INVESTIGATING THE ROLE OF SHORT - CHAIN FATTY ACIDS IN GLYCAEMIC REGULATION IN HUMANS

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

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For my kids,

Shaika, Maryam, Rashed and Ahmed

ABSTRACT

Introduction

Obesity increases individual risk for cardiometabolic diseases, including type 2 diabetes. Dietary fibre has emerged as an important modifiable risk factor for obesity, partially due to its fermentation by the gut microbiota to produce SCFAs-primarily acetate, propionate and butyrate. Despite increasing interest in their metabolic effects, human evidence on the impact of SCFAs on glycaemic regulation remains limited and inconsistent. This thesis therefore aimed to evaluate the most recent evidence linking SCFAs with glycaemic control measures in humans.

Method

Three complementary studies were conducted. First, a systematic review and meta-analysis synthesized evidence published up to December 2021 on the effects of SCFAs on glycaemic outcomes. Second, a secondary analysis of data from the Low Caloric Diet Diabetes Mellitus (LCDDM) study examined associations between circulating acetate and glycaemic measures in obese type 2 diabetes undergoing a low-calorie dietary intervention. Third, a 12-month randomised double -blind parallel controlled study assessed the effects of inulin propionate ester (IPE) supplementation on weight and glucose regulations in young overweight adults.

Result

The systematic review included 46 studies (n=913). Vinegar intake significantly reduced acute postprandial glucose responses in individuals with impaired glucose tolerance, type 2 diabetes, and healthy participants. However, neither acute nor chronic administration of acetate, propionate or butyrate affected glycaemic measures. In the LCDDM analysis, no significant associations were found between plasma acetate and glycaemic measures, but a negative association with body weight and body mass index was observed in specific subgroups. The IPE trial showed no significant effect on weight gain prevention and a small increase in blood glucose, despite good compliance.

Conclusion

Overall, SCFAs had no effect on the glycaemic measures. This thesis provides evidence from specific populations and highlights the need for further investigation to clarify the underlying mechanisms and the reasons for the divergence in findings.

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DECLARATION OF CONTRIBUTIONS.

The work described in this thesis is original, and most of it was performed by the author. Everything else is appropriately cited. Credit statements were also added after each chapter. All other collaborations and assistance are listed below.

Chapter 2:

The systematic review was performed in a team with Anna Cherta-Murillo, Jennifer E Pugh and Dana Hajjar. I primarily designed the research and defined PICO. I contributed to the database search, abstract and full article screening, data entry and data cleaning. I also assisted with the interpretation of the results, study outcomes and writing the discussion and the manuscript.

Chapter 3:

The date used in this chapter was extracted from the LCDDM study, where primary outcome results have already been published (Brown, et al. 2020). Analysis of the samples for acetate was carried out by Dr. Isabel Garcia Perez and Vally Wu.

I primarily extracted the data from the original LCDDM study data. I undertook the data entry, cleaning, statistical analysis of the study outcomes and interpretation of the results. I also prepared the tables and figures presented in this chapter.

Chapter 4:

All clinical trials were conducted at the NIHR Imperial Clinical Research Facility (ICRF) and University of Glasgow Clinical Research Facility.

Clinical visits:

All clinical visits at the London clinical site were carried out at NIHR/ICRF. I conducted the clinical visits and study procedures with the help of Dr Martina Tashkova, Dr. Katerina Petropoulou and Jennifer E. Pugh.

Study supplements:

Inulin was supplied by HP inulin, Beneo-Orafti, Tienen, Belgium, and was sourced commercially (Kreglinger, Chesham, United Kingdom).

Inulin Propionate Ester was established in collaboration with Moorepark Technology Limited (MTL, Fermoy, Ireland).

Data analysis:

Data analysis was primarily carried out by Joana C. Vasconcekos and Dr. A. Toby Prevost, clinical trial statisticians from Clinical Trial & Epidemiology Unit, at King's College, London, UK.

Blood Sample analysis:

I conducted the analysis of glucose and Insulin samples with the help of Jennifer E. Pugh and Wenan Zhang.

Gut hormones samples analysis:

I conducted the analysis of gut hormones samples with the help of Dr Katerina Petropoulou, Jennifer E. Pugh, Mingzhu Cai and Wenan Zhang.

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2. Pugh, J., Anjum, A., Petropoulou, K., Thom, G., Mccombie, L., Tashkova, M., Alaraj-Alshehhi, S., Babalis, D., Prechtl, C., Lean, M., Prevost, A., Vasconcelos, J., 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): A multi-centre, double-blind, randomised, parallel-group study to investigate the efficacy of inulin-propionate ester versus inulin (control) in the prevention of weight gain over 12 months." https://doi.org/10.12688/f1000research.125950.1

3. 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*, Article 102844. https://doi.org/10.1016/j.eclinm.2024.102844

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Finally, this thesis has come to an end, and it's time to write a few final personal words.

Firstly, I would like to express my deepest love and gratitude to **my kids - Shaikha, Mayam, Rashed and Ahmed**, who have been part of my doctoral journey despite the physical distance between us. As they grew, they became more understanding of the challenges I faced. Thank you, and I am sorry if I was not always there when you needed me. I love you the most.

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Finally, I am grateful to each and every person who has taken the time to read my thesis.

ABBREVIATIONS

- A. muciniphila: Akkermansia muciniphila
- AC: Acetate
- ADA: American Diabetes Association
- AgRP: Agouti-related Protein
- AMPK: AMP-Activated Protein Kinase
- ARC: Arcuate Nucleus
- AT: Adipose Tissue
- AUC: Area Under the Curve
- BID: Bioelectrical Impedance Device
- BMI: Body Mass Index
- BP: Blood Pressure
- CAD: Coronary Artery Disease
- CaMKKβ: Calmodulin-Dependent Protein Kinase Kinase Beta
- CCK: Cholecystokinin
- CHD: Coronary Heart Disease
- CI: Confidence Interval
- CRF: Clinical Research Facility
- CRP: C-Reactive Protein
- CVD: Cardiovascular Disease
- CWP: Cambridge Weight Plan
- CV: Coefficient of Variation
- D1-D5: Bias Domains 1 to 5
- DFs: Dietary Fibers
- DI: Dietary Intake
- DNL: De Novo Lipogenesis
- DM: Diabetes Mellitus
- DPP-4: Dipeptidyl Peptidase-4
- DVC: Dorsal Vagal Complex
- D2O: Deuterium Oxide

- EE: Energy Expenditure
- EDC: Electronic Data Capture
- EFSA: European Food Safety Authority
- ELISA: Enzyme-Linked Immunosorbent Assay
- FABP4: Fatty Acid Binding Protein 4
- FBC: Full Blood Count
- FDA: Food and Drug Administration
- FBG: Fasting Blood Glucose
- FFAR: Free Fatty Acid Receptor
- FFAR2: Free Fatty Acid Receptor 2
- FFAR3: Free Fatty Acid Receptor 3
- FFAs: Free Fatty Acids
- FI: Fasting Insulin
- FM: Fat Mass
- FOS: Fructooligosaccharides
- FXR: Farnesoid X Receptor
- GIT: Gastrointestinal Tract
- GLP-1: Glucagon-Like Peptide-1
- GM: Gut Microbiota
- GRADE: Grading of Recommendations, Assessment, Development, and Evaluation
- GPR: G-Protein Coupled Receptor
- GPR43: G-Protein Coupled Receptor 43
- GSIR: Glucose-Stimulated Insulin Release
- HbA1C: Haemoglobin A1c (Glycated Haemoglobin)
- HCAR: Hydroxycarboxylic Acid Receptor
- HDAC: Histone Deacetylases
- HDL: High-Density Lipoprotein
- HFD: High-Fat Diet
- HMW: High Molecular Weight

- HOMA: Homeostasis Model Assessment
- HOMA-B: Homeostasis Model Assessment of Beta-Cell Function
- HOMA-IR: Homeostasis Model Assessment of Insulin Resistance
- HOMA-S: Homeostasis Model Assessment of Insulin Sensitivity
- HSL: Hormone Sensitive Lipase
- HTN: Hypertension
- HV: Healthy Volunteers
- iAUC: Incremental Area Under the Curve
- IGT: Impaired Glucose Tolerance
- IL-6: Interleukin 6
- IN: Inulin
- IPE: Inulin Propionate Ester (or Intervention Arm)
- IR: Insulin Resistance
- IRB: Institutional Review Board
- KO: Knockout
- LCD: Low Calorie Diet
- LCDDM: Low-Calorie Diet in Diabetes Mellitus
- LC-MS: Liquid Chromatography-Mass Spectrometry
- LDL: Low-Density Lipoprotein
- LDL-C: LDL-Cholesterol
- LME: Linear Mixed Effects Model
- LR: Leptin Receptor
- MATSUDA: Matsuda Insulin Sensitivity Index
- MCP: Monocyte Chemoattractant Protein
- MCT-1: Monocarboxylate Transporter-1
- MEPS: Medical Expenditure Panel Surveys
- Metformin: An oral diabetes medication used to manage blood sugar levels
- NaCl: Sodium Chloride
- NCDs: Non-Communicable Diseases
- NDC: Non-Digestible Carbohydrates

- NFκB: Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells
- NFP: Non-Fibrous Polysaccharides
- NMR: Nuclear Magnetic Resonance
- NPC1L1: Niemann-Pick C1-Like 1
- NPY: Neuropeptide Y
- NPS: Non-Starch Polysaccharides
- NTS: Nucleus of the Tractus Solitarius
- OA: Osteoarthritis
- Olfr: Olfactory Receptor
- ORCs: Obesity-Related Complications
- ORPs: Obesity-Related Problems
- OXM: Oxyntomodulin
- PEPCK: Phosphoenolpyruvate Carboxy kinase
- PET-CT: Positron Emission Tomography-Computed Tomography
- PI: Postprandial Insulin
- PICOS: Patients, Intervention, Comparator, Outcome, Study Design
- POMC: Pro-opiomelanocortin
- PP: Pancreatic Polypeptide
- PPI: Postprandial Insulin
- PPAR: Peroxisome Proliferator-Activated Receptor
- PPG: Postprandial Glucose
- PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis
- PVN: Paraventricular Nucleus
- PYY: Peptide YY
- QUICKI: Quantitative Insulin Sensitivity Check Index
- RCT: Randomised Controlled Trial
- RCT, XO: Randomised Controlled Trial, Cross Over
- REC: Research Ethics Committee
- RE: Resting Energy
- RIA: Radioimmunoassay

