1016/j.jff.2013.09.025

Turton, M. D., O’Shea, D., Gunn, I., Beak, S. A., Edwards, C. M. B., Meeran, K., Choi, S. J., Taylor, G. M., Heath, M. M., Lambert, P. D., Wilding, J. P. H., Smith, D. M., Ghatei,

M. A., Herbert, J., & Bloom, S. R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*, *379*(6560), 69–72. https://doi.org/10.1038/379069a0

University of Sydney. (n.d.). *Glycemic Index Database*. Retrieved 10 September 2024, from https://glycemicindex.com/gi-search/

van der Beek, C. M., Bloemen, J. G., van den Broek, M. A., Lenaerts, K., Venema, K., Buurman, W. A., & Dejong, C. H. (2015). Hepatic Uptake of Rectally Administered Butyrate Prevents an Increase in Systemic Butyrate Concentrations in Humans. *The Journal of Nutrition*, *145*(9), 2019–2024. https://doi.org/10.3945/jn.115.211193

Van der Beek, C. M., Canfora, E. E., Lenaerts, K., Troost, F. J., Damink, S. W. M. O., Holst, J. J., Masclee, A. A. M., Dejong, C. H. C., & Blaak, E. E. (2016). Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clinical Science (London, England: 1979)*, *130*(22), 2073–2082. https://doi.org/10.1042/CS20160263

Van der Beek, C. M., Canfora, E. E., Lenaerts, K., Troost, F. J., Holst, J. J., Masclee, A. A. M., Dejong, C. H. C., & Blaak, E. E. (2014). Colonic Acetate Infusions Promote Fat Oxidation and Improve Metabolic Parameters in Overweight Males. *Clinical Nutrition*, *33*, S16–S17. https://doi.org/10.1016/S0261-5614(14)50039-8

van der Klaauw, A. A., Keogh, J. M., Henning, E., Trowse, V. M., Dhillo, W. S., Ghatei, M. A., & Farooqi, I. S. (2013). High protein intake stimulates postprandial GLP1 and PYY release. *Obesity (Silver Spring, Md.)*, *21*(8), 1602–1607. https://doi.org/10.1002/oby.20154

van Munster, I. P., Tangerman, A., & Nagengast, F. M. (1994). Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal proliferation. *Digestive Diseases and Sciences*, *39*(4), 834–842. https://doi.org/10.1007/BF02087431

Varghese, S., Rao, S., Khattak, A., Zamir, F., & Chaari, A. (2024). Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. *Nutrients*, *16*(21), Article 21. https://doi.org/10.3390/nu16213663

Vasconcelos, J., Toby, P., Morrison, D., & Frost, G. (2022). *Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults: IPREVENT Clinical Trial Statistical Analysis Plan*. *2*. https://doi.org/10.17632/n33kky5dww.2

Venter, C. S., Vorster, H. H., & Cummings, J. H. (1990). Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *The American Journal of Gastroenterology*, *85*(5), 549–553.

Verbeke, K., Ferchaud-Roucher, V., Preston, T., Small, A. C., Henckaerts, L., Krempf, M., Wang, H., Vonk, R. J., & Priebe, M. G. (2010). Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. *European Journal of Clinical Nutrition*, *64*(7), 678–684. https://doi.org/10.1038/ejcn.2010.92

Vergnaud, A.-C., Norat, T., Romaguera, D., Mouw, T., May, A. M., Romieu, I., Freisling, H., Slimani, N., Boutron-Ruault, M.-C., Clavel-Chapelon, F., Morois, S., Kaaks, R., Teucher, B., Boeing, H., Buijsse, B., Tjønneland, A., Halkjær, J., Overvad, K., Jakobsen, M. U., … Peeters, P. H. (2012). Fruit and vegetable consumption and prospective weight change in participants of the European Prospective Investigation into Cancer and Nutrition–Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home, and Obesity study. *The American Journal of Clinical Nutrition*, *95*(1), 184–193. https://doi.org/10.3945/ajcn.111.019968

Wajchenberg, B. L. (2007). β-Cell Failure in Diabetes and Preservation by Clinical Treatment. *Endocrine Reviews*, *28*(2), 187–218. https://doi.org/10.1210/10.1210/er.2006-0038

Walker, A. W., Duncan, S. H., Harmsen, H. J. M., Holtrop, G., Welling, G. W., & Flint, H. J. (2008). The species composition of the human intestinal microbiota differs between particle-associated and liquid phase communities. *Environmental Microbiology*, *10*(12), 3275–3283. https://doi.org/10.1111/j.1462-2920.2008.01717.x

Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., Brown, D., Stares, M. D., Scott, P., Bergerat, A., Louis, P., McIntosh, F., Johnstone, A. M., Lobley, G. E., Parkhill, J., & Flint, H. J. (2011). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *The ISME Journal*, *5*(2), Article 2. https://doi.org/10.1038/ismej.2010.118