- RER: Respiratory Exchange Ratio
- RRID: Research Resource Identifier
- RS: Resistant Starch
- SAE: Serious Adverse Event
- SCD: Standard Chow Diet
- SCFAs: Short-Chain Fatty Acids
- SCMT-1: Sodium-Coupled Monocarboxylate Transporter-1
- SMD: Standard Mean Difference
- SSTR: Subscapular/Triceps Ratio
- T2DM: Type 2 Diabetes Mellitus
- TC: Total Cholesterol
- TCA: Tricarboxylic Acid Cycle
- TG: Triglycerides
- TNF-α: Tumor Necrosis Factor Alpha
- UCP1: Uncoupling Protein 1
- UCP2: Uncoupling Protein 2
- VAS: Visual Analogue Scale
- VAT: Visceral Adipose Tissue
- VLDL: Very Low-Density Lipoprotein
- WC: Waist Circumference
- WHR: Waist-to-Hip Ratio
- WM: Water Mass
- αMSH: Alpha-Melanocyte Stimulating Hormone

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CHAPTER 1:
GENERAL INTRODUCTION

OBESITY

Obesity Definition

An increase in the prevalence of obesity has become a major global health problem in adults, as well as among children and adolescents. Obesity is a disease in which excess body fat has accumulated and health is adversely affected (Kopelman, 2000). According to the Obesity Medical Association, obesity is defined as:

> "a chronic, progressive, relapsing, and treatable multi-factorial, neurobehavioral disease, wherein an increase in body fat promotes adipose tissue dysfunction and abnormal fat mass physical forces, resulting in adverse metabolic, biomechanical, and psychosocial health consequence" (Fitch & Bays, 2022).

Obesity is internationally computed using the *Body Mass Index (BMI)* formula combining body weight and height. It is calculated as body weight in kilograms divided by the height squared (kg/m^2). Individuals are grouped into five groups as shown in Table 1.1. A lower cut-off point is used for south Asian and Chinese ethnicities because they have higher visceral adiposity and an increased predisposition to cardiometabolic disease (Mallik et al., 2023).

There are five BMI groups in the general population: *normal*; ranging from 18.5-24.9 kg/m^2, *overweight*; ranging from 25.0-29.9 kg/m^2, *class 1 obesity*; ranging from 30.0-34.9 kg/m^2, *class 2 obesity*; ranging from 35-39.9, and *class 3 obesity* equal to or greater than 40 kg/m^2 (Table 1.1). Other methods to assess fat mass in clinical practice include waist circumference (WC), skin fold thickness and bio impedance. These methods allow the comparison of weight status within and between populations and identify groups that are at higher risk of developing comorbidities and those who may need early intervention. There is a close relationship between an increase in BMI and the incidence of chronic

conditions such as type 2 diabetes mellitus (T2DM), hypertension (HTN), and coronary heart disease (CHD) (Kopelman, 2000; Fitch & Bays, 2022) .

Table 1.1: Classification of obesity using body mass index (BMI).

	Body mass index (Kg/m^2)	
	General population	**Certain ethnic populations [a]**
Normal body weight	18.5 – 24.9	18.5 – 22.9
Overweight	25.0 – 29.9	23.0 – 27.4
Class 1 Obesity	30.0 – 34.9	27.5 - 32.4
Class 2 Obesity	35.0 – 39.9	32.5 – 37.4
Class 3 Obesity	≥ 40	≥ 37.5

[a]South Asian, Chinese, other Asian, Middle Eastern, Black African and African Caribbean.
Derived and adopted from Mallik et al., (2023).

Prevalence of Overweight and Obesity

Epidemiological reports show that the rate of weight gain is higher within the first to five years of adulthood (Truesdale et al., 2006). National surveys in the United States have shown that there is twofold increase in the prevalence of obesity over time; from 17% in the ages of 6-11 years to 34% in the ages of 20-39 years (Williamson, 1990). The Health Survey for England 2021 estimates that 25.9% of adults in England are obese and 37.9% are overweight. Adult men and women are more likely to be overweight or obese (Health Survey for England, 2021 Part 1). In England and Wales, health surveys confirm an increase in the prevalence of obesity in adults from 6% and 8% in 1980 to 17% and 20% in 1997 in men and women respectively (Health survey for England – 2012, NHS England Digital). The estimated total number of overweight and obese people worldwide in 2005 was 937 million and 396 million respectively and it is predicted that these numbers will grow in 2030 to 1.35 billion overweight and 573 million (Kelly et al., 2008)

There has been a steady increase in the prevalence of obesity among young people, especially among college and university students (Telleria-Aramburu & Arroyo-Izaga, 2022), although it is believed that individuals in their middle years are most likely to suffer from obesity. Many adolescents go through physiological and social transitions that make them more prone to developing obesity in later life. During these crucial periods of growth, young individuals are more prone to energy imbalances, which often result in weight gain (Ayogu et al., 2022) . Positive and negative health behaviour that is acquired during this phase of transmission to adulthood have the potential to persist into later years (Kenzig,

2013), and reports showed that an obese adolescent carries a higher risk of being overweight or obese at 35 years of age (Engin, 2017). Studies have also shown that once an individual gains weight, the probability of returning to the normal body weight is very low: 1 in 210 for men and 1 in 124 for women (Fildes et al., 2015) .

Health Consequences of Obesity

Epidemiological studies show that people living with obesity are at an increased risk of developing Obesity-related problems (ORPs). Obese individuals had seven-fold risk of developing type 2 diabetes mellitus (T2DM) and a twofold risk of developing cardiovascular disease (CVD) (Pearson-Stuttard et al., 2024). Other ORPs, such as hypertension, hypercholesteremia, cholelithiasis, osteoarthritis, obstructive sleep apnea, cancer and psychological problems have been reported (Glenny et al., 1997). In a recent US cohort study, at baseline approximately 25% had one ORPs, and 30% had two or three. The highest costs over eight years follow-up were observed for chronic kidney disease (+78.8%) and T2DM (+47.8%) (Pearson-Stuttard et al., 2023). A retrospective cohort study using UK electronic databases between 2004 and 2019 found that ORP prevalence was higher in groups with larger BMIs groups and higher in obese than overweight groups (Pearson-Stuttard et al., 2024). The meta-analysis of 20 studies reported that heart disease was the most common underlying cause of death in patients with class obesity 3 (BMI >40 kg/m^2), followed by cancer and diabetes (Apovian, 2016). Other reports showed that for every 5-unit increase in BMI above 25 kg/m^2, there is an increase of 29%, 41%, and 21% in mortality rate, vascular mortality, and diabetes related mortality respectively (Whitlock et al., 2009). Due to the increased prevalence of ORPs and corresponding health costs in people living with obesity and overweight, prevention strategies and modalities are highly necessary.

Economic Consequences of Obesity

The increasing burden of non-communicable diseases (NCDs) attributable to obesity is considered a challenge to the health care infrastructures and health care delivery systems of developing and developed nations (BD Obesity Collaborators, 2020), where obesity accounts for 2% to 9% of the total healthcare expenditure budget. Medical Expenditure

Panel Surveys (MEPS) estimate that obesity can be attributed to more than $40 billion in medical spending. The cost for Medicare patients who were obese was $600 higher per year compared to the patients who were of normal weight (Apovian, 2016).

The cost of the obesity can be direct or indirect; direct costs include pharmaceutical treatments, surgeries and the medical treatment of the complications and chronic diseases associated with obesity. Indirect costs include lost productivity due to morbidity and mortality, the cost of weight loss programs, and the cost of the excess food consumed (Rosin, 2008). Reports also show that obesity is associated with annual increases in health care costs of 36% and medication costs of 77%, compared to normal weight costs (Kopelman, 2000) . It is estimated that if the number of individuals between 16 and 17 who are overweight or obese can be reduced by 1%, this would lead to a reduction of 52,812 obese adults, leading to a decrease in lifetime medical costs of $586 million (Wang et al., 2010). Addressing obesity prevention in early life would therefore have a considerable impact on health care costs.

Factors in the Development of Obesity

Obesity is a multifactorial disease that results from an imbalance between energy intake and energy expenditure, both of which are basically determined by the interaction between an individual's genome and environmental factors.

Non-modifiable factors.

Twin and adaptation studies found that genetic factors account for 40-70% of the inter-individual variation in BMI and susceptibility to obesity (McPherson, 2007) . Genetic obesity is classified into monogenic, syndromic, and polygenic types. However, most people with obesity have a complex multifactorial interaction. Research has found a link between epigenetic modifications and metabolic health in humans (Chiurazzi et al., 2020).

Monogenic Obesity

Monogenic obesity is caused by a deficiency of or mutation in a single gene. Most commonly, mutations in genes are involved in the hypothalamic leptin-melanocortin

energy balance system, such as mutations in leptin, leptin-receptor, POMC or MC4R (Chiurazzi et al., 2020). Monogenic obesity is mostly inherited as autosomal recessive, mostly because of consanguinity (Sivasubramanian & Malhotra, 2023). Individuals with these mutations often present with early onset and severe forms of obesity and hyperphagia. For example, leptin gene and Leptin receptor mutations exhibit the same phenotyping and clinical characteristics. People with such mutations are born with normal birth weights but gain rapid and progressive body weight and fat deposition, mainly in the trunk and limbs. They also exhibit hyperphagia and food seeking behaviour. In nutritional studies, Leptin gene mutation individuals were found to have a marked elevation in *ad libitum* meals, while leptin-deficient receptor individuals were found to have no detectable changes in resting metabolic rate measured by indirect calorimetry or total energy expenditure measured by chamber calorimetry (Farooqi & O'Rahilly, 2006). Some of the monogenic mutations are also described as syndromes they can be associated with other phenotypes such as neurological abnormalities or organic malformations including Prader-Willi syndrome, which is characterized by upper body obesity, short stature, mental retardation and hypogonadism, and is caused by deletion of the paternal segment of chromosome 15 (Arner, 2000) .

Polygenic obesity

This is caused by multiple gene variations interacting with environmental factors. Two common gene variants include the MC4R gene and the FTO-associated gene. A third gene has also recently been implicated in the regulation of body weight. This gene is the insulin-induced gene 2 (INSIG2). Further studies are needed to investigate these complex interaction mechanisms.

Epigenetic roles in obesity

Epigenetics is promising new field of medical research that could influence an individual's risk of developing obesity by providing a reversible change in gene expression without altering DNA sequences. An example of known epigenetic mechanisms is DNA methylation, which alters gene expression by adding a methyl group including Leptin and Adiponectin genes to a DNA sequence. Another epigenetic mechanism is the acetylation

and methylation of the genes involved in adipogenesis and appetite regulation. Meanwhile, non-coding RNAs that are not translated into proteins have also been implicated in the context of obesity, such as micro-RNA, which is involved in the process of adipogenesis, fat metabolism and inflammation (miR30 and miR21) (Mahmoud, 2022). These epigenetic mechanisms are dynamic and modifiable in response to the environmental factors and lifestyles. For example, exposure to the obesogenic substances disrupts methylation/histone acetylation, promoting fat deposition, obesity and metabolic disorders. Other environmental factors including high fat diets predispose people to obesity through epigenetic modulation while folate, vitamins (B12) and physical activity promote beneficial metabolic modifications (Ling & Rönn, 2019; Mahmoud, 2022).

In summary, obesity is a complex disease in which rare genetic mutations can cause obesity in a small subset of populations. However, it mostly results from interaction between genetic and environmental factors. Targeting epigenetic modifications as therapeutic interventions are nowadays part of wider genetic research studies.

Modifiable factors.

Many environmental factors affect body weight. They can be summarized into the following areas:

Physical activity

An individual's environment affects the amount and type of physical activity, which accounts for 20-50% of total energy expenditure. There are many factors that contribute to sedentary lifestyles, such as sitting in front of televisions, using smart phones, and playing video games. Studies found these behaviours more common in children and the elderly. For example, participants who watched TV for 3 hours per day almost doubled their overweight and were more likely to be obese later in life than non-TV watchers (Nicolaidis, 2019). Other cross-sectional studies found that the risk of obesity increases at a physical activity level ratio of less than 1.8 using a multivariate regression of BMI and physical activity (Ferro-Luzzi & Martino, 2007). A Finnish study found that those reporting

little or no physical activity had twice the risk of gaining 5kg or more compared to those who performed physical exercise three or more times each week (Rissanen et al. 1991).

Consumption of high energy food

Consumption of energy-dense foods is positively associated with weight gain. All media, including TV, the press and posters promote the concept of fast food or junk food, which is easily accessible and typically rich in obesogenic ingredients such as fat and refined carbohydrates (Nicolaidis, 2019). A 6-year longitudinal study found that women who consumed a highly energy dense diet had an increase of 2.4 units in their BMI, while women consuming a low energy diet had a lower increase of 0.9 units (Whitlock et al., 2009).

Cultural and Socioeconomic factors

Cultural and socioeconomic factors also play a role in developing obesity. Low income and low education levels have been implicated in the development of obesity. Meanwhile, investigations have been made into the way social and lifestyle changes impact the development of obesity and T2DM in the same ethnicity group. Research found that Indian Pima men and women who lived in the mountains of Northeastern Mexico had a lighter body weight and lower serum metabolic markers compared to those who had moved to Arizona. (Ravussin et al., 1994). The same trend was reported in African men and women moving to the US. A BMI of 21.7 and 22.6 was reported for Nigerian men and women living in Nigeria, while levels of 27.1 and 30.8 were recorded for men and women living in the US (Kopelman, 2000) .

Prevention of Obesity

Overweight and obesity represent a major public health problem. Although there has been a significant focus on treatments for obesity, there has been comparatively little on prevention.

Dietary treatment

Structural eating plans based on estimated energy needs are usually estimated by calculating base metabolism, adjusting for physical activity and subtracting the energy deficit (~600 kcal/day) to induce a weight loss of 0.5 kg/week. There are many other meal replacement approaches for weight loss such as low calorie diets, very low caloric diets, ketonic diet and Mediterranean diets (see Chapter 3). Despite the variation in the dietary pattern or approach to eating, meal patterns that reduce energy intake in relation to the energy expenditure also lead to weight loss (Smethers & Rolls, 2018). There is evidence that modest energy reduction of ~600 kcal/day results in the maintenance of a modest weight. The overall recommendation of dietary modification for weight loss is a diet with a low fat content (especially saturated fat) and high content of wholegrain fibre carbohydrate and fruit and vegetables (Ulijaszek, 2003).

Physical activity

Exercise plays a major role in maintaining long term weight loss together with diet restriction. There is a positive association between activity level and the degree of sustained weight loss. In long cohort follow up studies, men who maintained physical activity of more than 150 minutes per week had less weight gain compared to less active men (5.6 vs 9.1 kg), with an even more significant trend (3.8 vs. 9.5 kg) among women (Cox, 2017).

Medical treatment

Despite the availability of many classes of anti-obesity drugs, they may not work for younger or older people, and the effects may wane over time. There are also concerns about the potential side effects of obesity drugs and interaction between drugs (Decaria et al., 2012). Evidence shows that current weight loss medications treatments are not as effective as weight loss surgeries (Bray & Ryan, 2021).

Surgical treatment

Bariatric surgery is the only treatment for obesity that shows long term weight loss. Common surgical procedures include gastric banding, Roux-en-Y gastric bypass and

sleeve gastrectomy. A retrospective analysis of 197 patients aged 65 years or older who underwent weight loss surgeries between January 2000 and December 2007 showed patients achieving an average excess weight loss of 44.5% and 55.3% at 6 months and 1 year respectively (O'Keefe et al., 2010). Systematic reviews have shown that weight loss surgeries are effective in reducing weight and medication use and improving quality of life (Decaria et al., 2012). However, concerns remain surrounding the risk of anaesthesia and postoperative complications, while weight loss surgeries remain expensive and might not be available for elderly obese people or those with comorbidities.

In summary, obesity is a chronic medical condition which is steadily increasing worldwide. It is a multifactorial disease with multiple associated comorbidities and higher health costs. Despite the advances in the treatment choices available, uncertainties remain. This means that a greater emphasis on the prevention of obesity is urgently required, as obesity difficult and expensive to treat once it is established (Müller et al., 2001).

APPETITE REGULATION.

Appetite regulation or energy homeostasis is a complex balance between energy intake and expenditure that is predominantly coordinated by the brain (Bauer et al., 2016). The brain's appetite control system usually establishes a weight "set point" and tries to maintain body weight around it even if food intake varies (Arora & Anubhuti, 2006). The Hypothalamus and brainstem are the main regions responsible for the regulation of energy homeostasis.

Central Nutrient Control System

The Hypothalamus.

The hypothalamus plays a prominent role in coordinating neural, nutrient and hormonal signals, directly and indirectly forming a network of communication between the gut, pancreas, liver, adipose tissue, brainstem and hypothalamus. Within the hypothalamus, *Arcuate nucleus (ARC)* is the main region involved in feeding and satiety. Within the ARC, there are three main neural plexuses that regulate food appetite. These are ARC anorexigenic pro-opiomelanocortin (POMC), orexigenic neuropeptide Y(NPY) and agouti-related protein (AgRP). POMC neurons release α-melanocyte, stimulating hormone

αMSH, which directly activates downstream neuronal melanocortin receptors to inhibit food intake and increase energy expenditure. Meanwhile, AgRP neurons release AgRP, which antagonizes the αMSH effect and acts as an inhibitory neurotransmitter by stimulating food intake and suppressing energy expenditure (Hussain & Bloom, 2013; Joly-Amado et al., 2014).

Brainstem control

The brainstem has also demonstrated a role in regulating food intake. The Dorsal Vagal Complex (DVC) in the caudal brainstem is involved in communicating between the periphery and the hypothalamus to control food intake. The vagus nerve is an example of communication between the gastrointestinal system (GIT) and the nucleus of the tractus solitarius (NTS) in the DVC (Hussain & Bloom, 2013) .

Peripheral Nutrient Control System.

Gut hormones.

The GIT system is the largest endocrine organ in the body. It releases more than 20 regulator peptide gut hormones that influence appetite control and act as signals or communicators between the peripheral and central appetite centres. Gut hormones can be sub classified into the following groups:

Orexigenic hormones.

Ghrelin is the only known orexigenic gut hormone that is released into the circulation from the stomach. It is composed of 28 amino acids. Evidence suggests that it mediates orexigenic action via the stimulation of NPY/AgRP co-expressing neurons within the ARC of hypothalamus (Patterson et al., 2011).

Circulating ghrelin levels are increased by fasting after a meal. Ghrelin is known as “hunger hormone”. In rodents, the central and peripheral administration of ghrelin increases food intake and body weight as well as a reduction in fat utilisation. In humans, there is a negative correlation between circulating ghrelin levels and BMI. Fasting ghrelin levels were also found to be high in patients with anorexia nervosa (Murphy et al., 2006; Suzuki, Keisuke et al., 2011).

Anorectic Hormones

Peptide YY (PYY) is a member of the pancreatic polypeptide (PP) family, which is related to the Neuropeptide Y (NPY) (Murphy et al., 2006). These peptides have a similar PP-fold structural motif and exert their effects by binding to the Y family receptors. The most common circulating form of PYY is PYY_{3-36}, which binds to the Y_2 receptor preferentially (Hussain & Bloom, 2013). PYY is secreted by L-cells of the GIT tract, and its level rises within 15 minutes of food intake and remains elevated for several hours, peaking 1-2 hours after food intake (Bauer et al., 2016). PYY induces an anorectic effect directly via the hypothalamus in the ARC and indirectly via the vagus through its effect on Y_2 receptors. This anorectic effect has suggested that Y_2 receptors may be targeted for the treatment of obesity (Hussain & Bloom, 2013).

Cholecystokinin (CCK)

Cholecystokinin was first discovered in 1973 and plays a role in food intake. It is secreted by l-intestinal cells and peaks within 15 minutes after eating with a very short plasma half-life of a few minutes (Suzuki, Keisuke et al., 2011). CCK stimulates pancreatic secretion and gall bladder contraction as well as controlling meal size. It acts by binding to the CCK receptors (CCKr) and mediates its satiety action via CCKA receptors on the vagal nerve. Reports show that intermittent CCK infusion reduces meal sizes, but this effect is compensated for by an increase in the frequency of food intake (Arora & Anubhuti, 2006; Murphy et al., 2006; Suzuki et al., 2012).