Wang, S., Chao, C., Cai, J., Niu, B., Copeland, L., & Wang, S. (2020). Starch–lipid and starch–lipid–protein complexes: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(3), 1056–1079. https://doi.org/10.1111/1541-4337.12550

Weir, G. C., Gaglia, J., & Joslin, S. B.-W. (2020). Inadequate β-cell mass is essential for the pathogenesis of type 2 diabetes. *The Lancet. Diabetes & Endocrinology*, *8*(3), 249–256. https://doi.org/10.1016/S2213-8587(20)30022-X

Weir, J. B. de V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, *109*(1–2), 1–9. https://doi.org/10.1113/jphysiol.1949.sp004363

Wesolowska-Andersen, A., Brorsson, C. A., Bizzotto, R., Mari, A., Tura, A., Koivula, R., Mahajan, A., Vinuela, A., Tajes, J. F., Sharma, S., Haid, M., Prehn, C., Artati, A., Hong, M.-G., Musholt, P. B., Kurbasic, A., De Masi, F., Tsirigos, K., Pedersen, H. K., … Brunak, S. (2022). Four groups of type 2 diabetes contribute to the etiological and clinical heterogeneity in newly diagnosed individuals: An IMI DIRECT study. *Cell Reports Medicine*, *3*(1), 100477. https://doi.org/10.1016/j.xcrm.2021.100477

White, A. M., & Johnston, C. S. (2007). Vinegar Ingestion at Bedtime Moderates Waking Glucose Concentrations in Adults With Well-Controlled Type 2 Diabetes. *Diabetes Care*, *30*(11), 2814–2815. https://doi.org/10.2337/dc07-1062

White, U., Peterson, C. M., Beyl, R. A., Martin, C. K., & Ravussin, E. (2020). Resistant Starch Has No Effect on Appetite and Food Intake in Individuals with Prediabetes. *Journal of the Academy of Nutrition and Dietetics*, *120*(6), 1034–1041. https://doi.org/10.1016/j.jand.2020.01.017

Williams, E. K., Chang, R. B., Strochlic, D. E., Umans, B. D., Lowell, B. B., & Liberles, S. D. (2016). Sensory neurons that detect stretch and nutrients in the digestive system. *Cell*, *166*(1), 209. https://doi.org/10.1016/j.cell.2016.05.011

Wolever, T. M. S. (2004). Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition*, *91*(2), 295–300. https://doi.org/10.1079/BJN20031054

Wolever, T. M. S. (2006). *The Glycaemic Index: A Physiological Classification of Dietary Carbohydrate*. CABI.

Wolever, T. M. S., Brighenti, F., Royall, D., Jenkins, A. L., & Jenkins, D. J. (1989). Effect of rectal infusion of short chain fatty acids in human subjects. *The American Journal of Gastroenterology*, *84*(9), 1027–1033.

Wolever, T. M. S., Jenkins, D. J., Ocana, A. M., Rao, V. A., & Collier, G. R. (1988). Second-meal effect: Low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. *The American Journal of Clinical Nutrition*, *48*(4), 1041–1047. https://doi.org/10.1093/ajcn/48.4.1041

Wolever, T. M. S., Spadafora, P., & Eshuis, H. (1991). Interaction between colonic acetate and propionate in humans. *The American Journal of Clinical Nutrition*, *53*(3), 681–687. https://doi.org/10.1093/ajcn/53.3.681

Wolfe, B. E., & Dutton, R. J. (2015). Fermented Foods as Experimentally Tractable Microbial Ecosystems. *Cell*, *161*(1), 49–55. https://doi.org/10.1016/j.cell.2015.02.034

World Health Organization. (2011). *Global status report on noncommunicable diseases 2010*. https://iris.who.int/handle/10665/44579

World Health Organization. (2023a, April 23). *Diabetes*. https://www.who.int/news-room/fact-sheets/detail/diabetes

World Health Organization. (2023b, September). *Non communicable diseases*. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

World Health Organization. (2024a). *Mean fasting blood glucose*. https://www.who.int/data/gho/indicator-metadata-registry/imr-details/2380

World Health Organization. (2024b, March 1). *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

World Health Organization. (2024c, June 26). *Physical activity*. https://www.who.int/news-room/fact-sheets/detail/physical-activity

World Health Organization & International Diabetes Federation. (2006). *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: Report of a WHO/IDF consultation*. WHO IRIS. https://iris.who.int/handle/10665/43588

World Obesity Federation. (2024). *World Obesity Atlas 2024: No area of the world is unaffected by the consequences of obesity*. World Obesity Federation. https://www.worldobesity.org/news/world-obesity-atlas-2024