Glucagon-Like Peptide-1 (GLP-1)

GLP-1 is produced from L-cells in the GIT, and synthesizes a large precursor known as Pre-Proglucagon, which is processed further to produce a few active gut peptides including GLP-1, GLP-2 and oxyntomodulin. GLP-1 is rapidly degraded by the Dipeptidyl peptidase-4 (DPP-4) enzyme, resulting in only 25% of GLP-1 reaching the hepatorenal circulation and less than 10% reaching the systemic circulation. GLP-1 nerve fibres and receptors are found in the hypothalamus. After a meal, GLP-1 and GLP-2 are involved in a variety of functions such as glucose homeostasis, delayed gastric emptying and the

enhancement of intestinal growth. They also stimulate insulin secretion and the regulation of food intake. The peripheral and central administration of GLP-1 results in reduced food intake. There are two main bioactive forms of GLP-1: GLP-$1_{7\text{-}36}$ and GLP-$1_{7\text{-}37}$. (Arora & Anubhuti, 2006; Suzukiet al., 2012; Murphy et al., 2006). GLP-1 incretin properties have been used for the development of T2DM treatments such as Liraglutide (Bauer et al., 2016).

Oxyntomodulin (OXM)

like GLP-1, oxyntomodulin is also produced from pre-proglucagon precursor, and is released after a meal by the L-cells in response to food intake. It exerts its effect via GLP-1 receptors with low affinity to the glucagon receptor. Like GLP-1, OXM reduces food intake and delays gastric response. It also increases energy expenditure and suppresses ghrelin. OXM is deactivated by DPPIV, and OXM analogues are therefore being developed as a potential obesity treatment (Arora & Anubhuti, 2006; Murphy et al., 2006; Suzuki, Keisuke et al., 2011).

Leptin

Leptin is a peptide hormone (146 amino acids) that is released from adipose tissue and encoded by the obese gene (ob). The amount produced is directly proportional to the adipose tissue and it induces its effects by binding to Leptin receptors (LR). LRs are found in neuronal, hepatic, pancreatic, cardiac and intestine tissues (Frühbeck et al., 1998). Circulating leptin is transported across the blood-brain barrier, and after binding to its receptors in the hypothalamus, it stimulates a cascade of signalling pathways which results in the inhibition of several orexigenic peptides and the stimulation of other anorectic peptides (Arora & Anubhuti, 2006).

Mutations in the ob gene in humans results in severe obesity and hypogonadism, which can be treated with recombinant leptin therapy in children and adults (Frühbeck et al., 1998; Arora & Anubhuti, 2006).

Other gut hormones.

There are several other gut hormones that have been discovered to control appetite and food intake. Pancreatic polypeptide is released from the pancreas after a meal and reduces appetite. Amylin is released along with insulin from pancreatic B-cells in response to food ingestion. It is believed that its main function is glucose homeostasis, but amylin may also reduce food intake (Murphy et al., 2006). Meanwhile, Neurotensin and Xenin are gut hormones that are thought to regulate several digestive processes and control food intake. However, studies in rodents have not shown any anorexigenic effect. Further research is required to investigate these hormones and their role in food intake (Hussain & Bloom, 2013).

In summary, food intake is tightly controlled by a complex group of central and peripheral neural regulatory systems, involving the hypothalamus and brainstem, which integrate neural input from the higher and peripheral body's nutrient status. The gastrointestinal system (GIT) is the largest endocrine system, and it senses the presence of food and signals to the brain via neural and endocrine mechanisms that regulate short term appetite and satiety through the secretion of peptides and hormones that play a role in energy homeostasis in the human body (Figure 1.1). Hormonal imbalance can result in excessive weight gain and obesity, or unwanted weight loss. Gut peptides also represent an important pharmaceutical target for possible anti-obesity drugs.

OBESITY AND ENERGY HOEMOSTASIS

Evidence shows that energy homeostasis involves a complex interaction of neuroendocrine signals that originate from the GIT, the hypothalamus and adipose tissue to offer short- and long-term balance between energy intake and expenditure (Susan et al., 2004). To regulate food intake, the brain must modulate appetite, and this is achieved via an interplay between GIT hormones and the central nervous system (CNS). It is believed that information about general health is transmitted through long term humoral signals, while the process of meal initiation and termination is mediated via short term signals such as neural signals from brain and humoral signals from GIT. The receipt and integration of these signals take place at the hypothalamus Arcuate Nucleus (ARC) (Feinle-Bisset et al., 2021; Perry & Wang, 2012). Adipose tissue also acts as an endocrine

organ that affects energy homeostasis and cardiovascular health through the release of adipokines that regulate energy intake, energy expenditure, insulin sensitivity and inflammation (Korner et al., 2009). Adipose tissue regulates neuroendocrine function and metabolism via the production of peptides such as Leptin and adiponectin (discussed above). Leptin contributes to the overall energy balance by suppressing appetite and affecting energy expenditure. Adiponectin decreased insulin resistance while Resistin increased insulin resistance in rodents, although its action has yet to be established in humans (Korner et al., 2009; Susan et al., 2004). Energy homeostatic regulation therefore involves feeding, energy intake and expenditure regulations and body weight. Figure 1.1 summarizes the central and peripheral signals that regulate energy homeostasis.

Figure 1.1: Summary of the physiological regulation of food intake.

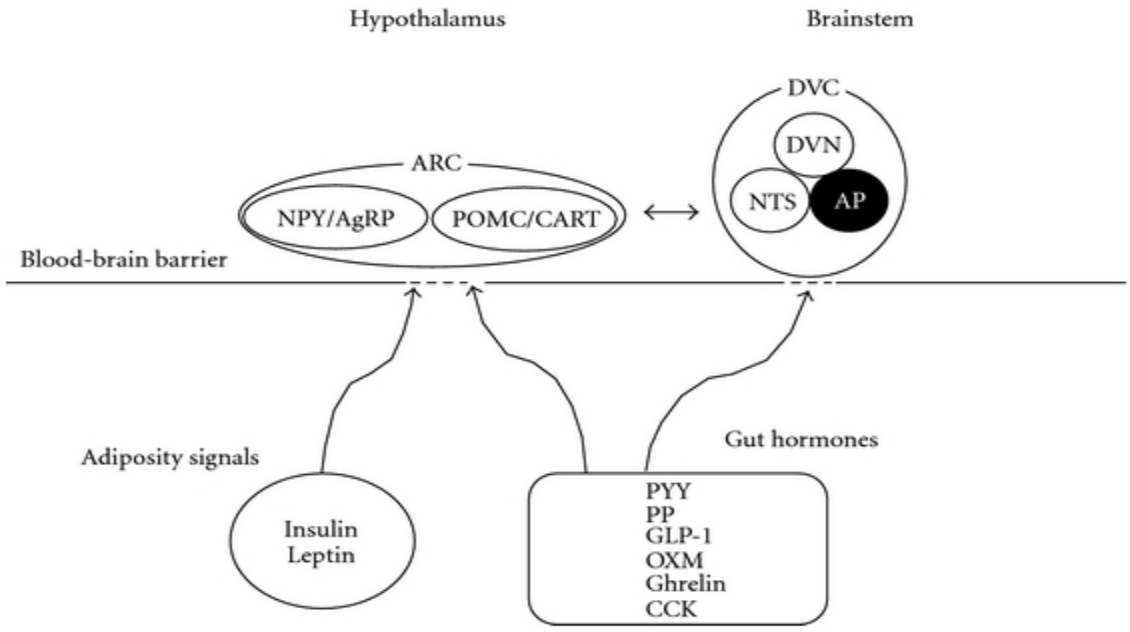


Figure1.1. A summary of the signalling pathways that unite at the hypothalamus and brainstem to regulate food intake. ARC, arcuate nucleus; NPY/AgRP, neuropeptide Y and agouti-related peptide; POMC/CART, proopiomelanocortin and cocaine- and amphetamine-regulated transcript; DVC, dorsal vagal complex; DVN, the dorsal motor nucleus of vagus; NTS, the nucleus of the tractus solitarius; AP, area postrema; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin; PP, pancreatic polypeptide; PYY, peptide YY; OXM, oxyntomodulin. (*Adopted from* **Suzuki et al., 2011).**

OBESITY AND DIABETES RISK

Obesity is characterized by chronic systemic inflammation that is derived mainly from adipose tissue inflammation and the overproduction of inflammatory cytokines such as tumour necrosis factor α (TNF- α), monocyte chemoattractant (MCP) and interleukin 6 (IL-6). The nuclear factor kappa of activated B-dependent pathway (NFk B) is strongly associated with insulin systemic resistance. Although there is a complex interaction between genetic and environmental factors in the development of obesity, imbalance between food intake and energy expenditure contributes largely to the deposition of adipose tissue. This expansion or hyperplasia results in the dysfunction of adipose tissue and the deposit of fat outside it. This deposition of fat extends to the ectopic areas and around vital organs such as the liver, heart, kidney and intestines, which is referred to as visceral fat (Farb & Gokce, 2015; González et al., 2017). Visceral adiposity is associated with vascular and insulin systemic inflammation, which promotes the atherosclerotic process. This increases the risk of cardiovascular disease (Engin, 2017) because these organs store excess energy and interfere with energy consumption in comparison to fat depots in the subcutaneous parts of the lower body that carry less risk of cardiometabolic disease.

Changes in body weight and body fat distribution are significantly associated with the risk of diabetes, as it is associated with insulin resistance (IR). In a survey of US adults, the risk of diabetes increased by a factor of 4.5% for every kilogram increase in body weight, and this association was consistent across various levels of age as well as gender and ethnicity (Ford et al., 1997). The Health Professional Follow up Study examined the relationship between changes in body weight and body fat distribution (1986-1996) and subsequent risk of diabetes (1996-2000) among 22,171 men. The study found that for every kilogram of weight gained, the risk of diabetes mellitus increased by 7.3%. More specifically, abdominal fat was positively associated with risk of diabetes, independent of the weight gain (Koh-Banerjee et al., 2004). The study also reported that 56% of new diabetes cases and 20% of existing cases were related to a weight gain of 7kg and a waist circumference increase of 2.5cm (Koh-Banerjee et al., 2004). Meanwhile, a review

of 216 cohort studies reported a 1.72 increase in the relative risk of developing type 2 DM for every 5 unit increase in BMI (Jayedi et al., 2022).

Given the growing evidence of weight gain linked to increased insulin resistance and diabetes risk, measures for obesity prevention are extremely important.

DIETARY FIBRE

Definition

Definitions of dietary fibre (DF) have evolved over a past century. Numerous definitions exist worldwide and there is no unified agreement as to the correct one. However, establishing a unified definition for dietary fibre is necessary for health regulations, nutritional labelling messages, dietary fibre intake comparisons and medical research. The term “Dietary Fibre” was first introduced in 1953, described by Hipsley to include intrinsic and non-digestible components such as cellulose, hemicellulose and lignin. Later, the definition was expanded by Trowell to include all plant components that are resistant to hydrolysis by gut enzymes. The definition was further expanded in eighties and nineties to include all plant components, including pectins, gums and other oligosaccharides that are resistant to digestion by human enzymes (Stribling & Ibrahim, 2023; Ester et al., 2025).

The widely accepted definition of DF was established by the 2001 National Academy of Sciences Expert Panel, which defined DF as intrinsic and intact fibre from plant sources and added fibre from isolated or synthesized sources. However, there are a few concerns regarding this definition from various regulatory institutions. The US Food and Drug Administration’s (FDA) requirements for proof of physiological effects, for example, have complicated the scientific understanding of DF. The inconsistency of the definition across countries has also made it difficult to interpret scientific research work. The US FDA excluded fermentation as an effect, while Canada’s Health Authority accepted fermentation as a physiological effect (Ester et al., 2025; Stribling & Ibrahim, 2023; Williams, B. A. et al., 2019).

The 2009 codex definition describes DF as carbohydrate polymers with 10 or more monomeric units. These are not hydrolysed by endogenous enzymes in the human small

intestine and are either naturally occurring or extracted from food by physical, chemical or enzymatic means with proven health benefits, or synthetic carbohydrates that are shown to have health benefits. All current definitions recognize DF as a group of carbohydrate polymers and oligosaccharides that escape digestion in the small intestine and pass into the large bowel, where they are partially or completely fermented by gut microbiota. The differences arise over areas such as the degree of polymerization (the debate on whether to include or exclude carbohydrates with DP 3-9) which was left to national authorities. There is also a second debate about whether fibre must be intrinsic or extrinsic and about the physiological health benefit of DFs (Fuller et al., 2016).

While there is no unified accepted definition of DF, the FDA released an official definition in 2015, as:

> "un-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignins that are intrinsic and intact in plants; isolated or synthetic non digestible carbohydrates (with 3 or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health" (Ester et al., 2025).

Classification

There is still uncertainty about DF classification, but they are usually classified based on sources, solubility, fermentability and health benefits. The most widely accepted method of classification is based on the degree of polymerization (DPs): simple sugars (1-2) are considered digestible and non-DF, while non-digestible carbohydrates are divided into low molecular weight (LMW) DFs with a DP of 3-9 and high molecular weight (HMW) long chain DFs with a DP of 10 to 15,000. The LMW DF fraction includes oligosaccharides and inulin, even though their DP can be greater than 10. The HMW fraction includes non-starch polysaccharides such as cellulose, hemicellulose, gums and pectin, as well as resistant starch (types 1-5), and other associated substances such as lignin (Stribling & Ibrahim, 2023). (Figure 1.2)

Figure 1.2: Classification of Dietary Fibres according to molecular weight.

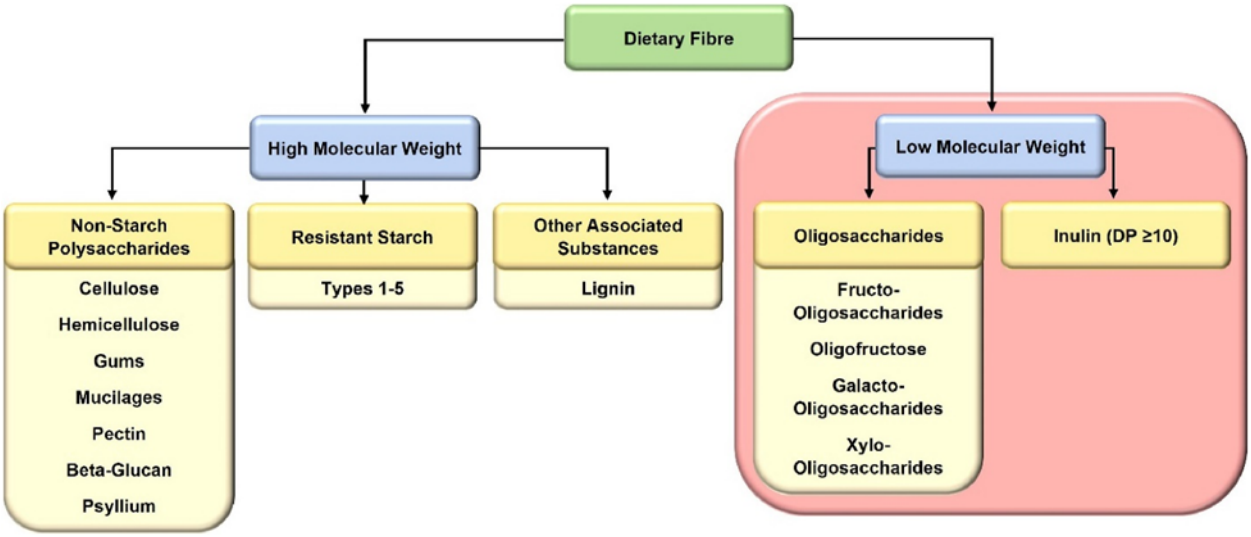


Figure 1.2. Classification of SCFAs based on the degree of polymerization (DP); they are classified into low molecular weight (DP3-9), and high molecular weight (DP≥10). *Derived from Tribling & Ibrahim (2023).*

Dietary Fibre, Weight Regulation and Appetite

Diet and lifestyle are important modulators of weight regulation and appetite, and studies have linked fibre intake to the levels of overweight and obesity. Recently, there has been an increase in the consumption of processed food and an inadequate consumption of high fibre food. (Chambers et al., 2015; Byrneet al., 2019). ADA recommends a daily standard consumption of ~ 25 g/d for women and ~ 38 g/day for men to increase fibre intake by 14 g fibre/1000 Kcal daily (Weickert & Pfeiffer, 2018) .

Several animal and human studies have shown that the increased consumption of dietary fibres is associated with reduced appetite and lower body weight, and reduces cardio metabolic risk (Chambers, E. et al., 2019). Research shows an inverse relationship between DF intake and change in body weight. A meta-analysis by Hong et al. (2015) found that high consumption of cereals, fibre or whole grains was associated with

significant reduction in all-cause mortality and death from CVD, T2DM, cancer, respiratory disease, and other illnesses, suggesting that the protective effect of the whole grain is due mainly to its high content of cereal fibre. Fibre reduces the energy density of the food due to its bulking effects and promotes satiety (Huang et al., 2015). An increase of 8g of dietary fibre per 1000 kcal over a 20 months longitudinal study of middle-aged women resulted in an average weight loss of 4.4lbs, which was mainly due to decreased body fat (Lattimer & Haub, 2010). Similarly, Koh-Banerjee et al. (2004) reported that for every 40 g/d increase in whole grain intake, weight gain decreased by 1.1lbs, suggesting a dose-response relationship. Among various fibre sources, bran showed the largest contribution to the reduction of weight gain by 0.8lbs per 20 g/d of bran intake (Koh-Banerjee, P. et al., 2004). Oat B-glucan has also been shown to reduce body weight, BMI, body fat and WHR, emphasising its role in reducing abdominal fat and obesity. B-glucan also plays helps to improve postprandial satiety. This soluble fibre contributes to the production of Cholecystokinin (CCK) that results in prolonged satiety (Fuller et al., 2016). Dextrin and Inulin-type fructans also have positive effects on energy homeostasis and glucose metabolism. Research studies reported a reduction of BMI, body fat, fasting glucose and fasting insulin (Martinez et al., 2021) ,

There are several hypothesized mechanisms by which DFs exert their beneficial effects on body weight. Firstly, DFs shift gut bacterial taxa such as *Bifidobacterium* and *Lactobacillus*, which are associated with beneficial effects on gut barrier, metabolic endotoxemia and ultimately body weight. Secondly, DF is known to produce short chain fatty acids (SCFAs), which stimulate the release of satiety hormones like Glucagon–Like Peptide (GLP-1) and Peptide YY (PYY), enhancing satiety. Additionally, DF increases post meal satiety and decreases hunger. It also delays gastric emptying and is well known for its bulking and laxative properties (Martinez et al., 2021; Fuller et al., 2016) .

Overall, an increase in dietary fibre consumption improves weight gain, although various fibre types have different beneficial effects on weight regulation due to their composition and solubility. Further studies are therefore required to understand their various contributions to host energy homeostasis.

Dietary Fibre, postprandial Glucose and Insulin

Dietary fibre plays a role in reducing insulin resistance and the risk of diabetes. Various DFs are fermented into different types and amounts of SCFA, inducing different effects on glycaemic responses. Soluble fibres have been shown to affect the post prandial glucose and insulin resistance positively. This effect has been linked to their high viscosity, which increases the viscosity of the stomach contents, prolonging gastric emptying, slowing transit time and reducing rate of starch digestion and glucose absorption (Gao et al., 2019) .

A study of healthy subjects consuming 20g of Fructoologosaccharides (FOS) per day reported a moderate decrease in hepatic glucose production, although no reduction in fasting plasma glucose concentration was observed (Luo et al., 2000). In a randomised controlled trial, women with T2DM consumed either 10g daily of resistant dextrin as an intervention or a similar amount of maltodextrin as a placebo for 8 weeks. Results showed that the intervention group showed a significant improvement in fasting insulin, insulin resistance and sensitivity markers, as well as pro inflammatory cytokines (TNF-α, IL-6), while no change was seen in fasting glucose and HbA1C% (Aliasgharzadeh et al., 2015). Mayer et al. (2000), found a 22% lower risk of developing diabetes in women who consumed 26g/d of DF, compared to women who only consumed 13 g (Meyer et al., 2000). Schulz et al.'s study (2004) supported these findings, with men and women showing a lower risk of diabetes ($p<0.001$) with the consumption of an additional 12g/d of DFs (Schulze, Matthias et al., 2004). It is worth mentioning that the inverse relationship between DFs and diabetes risk observed by Meyer et al. and Schulze et al. was not connected with age and body weight.