Wu, L., Fritz, J. D., & Powers, A. C. (1998). Different Functional Domains of GLUT2 Glucose Transporter Are Required for Glucose Affinity and Substrate Specificity**This work was supported by grants from the Department of Veterans Affairs Research Service (Career Development Award and Merit Review Award), a fellowship award from the Juvenile Diabetes Foundation International (to L.W.), and the Vanderbilt Diabetes

Research and Training Center (NIH Grant DK-20593). *Endocrinology*, *139*(10), 4205–4212. https://doi.org/10.1210/endo.139.10.6245

Wu, T., Rayner, C. K., Jones, K., & Horowitz, M. (2010). Chapter Three—Dietary Effects on Incretin Hormone Secretion. In G. Litwack (Ed.), *Vitamins & Hormones* (Vol. 84, pp. 81–110). Academic Press. https://doi.org/10.1016/B978-0-12-381517-0.00003-5

Wyatt, G. M., & Horn, N. (1988). Fermentation of resistant food starches by human and rat intestinal bacteria. *Journal of the Science of Food and Agriculture*, *44*(3), 281–288. https://doi.org/10.1002/jsfa.2740440309

Wysham, C., & Shubrook, J. (2020). Beta-cell failure in type 2 diabetes: Mechanisms, markers, and clinical implications. *Postgraduate Medicine*, *132*(8), 676–686. https://doi.org/10.1080/00325481.2020.1771047

Xiong, W., Devkota, L., Zhang, B., Muir, J., & Dhital, S. (2022). Intact cells: "Nutritional capsules" in plant foods. *Comprehensive Reviews in Food Science and Food Safety*, *21*(2), 1198–1217. https://doi.org/10.1111/1541-4337.12904

Xu, J., Ma, Z., Li, X., Liu, L., & Hu, X. (2020). A more pronounced effect of type III resistant starch vs. Type II resistant starch on ameliorating hyperlipidemia in high fat diet-fed mice is associated with its supramolecular structural characteristics. *Food & Function*, *11*(3), 1982–1995. https://doi.org/10.1039/C9FO02025J

Yamada, Y., Hosoya, S., Nishimura, S., Tanaka, T., Kajimoto, Y., Nishimura, A., & Kajimoto, O. (2005). Effect of Bread Containing Resistant Starch on Postprandial Blood Glucose Levels in Humans. *Bioscience, Biotechnology, and Biochemistry*, *69*(3), 559–566. https://doi.org/10.1271/bbb.69.559

Yamashita, H., Fujisawa, K., Ito, E., Idei, S., Kawaguchi, N., Kimoto, M., Hiemori, M., & Tsuji, H. (2007). Improvement of Obesity and Glucose Tolerance by Acetate in Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) Rats. *Bioscience,*

Biotechnology, and Biochemistry, *71*(5), 1236–1243. https://doi.org/10.1271/bbb.60668

Yamashita, H., Maruta, H., Jozuka, M., Kimura, R., Iwabuchi, H., Yamato, M., Saito, T., Fujisawa, K., Takahashi, Y., Kimoto, M., Hiemori, M., & Tsuji, H. (2009). Effects of Acetate on Lipid Metabolism in Muscles and Adipose Tissues of Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) Rats. *Bioscience, Biotechnology, and Biochemistry*, *73*(3), 570–576. https://doi.org/10.1271/bbb.80634

Yao, H., Zhang, A., Li, D., Wu, Y., Wang, C.-Z., Wan, J.-Y., & Yuan, C.-S. (2024). Comparative effectiveness of GLP-1 receptor agonists on glycaemic control, body weight, and lipid profile for type 2 diabetes: Systematic review and network meta-analysis. *BMJ*, *384*, e076410. https://doi.org/10.1136/bmj-2023-076410

Yoon, J., Thompson, L., & Jenkins, D. (1983). The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *The American Journal of Clinical Nutrition*, *38*(6), 835–842. https://doi.org/10.1093/ajcn/38.6.835

Yu, J. H., & Kim, M.-S. (2012). Molecular Mechanisms of Appetite Regulation. *Diabetes & Metabolism Journal*, *36*(6), 391. https://doi.org/10.4093/dmj.2012.36.6.391

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., Suez, J., Mahdi, J. A., Matot, E., Malka, G., Kosower, N., Rein, M., Zilberman-Schapira, G., Dohnalová, L., Pevsner-Fischer, M., … Segal, E. (2015). Personalized Nutrition by Prediction of Glycemic Responses. *Cell*, *163*(5), 1079–1094. https://doi.org/10.1016/j.cell.2015.11.001