Most human studies involving resistant starch (RS) have shown a decrease in postprandial blood glucose and insulin levels. However, there was a variation in the carbohydrate content in the meals tested. Al-Tamimi et al. (2010) removed this variation by controlling for non-starch ingredients and available carbohydrates and concluded that postprandial glucose and insulin levels were significantly reduced with the

supplementation of 30 g of RS4 (Al-Tamimi et al., 2010). Inulin-type fructans were found to have beneficial effects on glucose metabolism, including an improvement in fasting glucose, fasting insulin, HOMA-IR and HbA1C (Régnier et al., 2021). In contrast, a crossover randomised study, which included overweight and normal weight men and women who consumed either 300ml water containing 75 g glucose as control or 24 g inulin (IN) or 28g RS for 6 hours on three separate days, found that IN significantly increases SCFA ($P<0.001$) but had no effect on free fatty acids (FFAs) or glucose and insulin responses. RS had no significant effect on SCFA, but reduced glucose ($P=0.05$) and insulin responses ($P=0.024$) (Rahat-Rozenbloom et al., 2017).

Looking at the mechanisms involved in the lowering effects of DFs on glucose, insulin and diabetes risk, understanding the absorption and solubility properties of DF gives some insight on how DF affects glucose and insulin level. Soluble DF demonstrates delayed gastric emptying and the decreased absorption of macronutrients, resulting in lower postprandial blood glucose and insulin levels. This is most likely attributable to the viscosity of soluble fibre inside the GIT. However, insoluble fibre has only a small effect on macronutrient absorption. It has been suggested that insoluble fibre increases the passage rate of foodstuffs through the GIT, resulting in a decreased absorption of nutrients. Other well studied mechanisms suggest that DFs stimulate the secretion of anorectic gut peptides, including GLP-1 and GIP (glucose-dependent insulinotropic polypeptide) and reduce appetite and food intake through the production of SCFAs, which will be discussed in more detail below (Lattimer & Haub, 2010). It is worth noting that the type of carbohydrates (NSC or DF) is more closely related to the risk of diabetes than the total carbohydrates, so it is important to understand the glycaemic index and glycaemic load of various carbohydrates in order to predict immediate postprandial glucose level.

Carbohydrates and diabetes risk.

Carbohydrates represent a heterogenous group with various metabolic effects, which means that not all carbohydrates in food and drink produce the same blood-glucose raising potential. They can be classified into simple or complex carbohydrates. Complex carbohydrates are less likely to cause blood sugar spikes because they take longer to break down, and as a result, blood sugar levels remain stable and fullness lasts longer. It is therefore important to understand food glycaemic index (GI) and glycaemic load (GL)

to address diabetes and metabolic risk. Glycaemic index (GI) is a relative measure of the incremental glucose response per gram of carbohydrates or the immediate postprandial glucose response in comparison to a reference group such as glucose or white bread. The glycaemic load is the glycaemic index multiplied by the amount of carbohydrate. Carbohydrates with low GI cause a smaller glucose/insulin response, while carbohydrates with high GI cause a greater glucose/insulin response (Lattimer & Haub, 2010; Chiu & Taylor, 2011).

Research studies have shown inconsistent results in terms of the potential of GI/GL to predict postprandial glucose and diabetes risk. This inconsistency may be related to the diverse components and their effect on digestion, absorption and glucose metabolism (Delzenne & Cani, 2005; Lattimer & Haub, 2010).

A large systematic review, which included 40 prospective observational studies to examine the effect of GI/GL for T2DM, CHD and strokes, found that the GI/GL in overweight/obese females had the strongest magnitude of risks in both US and Asian studies, and reported that eating more fibre and cereal decreased risk of T2DM (Hardy et al., 2020). Schulze et. al. (2004) reported 741 new incident cases of T2DM among 91,249 female nurses, after an eight-year follow up. After adjustment for confounding variables such as age, BMI and family history, they found a significant positive association between glycaemic index and the risk of T2DM and concluded that a higher glycaemic index was related to higher carbohydrate intake, higher glycaemic load, higher trans fatty acids and lower total fibre variables (Schulze et al., 2004). Mayer examined the relationship between diabetes incidents and several variables, including carbohydrate-related dietary variables in a large cohort women's health study in which detailed dietary information was collected at baseline (Meyer et al., 2000). After the adjustment of confounding variables, total carbohydrates, starch, lactose and maltose were found to be unrelated to the incidence of diabetes. Sucrose was inversely associated with incidence of diabetes, while glucose and fructose were positively associated with diabetes risk. However, the risk of diabetes across different glycaemic index and load was inconsistent (Meyer et al., 2000).

Complex fermentable carbohydrates offer the potential to lower diabetes risk. Short term consumption of 60g of resistant starch over 24 hours results in a postprandial decrease

in glucose and insulin, and an increase in postprandial insulin sensitivity in healthy subjects (Robertson et al., 2003). The addition of 40g of resistant starch to the diet of insulin resistant subjects for 12 weeks resulted in a significant increase in peripheral insulin sensitivity (P = 0.023) (Johnston et al., 2010). Research studies conducted on fermentable carbohydrates and diabetes risk so far have been promising, and this type of carbohydrate has the potential to prevent weight gain.

Dietary Fibre and Cholesterol Lowering Effect.

Elevated total and LDL-cholesterol levels are considered major risk factors for CVD. Gel-forming fibres that are carried by low-density lipoproteins (LDL) can lower high levels of cholesterol while leaving the HDL fraction unchanged (Cassidy & Vahouny, 1987). The proposed mechanism by which soluble fibres lower cholesterol claims that soluble fibre forms a viscous layer and increases gut viscosity, resulting in a lower reabsorption of bile acids while increasing the synthesis of bile acids from cholesterol and reducing circulating blood cholesterol.

Many studies support the positive effect of B-glucan in reducing blood cholesterol level, and subsequent reduction the risk of CAD. Studies in both hypercholesterolemic and healthy subjects found that the daily consumption of 5g of B-glucan significantly decreased serum totals and LDL cholesterol (Lattimer & Haub, 2010). Meanwhile, Beta Glucan (3g daily) reduced total cholesterol by 8.9% and non-high-density lipoprotein cholesterol levels by 12.1% over 8 weeks (Free et al., 2020) Based on this, The US FDA and the European Food Safety Authority (EFSA) have recommended a minimum consumption of 3g per day of oat or barley B-glucan to reduce blood cholesterol.

GLUCOSE METABOLISM REGULATION.

Circulating glucose in the body is derived mainly from intestinal absorption after feeding, glycogenolysis (the breakdown of glycogen to glucose) and gluconeogenesis (the formation of glucose). How fast glucose appears in the circulation depends on the type of food ingested and the rate of gastric emptying.

Glucose homeostasis is maintained by the bi-hormonal model through insulin and glucagon. In the fed state, glucose is absorbed from the ingestion of nutrients, and its concentration rises in the circulation. At this stage, the pancreas stimulates insulin secretion while glucagon secretion is supressed. Insulin also supresses gluconeogenesis and glycogenolysis in the liver and increases glucose uptake by peripheral tissues (skeletal, liver and fat) by binding to the cell receptor GLUT4, causing an influx of glucose. Meanwhile, insulin stimulates the process of glycogen and the synthesis of triglycerides, ultimately reducing plasma glucose.

In contrast, during fasting states, plasma glucose level remains between 4-5mM, and is highly regulated by the liver under the direction of glucagon. At this stage, the liver provides glucose as a fuel for other tissues including the brain, red blood cells and muscles. Glucagon promotes the cascade of kinase action that releases glucose from stored glycogen through a process called glycogenolysis, which is critical for maintaining glucose homeostasis during the overnight fasting period. After prolonged fasting (~30 hours), de novo glucose synthesis, or gluconeogenesis, is responsible for the generation of glucose for vital tissues. If the period of fasting extends further, the major non-carbohydrates precursors for this process are lactate, which is transported from skeletal muscles or red blood cells, and glycerol (Han et al., 2016; Petersen et al., 2017). Prolonged fasting also triggers ketosis within 2-4 days. The degree of ketosis and how fast the body begins the ketosis process varies according to age and individual health (Solianik et al., 2023; Grundler et al., 2024).

SHORT CHAIN FATTY ACIDS (SCFAs).

SCFA Definition.

The short chain fatty acids (SCFAs), or volatile fatty acids, are defined as saturated fatty acids made of 1 to 6 carbon atoms produced by the fermentation of colonic microflora. SCFAs are week organic acids, and each molecule of SCFA has only one carboxylic organic acid function (-COOH) (Bornet et al., 2002). SCFAs are major metabolites produced in the colon through the anaerobic gut bacterial fermentation of non-digestible

carbohydrates (NDC) or Complex carbohydrates such as FOS, RS, IN and polysaccharides, which escape digestion and absorption in the small intestine. Other metabolites, including hydrogen, methane and carbon dioxide are also released through NDC fermentation (Nogal, Valdes et al., 2021). The most abundant SCFAs in the gut are acetate (C2), propionate (3) and butyrate(C4) which constitute >95% of the SCFAs, while <5% are made up of formate, valerate and caproate (Figure 1.3) (Canfora et al., 2017).

SCFA Production.

Most SCFA production takes place in the large intestine, specifically the cecum. SCFAs are produced in relatively high quantities (70-140mM) in the proximal colon, followed by the distal colon (20 to 70 mM) and the distal ilium (20 to 40mM) (Rauf et al., 2022). It is estimated that fermentation of 50-60g of carbohydrates per day yields approximately 500-600mmol/l of SCFA in the gut (Nogal, Valdes et al., 2021). The major SCFAs formed are acetate, propionate and butyrate that are found in the colon and stool with an approximate molar ratio of 60:20:20 respectively. These values may vary based on the physicochemical nature of fibres and the fermentable substrate that affects the amount and the profile of the SCFA produced (Bornet et al., 2002). For example, the fermentation of oat bran, which contains high amount of ß-glucan, yields a larger amount of major SCFA (Canfora et al., 2017). Other factors include specific species of microbiota, diversity and absolute amount of microbiota in the intestine of the host, the Ph of the colon and gut transit time (Bornet et al., 2002; Rauf et al., 2022). Metagenomic approaches have facilitated the identification of the bacterial species responsible for SCFA production, proving that the acetate formation pathway is widely distributed among the bacterial species, whereas propionate and butyrate production appear more conserved (Blaak et al., 2020). Complex enzymatic pathways are involved in the production of SCFAs from DFs. The glycolytic pathway is known to be the most predominant pathway (Figure 1.4).

Acetate formation

Acetate production occurs via two different pathways. Firstly, acetyl-coenzyme A (acetyl-CoA) is produced by the decarboxylation of pyruvate, then acetyl-CoA is hydrolysed to acetate by an acetyl-CoA hydrolase. The most common bacterial species that can

produce acetate are *Prevotella* spp., *Ruminococcus* spp., *Bifidobacterium* spp., *Bactriodes* spp. and C. *muciniphila*. Secondly, the Wood-Ljungdahl pathway, where the reduction of carbon dioxide to carbon monoxide reacts with acetyl-CoA to produce acetate later (Nogal et al., 2021).