Zenel, A. M., & Stewart, M. L. (2015). High Amylose White Rice Reduces Post-Prandial Glycemic Response but Not Appetite in Humans. *Nutrients*, *7*(7), 5362–5374. https://doi.org/10.3390/nu7075225

Zhai, F., Wang, H., Wang, Z., Popkin, B. M., & Chen, C. (2008). Closing the energy gap to prevent weight gain in China. *Obesity Reviews*, *9*(s1), 107–112. https://doi.org/10.1111/j.1467-789X.2007.00450.x

Zhang, C., Dong, L., Wu, J., Qiao, S., Xu, W., Ma, S., Zhao, B., & Wang, X. (2020). Intervention of resistant starch 3 on type 2 diabetes mellitus and its mechanism based on urine metabonomics by liquid chromatography-tandem mass spectrometry. *Biomedicine & Pharmacotherapy*, *128*, 110350. https://doi.org/10.1016/j.biopha.2020.110350

Zhang, W., Kong, L., Zhong, Z., Lin, L., Li, J., & Zheng, G. (2023). Short chain fatty acids increase fat oxidation and promote browning through β3-adrenergic receptor/AMP-activated protein kinase α signaling pathway in 3T3-L1 adipocytes. *Journal of Functional Foods*, *103*, 105488. https://doi.org/10.1016/j.jff.2023.105488

Zhang, Y., Wang, H., Zhang, M., & Wang, Y. (2007). Effects of resistant starch on insulin resistance of type 2 diabetes mellitus patients. *Chinese Journal of Preventive Medicine*, *41*(02), Article 02. https://doi.org/10.3760/j.issn:0253-9624.2007.02.005

Zhao, W., Wang, L., Fan, Z., Lu, J., Zhu, R., Wu, Y., & Lu, X. (2020). Co-ingested vinegar-soaked or preloaded dried apple mitigated acute postprandial glycemia of rice meal in healthy subjects under equicarbohydrate conditions. *Nutrition Research*, *83*, 108–118. https://doi.org/10.1016/j.nutres.2020.09.003

Zhao, Y., Liu, Y., Tao, T., Zhang, J., Guo, W., Deng, H., Han, M., Mo, H., Tong, X., Lin, S., Yang, J., Zhai, H., Wang, Q., Hu, Z., Zhang, W., Chen, H., & Xu, G. (2024). Gastric mechanosensitive channel Piezo1 regulates ghrelin production and food intake. *Nature Metabolism*, *6*(3), 458–472. https://doi.org/10.1038/s42255-024-00995-z

Zhou, J., Martin, R. J., Tulley, R. T., Raggio, A. M., McCutcheon, K. L., Shen, L., Danna, S. C., Tripathy, S., Hegsted, M., & Keenan, M. J. (2008). Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through

fermentation in rodents. *American Journal of Physiology. Endocrinology and Metabolism*, *295*(5), E1160-1166. https://doi.org/10.1152/ajpendo.90637.2008

Zhou, T., Xu, X., Du, M., Zhao, T., & Wang, J. (2018). A preclinical overview of metformin for the treatment of type 2 diabetes. *Biomedicine & Pharmacotherapy*, *106*, 1227–1235. https://doi.org/10.1016/j.biopha.2018.07.085

Zhu, L., Gu, M., Meng, X., Cheung, S. C. K., Yu, H., Huang, J., Sun, Y., Shi, Y., & Liu, Q. (2012). High-amylose rice improves indices of animal health in normal and diabetic rats. *Plant Biotechnology Journal*, *10*(3), 353–362. https://doi.org/10.1111/j.1467-7652.2011.00667.x

Zijlstra, N., de Wijk, R., Mars, M., Stafleu, A., & de Graaf, C. (2009). Effect of bite size and oral processing time of a semisolid food on satiation1. *The American Journal of Clinical Nutrition*, *90*(2), 269–275. https://doi.org/10.3945/ajcn.2009.27694

Zijlstra, N., Mars, M., de Wijk, R. A., Westerterp-Plantenga, M. S., & de Graaf, C. (2008). The effect of viscosity on ad libitum food intake. *International Journal of Obesity*, *32*(4), 676–683. https://doi.org/10.1038/sj.ijo.0803776

Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: Diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology*, *16*(1), 35–56. https://doi.org/10.1038/s41575-018-0061-2

Zwirska-Korczala, K., Konturek, S. J., Sodowski, M., Wylezol, M., Kuka, D., Sowa, P., Adamczyk-Sowa, M., Kukla, M., Berdowska, A., Rehfeld, J. F., Bielanski, W., & Brzozowski, T. (2007). BASAL AND POSTPRANDIAL PLASMA LEVELS OF PYY, GHRELIN, CHOLECYSTOKININ, GASTRIN AND INSULIN IN WOMEN WITH MODERATE AND MORBID OBESITY AND METABOLIC SYNDROME. *Journal of Physiology and Pharmacology*, *58*, 13–35.

Appendices

Appendix 1: Database search strategy for systematic review (Chapter 2)

Supplemental Table 1. Search algorithms for each database.	
Database	Search Algorithm
PubMed	("short-chain fatty acids"[Title/Abstract] OR "short-chain fatty acids"[Title/Abstract] OR "short-chain fatty acid"[Title/Abstract] OR "short-chain fatty acid"[Title/Abstract] OR "acetate"[Title/Abstract] OR "acetic acid"[Title/Abstract] OR "vinegar"[Title/Abstract] OR "propionate"[Title/Abstract] OR "propionic acid"[Title/Abstract] OR "butyrate"[Title/Abstract] OR "butyric acid"[Title/Abstract]) AND "clinical trial"[Publication Type] AND (("humans"[Title/Abstract] OR "human"[Title/Abstract] OR "people"[Title/Abstract] OR "adults"[Title/Abstract] OR "adult"[Title/Abstract] OR "subjects"[Title/Abstract] OR "volunteers"[Title/Abstract] OR "participants"[Title/Abstract] OR "patients"[Title/Abstract] OR "men"[Title/Abstract] OR "man"[Title/Abstract]) AND "clinical trial"[Publication Type]) AND ("glucose"[Title/Abstract] OR "glycem*"[Title/Abstract] OR "glycaem*"[Title/Abstract] OR "insulin"[Title/Abstract] OR "insulin*"[Title/Abstract] OR "metabolic"[Title/Abstract] OR "metabolism"[Title/Abstract] OR "homeostatic model assessment"[Title/Abstract] OR "glycated haemoglobin"[Title/Abstract] OR "glycated hemoglobin"[Title/Abstract] OR "glycosylated haemoglobin"[Title/Abstract] OR "a1c"[Title/Abstract] OR "a1c"[Title/Abstract]) AND "clinical trial"[Publication Type]
EMBASE	("short chain fatty acids" or "short-chain fatty acids" or "short chain fatty acid" or "short-chain fatty acid" or acetate or "acetic acid" or "vinegar" or propionate or "propionic acid" or butyrate or "butyric acid").ti. And (humans or human or people or adults or adult or subjects or volunteers or participants or patients or men or man).ti. and (glucose or glycaem* or glycem* or insulin or insulin* or metabolic or metabolism or "homeostatic model assessment" or "glycated haemoglobin" or "glycated hemoglobin" or "glycosylated haemoglobin" or A1c or A1C).ab.

Web of Science	TITLE(("short chain fatty acids" OR "short-chain fatty acids" OR "short chain fatty acid" OR "short-chain fatty acid" OR acetate OR "acetic acid" OR "vinegar" OR propionate OR "propionic acid" OR butyrate OR "butyric acid") AND (humans OR human OR people OR adults OR adult OR subjects OR volunteers OR participants OR patients OR men OR man) AND (glucose OR glycaem* OR glycem* OR insulin OR insulin* OR metabolic OR metabolism))
Scopus	TITLE(("short chain fatty acids" or "short-chain fatty acids" or "short chain fatty acid" or "short-chain fatty acid" or acetate or "acetic acid" or "vinegar" or propionate or "propionic acid" or butyrate or "butyric acid") AND (humans or human or people or adults or adult or subjects or volunteers or participants or patients or men or man) AND (glucose or glycaem* or glycem* or insulin or insulin* or metabolic or metabolism))
Cochrane CENTRAL	(short chain fatty acids or short-chain fatty acids or short chain fatty acid or short-chain fatty acid or acetate or acetic acid or vinegar or propionate or propionic acid or butyrate or butyric acid) in Title Abstract Keyword AND (humans or human or people or adults or adult or subjects or volunteers or participants or patients or men or man) in Title Abstract Keyword AND (glucose or glycaem* or glycem* or insulin or insulin* or metabolic or metabolism)

Appendix 2: Database search strategy for systematic review (Chapter 5)

Supplemental Table 1. Search algorithms for each database.	
Database	Search Algorithm
PubMed	((("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D")) AND (("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c"))) AND (("resistant starch" or "high amylose" or "indigestible starch" or "type 1 resistant starch" or "type 2 resistant starch" or "type 3 resistant starch" or "type 4 resistant starch" or "type 5 resistant starch")).) AND ((("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D")) AND ("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c")) AND ("food structure" or "food matrix" or "food composition" or "food microstructure" or " food macrostructure"))
OVID	(("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D") and ("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c") and ("resistant starch" or "high amylose" or "indigestible starch" or "type 1 resistant starch" or "type 2 resistant starch" or "type 3 resistant starch" or "type 4 resistant starch" or "type 5 resistant starch")).af. AND (("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D") and ("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c") and ("food structure" or "food matrix" or "food composition" or "food microstructure" or " food macrostructure")).ab.