Propionate formation

Propionate is produced via the succinate, acrylate or propanediol pathways. The succinate pathway predominates, whereby succinate is converted to methylmalonate, then to propionate and carbon dioxide. The most common bacterial species that use this pathway are *Bacteroidetes* and *Firmicut*es. Secondly, the Acrylate pathway, in which lactate is reduced to propionate by acetyl-CoA dehydratase. This pathway is restricted to very few species like *Cooprococcus catus*, *Lachnospiraceae* and *Negativicutes*. Lastly, the propanediol pathway uses deoxy sugars such as fucose and rhamnose. In the same way, 1,2 propandediol is converted into pro-pionaldehyde and propionyl-CoA, which leads to the formation of propionate. This is more prevalent within the *Lachnospiraceae, Ruminococcus obeum and in Roseburia inulinivorans* (Nogal et al., 2021; Louis & Flint, 2017).

Butyrate formation

Butyrate is formed by the combination of two molecules of acetyl-CoA to form acetoacetyl-CoA, which is followed by a stepwise reduction to *butyryl-CoA*. Two routes have been identified for the final step in butyrate formation, either via butyryl-CoA: acetate CoA-transferase or via phosphotransbutylase and butyrate kinase (Louis & Flint, 2017). Butyrate production is widely distributed among Gram-positive Firmicutes. Two abundant groups are related to *Eubacterium rectale/ Roseburia* spp and to *Faecalibacterium prausnitziilike* spp. (Blaak et al., 2020).

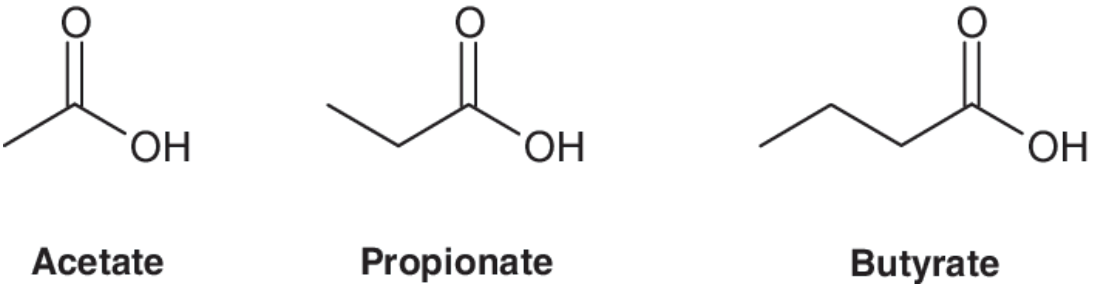


Figure 1.3. The chemical structure of the short chain fatty acids, acetate (C2), propionate (C3) and butyrate (C4). *From Darzi et al.2011.*

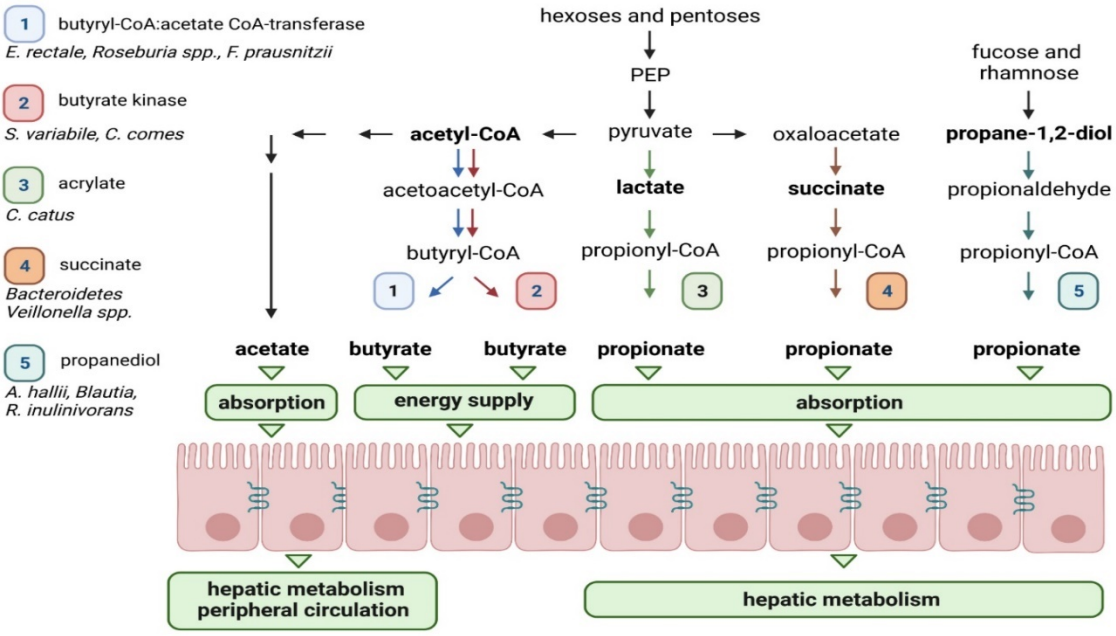


Figure 1.4. Short Chain Fatty Acids (SCFA) biosynthesis pathways from dietary carbohydrate fermentation and the major SCFA-producing bacteria. Acetate is formed mainly from pyruvate via acetyl-coenzyme A (CoA). Propionate is formed via three pathways: acrylate, succinate and propanediol. The first two pathways start from phosphoenolpyruvate (PEP) and the last uses deoxy sugars, such as fucose and rhamnose. Butyrate is produced via two pathways: the butyryl -CoA: acetate CoA-transferase and the butyrate kinase. *From Nutrients 2022*

SCFA absorption and Systemic Concentrations

Colonic absorption of SCFAs is effective and rapid in the colonocytes or through the portal vein into the liver, with only 5-10% excreted in the faeces (Blaak et al., 2020). Unionized SCFAs can easily cross the apical membrane of the colonic epithelium cells, while ionized SCFAs require specific transporters for absorption. Two main receptors have been identified: The monocarboxylate transporter-1 (MCT-1) and the sodium-coupled monocarboxylate transporter-1 (SCMCT-1). These receptors are present in colonocytes, cecum and the small intestine. They are also expressed in the lymphocytes, thyroid glands, and kidneys (Rauf et al., 2022; Canfora et al., 2017).

Most SCFAs are used by colonocytes as an energy source, mainly butyrate. SCFAs are then transported towards the hepatic portal vein. The estimated SCFA concentrations in portal blood (~ 375 Mmol/l) are almost 5 times higher than in peripheral venous blood (~ 79 Mmol/l), and the concentrations of the SCFAs in the hepatic vein are 39% of those found in portal blood, at around 148 Mmol/l (Blaak et al., 2020; Rauf et al., 2022).

Table 1.2 shows the concentration of SCFAs and molar ratio in the colon, portal and periphery.

SCFA Receptors

SCFAs act as signalling molecules on gut cells and other tissue cells, and act by binding to receptors, triggering a cascade of actions. The main receptors are free fatty acid receptor 3, previously known as G-protein coupled receptor (FFAR3 /GPR41), FFAR 2/ GRP43, G-protein coupled receptor 109a/ hydroxycarboxylic acid receptor 2 (GPR109a/ HCAR2) and Olfactory receptor-78 (Olfr78 in mice or OR51E2 in humans) (Nogal et al., 2021).

FFAR3, FFAR2 and GPR109a are expressed in numerous cells in the small intestine, colon, liver, spleen, heart, skeletal muscle, neurons, immune cells and adipose tissue. FFAR2 prefers to bind acetate and propionate, while FFAR3 binds propionate, butyrate, and acetate with lower affinity. Meanwhile, GPR109a mainly binds butyrate, which is the major intestinal fuel that supplies ~60-70% of the energy needs for colonocytes. Butyrate

also acts as a ligand for two transcription factors: Peroxisome proliferator-activated receptor ð (PPAR ð) and aryl hydrocarbon receptor. Olfr78 mainly binds acetate and propionate, leading to an increase in cyclic adenosine monophosphate (cAMP) and renin release, through which acetate and propionate are though to regulate blood pressure (Nogal et al., 2021; Canfora et al., 2015).

Table 1.2: Short Chain Fatty Acids (SCFAs) molar ration and concentration.

SCFA	Colon		Portal vein		Hepatic vein		peripheral	
	R	µmol/kg	R	µmol/kg	R	µmol/kg	R	µmol/kg
Acetate	60	43.5–63.4	71	182-263	81	71-220	90	5-220
Propionate	20	14.2-26.7	21	18-30	12	0.8-7	5	3-8
Butyrate	20	14.7-24.4	8	15-30	7	0.5-12	5	7-10

Table 1.2. The SCFA molar ratio (R) and concentration expressed as µmol/kg of luminal content in the colon, portal, hepatic and peripheral blood. Acetate is the most abundant SCFA in these areas. *From Black et al. 2020*

BIOLOGICAL FUNCTION OF SCFAs.

SCFAs result directly from the fermentation of NDCs in the colon. It has been shown that SCFAs have several biological functions for maintaining gut health, specifically butyrate, which is the preferred oxidative fuel for colonocytes. Butyrate improves the integrity of the epithelial barrier by regulating the tight junctions between intestinal epithelium cells. It is sometimes referred to as the butyrate paradox because it induces cell proliferation in healthy colon, but terminal differentiation and apoptosis in transformed cells (Blaak et al., 2020; Morrison & Preston, 2016). SCFAs have significant cellular effects on the mucosal immune system, such as regulating cellular pathways, affecting the immuno-metabolism and the epigenetic regulation of lymphocytes. This is achieved by inhibiting Histone Deacetylases (HDACs), resulting in a reduction in autoimmune activity and intestinal inflammatory effects (Liu, Xiao-Feng et al., 2023).

Several studies have shown the favourable effects of SCFAs on body weight control by regulating energy intake and energy expenditure. SCFAs, specifically butyrate and

propionate, have also been shown to improve glucose levels and increase glucose uptake, mediated by FFAR2 and FFAR3 activation. SCFAs can also regulate lipolysis and adipogenesis, resulting in a decrease in the circulating lipid plasma levels and bodyweight.

Effect of SCFAs on Weight and Energy Regulation.