Web of Science	ALL=(("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D") AND ("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c") AND ("resistant starch" or "high amylose" or "indigestible starch" or "type 1 resistant starch" or "type 2 resistant starch" or "type 3 resistant starch" or "type 4 resistant starch" or "type 5 resistant starch")). AND ALL=(("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D") AND ("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c") AND ("food structure" or "food matrix" or "food composition" or "food microstructure" or " food macrostructure")
Scopus	(ALL ("type 2 diabetes mellitus" OR "type 2 diabetes" OR "T2DM" OR "T2D") AND ALL ("Glucose homeostasis" OR "Glycaemic control" OR "Insulin resistance" OR "Insulin sensitivity" OR "Glucose" OR "Insulin" OR "Glucose tolerance" OR "Glycaemic index" OR "HbA1c") AND ALL ("resistant starch" OR "high amylose" OR "indigestible starch" OR "type 1 resistant starch" OR "type 2 resistant starch" OR "type 3 resistant starch" OR "type 4 resistant starch" OR "type 5 resistant starch")) AND (EXCLUDE (DOCTYPE , "re") OR EXCLUDE (DOCTYPE , "bk") OR EXCLUDE (DOCTYPE , "le") OR EXCLUDE (DOCTYPE , "cp") OR EXCLUDE (DOCTYPE , "no") OR EXCLUDE (DOCTYPE , "ch")) and (ALL ("type 2 diabetes mellitus" OR "type 2 diabetes" OR "T2DM" OR "T2D") AND ALL ("Glucose homeostasis" OR "Glycaemic control" OR "Insulin resistance" OR "Insulin sensitivity" OR "Glucose" OR "Insulin" OR "Glucose tolerance" OR "Glycaemic index" OR "HbA1c") AND ALL (("food structure" OR "food matrix" OR "food composition" OR "food microstructure" OR " food macrostructure"))) AND (EXCLUDE (DOCTYPE , "re") OR EXCLUDE (DOCTYPE , "ch") OR EXCLUDE (DOCTYPE , "cp") OR EXCLUDE (DOCTYPE , "bk") OR EXCLUDE (DOCTYPE , "ed"))

Cochrane CENTRAL	(("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D")) AND (("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c")) AND (("resistant starch" or "high amylose" or "indigestible starch" or "type 1 resistant starch" or "type 2 resistant starch" or "type 3 resistant starch" or "type 4 resistant starch" or "type 5 resistant starch")) AND (("type 2 diabetes mellitus" or "type 2 diabetes" or "T2DM" or "T2D")) AND (("Glucose homeostasis" or "Glycaemic control" or "Insulin resistance" or "Insulin sensitivity" or "Glucose" or "Insulin" or "Glucose tolerance" or "Glycaemic index" or "HbA1c")) AND (("food structure" or "food matrix" or "food composition" or "food microstructure" or " food macrostructure"))

Appendix 3: Meal preparation standard operating procedure for Chapter 8 study.

Breakfast 1 – Treatment A

Meal preparation Time: 5 minutes

Ingredients	Male quantities (g)	Female quantities (g)
whole oats (raw)	90	50
Whole milk	200.0	190.0
Butter	3.0	5.0
blueberries	45.0	45.0
Raspberries	80.0	80.0
Cashew nuts	20.0	28.0
Orange	100.0	50.0

1. Cut and peel orange place in a separate bowl
2. Melt butter in microwave for 10 seconds
3. Mix oats, melted butter and cold whole milk together
4. Add blueberries, raspberries and cashew nuts on top, serve immediately

Breakfast 1 – Treatment B

Meal preparation Time: 5 minutes

Ingredients	Male quantities (g)	Female quantities (g)
Ready brek	110.0	80.0
Whole milk	280.0	220.0
Butter	7.0	9.0
blueberries	30.0	45.0
Rasperries	60.0	40.0
Orange juice	120.0	80.0

1. Measure orange juice into a glass
2. Heat milk in microwave per readybrek pack instructions
3. Melt butter in microwave for 10 seconds
4. Blend berries using handheld blender until fully blended
5. Pour blended berries on top of readybreak, serve immediately

Breakfast 2 – Treatment A

Meal preparation Time: 10 minutes

Ingredients	Male quantities (g)	Female quantities (g)
Toasted grain rye bread	160.0	120.0
Butter	5.0	5.0
Sunflower seeds	40.0	35.0
green banana	80.0	50.0
crunchy peanut butter	15.0	15.0

1. Toast bread for 2.5 mins
2. Add butter and crunchy peanut butter
3. Chop banana into bite size pieces and place on top of bread
4. Add sunflower seeds on top

Breakfast 2 – Treatment B

Meal preparation Time: 10 minutes

Ingredients	Male quantities (g)	Female quantities (g)
White bread	130.0	110.0
Butter	10.0	5.0
ripe banana	70.0	50.0
Smooth peanut butter	40.0	45.0

1. Toast bread for 2.5 mins
2. Add butter and smooth peanut butter
3. Chop banana into bite size pieces and put on top of bread

Meal 1 – Treatment A

Meal preparation Time: 30 minutes

Ingredients	Male quantities (g)	Female quantities (g)
Cod	110.0	70
Chickpea flour	30	30
egg	15	15
Garden peas	130.0	130
potatoes boiled	230.0	180
mayonnaise	30.0	30.0
oil	12.0	7
Orange	150	130

5. Potatoes must be prepared the day before: leave peel on, wash thoroughly, cut into quarters. Boil for 10 minutes in unsalted water. Leave overnight in fridge to retrograde.
6. Crack egg and mix with fork. Weigh out egg and chickpea flour on two plates or flat bowls
7. Weigh fish and cut into large pieces
8. Roll in flour and egg until ALL is used
9. Add oil to pot, fry fish until fully cooked
10. Mix cold potatoes with mayonnaise
11. Put peas in microwave to warm for 1 minute and 30 seconds
12. Peel and cut up orange

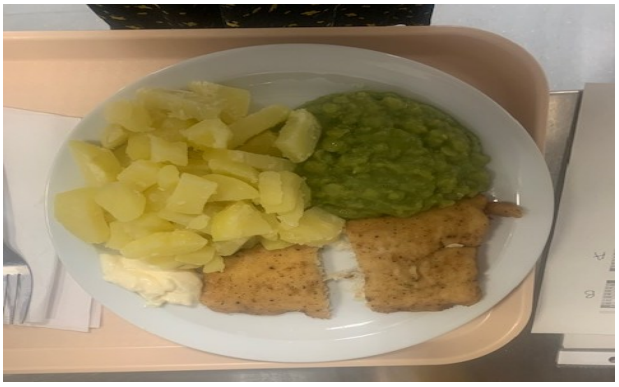


Meal 1 – Treatment B

Meal preparation Time: 30 minutes

Ingredients	Male quantities (g)	Female quantities (g)
lightly dusted cod	170.0	130
boiled potatoes	180.0	180.0
mushy peas	130.0	120.0
oil	15.0	10
mayonnaise	20.0	20.0
Orange juice	150	100

1. Peel potatoes, cut up into small pieces, boil for 10 minutes in unsalted water
2. Microwave cod for 2 minutes, ensure fully cooked
3. Mix mushy peas and oil, heat in microwave for 2 minutes
4. Place everything on plate, add mayonnaise on the side of the same plate
5. Weigh and pour orange juice



Meal 2 – Treatment A

Meal preparation Time: 20 minutes

Ingredients	Male quantities (g)	Female quantities (g)
Toasted grain rye bread	120.0	100.0
Butter	3.0	3.0
Cheddar cheese	50.0	35.0
Whole apple	100.0	50.0
chickpeas	120.0	100.0
garlic	1.0	1.0
olive oil	3.0	3.0
Sunflower seeds	28.0	30.0
carrots	45.0	30.0

1. Cut up apple and carrots into bite size pieces
2. Combine carrots, chickpeas, garlic, olive oil in small bowl
3. Toast bread for 3 minutes
4. Melt butter in microwave and spread on bread, add cheese, sliced
5. Put apple in another small bowl

Meal 2 – Treatment B

Meal preparation Time: 20 minutes

Ingredients	Male quantities (g)	Female quantities (g)
White bread	135.0	95.0
Butter	3.0	3.0
Cheddar cheese	85.0	70.0
apple juice	60.0	80.0
chickpea flour	30.0	30.0
garlic	1.0	1.0
olive oil	5.0	3.0
Carrots	20.0	45.0

1. Mix chickpea flour, garlic and olive oil thoroughly
2. Chop carrots into bite size pieces
3. Melt butter in microwave, spread on bread
4. Add cheese, make sandwich
5. Pour and weigh apple juice

Meal 3 – Treatment A

Meal preparation Time: 30 minutes

Ingredients	Male quantities (g)	Female quantities (g)
Toasted grain rye bread	140.0	120.0
SF baked beans	103.5	103.5
Butter beans	120.0	120.0
Cheddar cheese	50.0	30.0
butter	8.0	10.0
Spinach leaves salad	60.0	60.0
carrots (salad)	60.0	60.0
Parmesan	13.0	20.0
olive oil	10.0	5.0

1. chop up carrots and spinach
2. Grate cheddar and parmesan cheese separately
3. Mix butterbeans with baked beans and microwave for 1 minute
4. Heat butter in microwave for 10 seconds to soften
5. Toast rye bread for 3 minutes
6. Place salad on bottom of meal, add buttered toast
7. Add beans and cheese on top



Meal 3 – Treatment B

Meal preparation Time: 30 – 45 minutes

Ingredients	Male quantities (g)	Female quantities (g)
White bread	130.0	100.0
smashed sf baked beans	140.0	140.0
Cheddar cheese	50.0	35.0
butter	5.0	10.0
cooked carrots	60.0	60.0
Cooked spinach	75.0	150.0
olive oil	8.0	10.0

1. Use food mill to remove skin from baked beans
2. Cook spinach with half of the olive oil until completely cooked, approx. 3 minutes
3. Grate carrots and cook separately with rest of olive oil for approx. 3 minutes
4. Grate cheese
5. Heat beans in microwave for 1 minute
6. Melt butter in microwave for 10 seconds
7. Place bread on plate, spread butter, add carrots and spinach, then beans and cheese

Meal 4 – Treatment A

Meal preparation Time: 45 min – 1 hour

Ingredients	Male quantities (g)	Female quantities (g)
gnocchi	200.0	120.0
Tomato passata	200.0	200.0
green lentils	50.0	50.0
onion	20.0	20.0
garlic	2.0	2.0
celery	30.0	30.0
olive oil	10.0	10.0
Parmesan	48.0	45.0
Cinnamon	0.5	0.5
Pear	21.0	15.0
Almonds	50.0	50.0

1. Gnocchi: must be cooked the evening before for 2 minutes, per packet instructions and left in fridge overnight.
2. Chop garlic, onion and celery and add to pan with olive oil, lightly fry on low heat for 5 minutes
3. Add green lentils, passata and 100 ml water to pot, cover with lid and leave to simmer for 30 minutes. Stir regularly. Do not let it get too dry. Add 50 – 100 ml more water if needed.
4. Grate parmesan
5. Chop pear into bite size pieces, place in a small bowl and add almonds and cinnamon
6. Once the lentils are cooked (the only way to check is to try one), place reheated gnocchi (1 min in microwave) in a flat bowl.
7. Add half parmesan into lentil sauce and place half on top of pasta and sauce in bowl.



Meal 4 – Treatment B:

Meal preparation Time: 30 - 45 min

Ingredients	Male quantities (g)	Female quantities (g)
gnocchi	200.0	150.0
Tomato passata	200.0	200.0
red lentils puree	80.0	70.0
onion	20.0	20.0
garlic	2.0	2.0
celery	30.0	30.0
olive oil	20.0	15.0
Parmesan	60.0	48.0
Cinnamon	0.5	0.5
Baked Pear	50.0	50.0

1. Chop garlic, onion and celery and add to pan with olive oil, lightly fry on low heat for 5 minutes
2. Add red lentils, passata and 100 ml water, cover with lid and leave to simmer for 10 minutes. Stir regularly. Do not let it get too dry. Add 50 – 100 ml more water if needed.
3. Grate parmesan
4. Blend sauce with handheld blender
5. Boil gnocchi for 2 minutes. Add when water is boiling.
6. Chop pear up very small and add cinnamon before microwaving for 1 minute and 30 seconds.



Meal 5 – Treatment A

Meal preparation Time: 30 minutes

Ingredients	Male quantities (g)	Female quantities (g)
chicken sausage (raw weight)	113.0	113.0
Mashed cooked/cooled potatoes russet	200.0	200.0
Mashed butter beans	80.0	80.0
butter	30.0	30.0
whole milk	130.0	130.0
Baked beans	100.0	140.0

1. Potatoes must be prepared the day before: leave peel on, wash thoroughly, cut into quarters. Boil for 10 minutes in unsalted water. Leave overnight in fridge to retrograde.
2. Fry sausages on low-medium heat for 12 – 15 minutes, turn them regularly
3. Reheat potatoes and roughly mash with butter beans, milk and melted butter (microwave for 10 seconds).
4. Heat beans in microwave for 1 minute

Meal 5 – Treatment B

Meal preparation Time: 30 minutes

Ingredients	Male quantities (g)	Female quantities (g)
chicken sausage (raw weight)	113.0	90.0
potatoes boiled and smashed	250.0	240.0
butter	30.0	25.0
whole milk	130.0	100.0
baked beans (pureed)	180.0	120.0

1. Boil potatoes for 10 minutes
2. Fry sausages on low-medium heat for 12 – 15 minutes, turn them regularly
3. Use food mill to remove skin from baked beans
4. Puree potatoes with melted butter (microwave for 10 seconds), and milk
5. Heat beans in microwave for 1 minute