According to several studies, an increase in the daily consumption of dietary fibre helps prevent weight gain and control body weight. This is linked to an increase in the production of colonic SCFAs. As the main metabolites for NDC colonic fermentation, SCFAs have been associated with a positive effect on weight control by regulating energy intake and expenditure. Several studies have investigated the effect of SCFAs on body weight, concluding that SCFAs can prevent or attenuate weight gain via decreases in energy intake and increases in energy expenditure (Sukkar et al., 2019). The potential mechanisms through which SCFA induces this positive effect on body weight is by stimulating the anorectic hormones, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) and secretion from enteroendocrine L cells by binding to the GPCR 41/43, re-named FFAR3/ FFAR2 respectively (Nogal et al., 2021). The rise in GLP-1 and PYY secretion attenuates appetite by stimulating the POMC neuron in the hypothalamus, suppressing NYP and delaying gastric emptying (Nogal et al., 2021; Morrison & Preston, 2016). Butyrate also tends to inhibit HDAC, resulting in the stimulation of PYY secretion. Frost et al. (2014) demonstrated that acetate can cross the blood-brain barrier and is taken up the hypothalamus, resulting in a decrease in appetite and increases in γ-aminobutyric acid and lactate using ^{11}C-acetate and PET-CT (Frost et al., 2014). SCFAs can therefore modulate neuronal activity and visceral reflexes directly via receptors expressed in the neuron in the central, peripheral, autonomic and somatic nervous system, providing an additional mechanism for weight and appetite regulation (Morrison & Preston, 2016). SCFAs can also increase energy expenditure through increased lipid oxidation (Sukkar et al., 2019).

Data from animal studies have shown that treatment with SCFA can reduce body weight and adiposity. An oral administration of sodium butyrate in obese mice led to the loss of

body weight via increased energy expenditure and fat oxidation (Gao et al., 2009). In another study, researchers found a significant reduction in body weight gain (~8g) in mice fed a high fat diet (HFD) for 12 weeks with either oral administration of acetate, propionate and butyrate compared to HFD alone (P=0.05) (den Besten et al., 2015). A further study by Arnoldussen et al. (2017) confirmed the positive effect of SCFA on weight control in mice, in which 2 months of butyrate supplementation tended to reduce body weight by 16%, but 12-months supplementation in mice (>10 months old) showed a higher reduction in body weight of 23% (P<0.001). This highlights the potential for weight gain prevention through long term supplementation (Blaak et al., 2020). Knockout (KO) receptors of GPR 41/GPR43 also lowers energy expenditure in rodents (Kimura et al., 2011; Kimura et al., 2013)

Studies in humans indicate that SCFA could be used as energy intake regulator. For example, an intake of 10g of inulin propionate ester in an acute crossover study increased satiety and reduced appetite, measured by visual analogue scales (VAS), compared to inulin alone (Alhabeeb et al., 2014). Chambers et al. (2018) showed that the ingestion of 6.8g of Na propionate in healthy male volunteers showed a reduction in whole-body resting energy expenditure and lipid oxidation compared to Na chloride control (Chambers et al., 2018). Meanwhile, the consumption of 1.5g of acetate daily for 12 weeks in obese participants resulted in higher reduction in weight and in visceral fat percentage compared to a placebo group (Canfora et al., 2015). A further study with a mixture of SCFAs, rectally infused overnight in fasted normoglycemic participants, improved resting EE compared to a placebo. However, no significant differences between interventions were found in energy expenditure or substrate oxidation (Canfora et al., 2015). In contrast, no effect on energy expenditure was seen in normoglycemic overweight/obese subjects after distal colonic infusions of acetate (van der Beek et al., 2016).

In summary, the impact of SCFAs on weight regulation is promising. Most of the available human studies show similarities with studies on rodents, demonstrating a favourable effect on body weight and energy expenditure. However, more human studies over longer periods of supplementation are required in order to confirm the effects of SCFA on body weight and appetite regulation.

Effect of SCFAs on Glucose Metabolism

SCFAs, including acetate, propionate, and butyrate, are produced in the colon through the microbial fermentation of dietary fibres. Once produced, they are absorbed into the circulation and can induce systemic effects. Research studies show associations between higher colonic SCFA concentrations and improved insulin sensitivity and/or lower fasting glucose (Cherta-Murillo et al., 2022; Zhang et al., 2023). The potential mechanisms or pathways that are relevant to glucose regulations include gut hormone release, hepatic substrate metabolism, adipose tissue signalling and peripheral insulin sensitivity(Canfora et al., 2019; Chambers et al., 2018). However, no conclusive effect of SCFAs on glycaemia has been found, and this could be attributed to the type of SCFA, dose, route, individual gut microbiota composition and individual phenotypes and responses.

The widely studied mechanism by which SCFAs influence glucose regulation in humans is through the stimulation of anorectic gut hormones; mainly GLP-1 and PYY. SCFAs bind to the G-coupled receptors FFAR2/FFAR3 expressed in the enteroendocrine cells, stimulating the release of GLP-1 and PYY. This slows gastric emptying, reduces postprandial glucose excursion and stimulates insulin release (Byrne et al., 2015; Chambers et al., 2015; Tolhurst et al., 2012). Various studies of high DF supplementations have demonstrated improvements in glucose and insulin sensitivity through an increase in the production of SCFAs (Canfora et al., 2019). However, other studies have demonstrated that glucose and insulin are not affected after a rectal administration of acetate and propionate (Canfora et al., 2017; Freeland et al., 2009; Laurent et al., 1995; Wolever et al., 1989), although a decrease in circulating fatty acids and an increase in fasting PYY was reported (Freeland et al., 2009). Long-term studies appear to show the positive effects on glucose and insulin compared to acute studies. Venter et al. (1990) reported a decrease in fasting glucose level and an increase in insulin secretion during an oral glucose tolerance test after 7 weeks of propionate supplementation in healthy females (Venter et al., 1990). Subsequent daily propionate supplementation in bread for one week decreased glucose levels in comparison to propionate free bread (Todesco et al., 1991) . However, this was explained by lower digestion rates, as higher faecal bulk was found in the propionate treated group. Another mechanism is hepatic metabolism,

through which SCFAs reach the circulation after absorption. Human studies suggest that SCFAs may attenuate hepatic glucose output and modulate hepatic lipid oxidation, although the extent of these effects may vary between SCFAs and their concentration (den Besten et al., 2013). The same investigator found that dietary SCFAs in mice prevented weight gain and its associated metabolic changes by decreasing peroxisome proliferator-activated receptor γ (PPAR γ) expression and activity therapy and increasing the expression of Mitochondrial Uncoupling Protein 2 (MCP2) and the AMP to ATP ratio as well as enhancing the oxidative metabolism in hepatic and lipid tissues via AMPK pathway (den Besten et al., 2015). However, this mechanism needs further investigation in humans. Meanwhile, Tirosh et al. (2019) demonstrated that propionate supplementation in mice led to the rapid activation of the sympathetic nervous system and an increase in the fasting hormone glucagon and fatty acid binding protein 4 (FABP4) in the postprandial state. This finding was replicated in human studies, enhancing hepatic glycogenolysis by leading to hyperglycaemia and compensatory hyperinsulinemia (Tirosh et al., 2019).

Another proposed mechanism is adipose tissue signalling and insulin sensitivity, through which SCFAs have been found to regulate lipolysis and adipokine secretion via FFAR2/3, enhancing insulin sensitivity (Chambers et al., 2018). Other investigations have shown an improvement in peripheral and hepatic insulin sensitivity in healthy lean men compared to men with metabolic syndrome after daily supplementation of 4g of sodium butyrate for 4 weeks (Bouter et al., 2018). The administration of inulin propionate ester *in vitro* to human beta cells showed that propionate increased glucose stimulated insulin system (GSIS) and maintained beta-cell mass through the inhibition of apoptosis (Pingitore et al., 2017). Studies also showed that higher circulating SCFAs (not faecal) are associated with increased insulin sensitivity indices markers such as QUICKI (Müller et al., 2019). SCFAs may also indirectly affect glucose regulation through appetite suppression and the reduction of body weight (Byrne et al., 2015).

The most robust human study linking specific SCFA to metabolic benefits is a randomised controlled trial using the targeted colonic delivery of inulin-propionate ester (IPE), which showed reduced weight gain, improved insulin sensitivity, and increased GLP-1 secretion in overweight adults (Byrne et al., 2019; Chambers et al., 2015).

These studies provide direct and indirect evidence of the effect of SCFAs on glucose regulation in humans. It appears that the association between SCFA supplementation, whether through DFs intake or direct administration of SCFAs, remains correlative and inconclusive, while the heterogeneity of the results reflects interindividual gut microbiota composition and fermentability capacities. Short-term studies showed the favourable effect of SCFAs specifically on glucose levels, but longer-term studies are required to examine their effects on glucose and insulin sensitivity. In addition, not all SCFAs produce the same effect on glucose regulation, and different routes of administration of SCFAs – whether oral or intravenous – may have different effects. Other factors that might contribute to the uncertainty and variation in the effects include SCFA dose, period of exposure, participant phonotype, interindividual differences in diet and variability in terms of individual response (Cherta-Murillo et al., 2022). There is also an ongoing debate about whether faecal or serum SCFAs measurement better reflects an individual's metabolic status (Müller et al., 2019). Currently available mechanistic studies are derived from animal models that do not translate fully to human physiology. Future research should use well controlled human studies using different SCFAs and a wider range of doses for longer durations in different participant phenotypes in order to address short- and long-term glycaemic end points.

The Effect of SCFAs on Lipid Metabolism.

SCFAs affect lipid metabolism and adipose tissue at various levels. In the liver, propionate is gluconeogenic, whereas acetate and butyrate are lipogenic (Morrison & Preston, 2016). Circulating SCFAs can regulate lipolysis and adipogenesis. Acetate and propionate can inhibit endogenous lipolysis, whereas propionate can only inhibit extracellular lipolysis (Nogal et al., 2021). The inhibition of lipolysis lowers FFAs, which are the precursor for lipid synthesis in the liver. SCFAs were also found to inhibit insulin-stimulated lipid accumulation in adipocytes via FFAR2 signalling, resulting in smaller adipocytes and lower inflammatory markers. Acetate was found to stimulate leptin secretion, which is an important energy and appetite regulator (Morrison & Preston, 2016). Acetate and propionate play a crucial role in adipogenic differentiation via the overexpression of FFAR2 and PPARγ (Hong et al., 2005).

Several studies, *in vitro* and *in vivo*, have examined the effect of SCFAs on lipid metabolism. Oral/intravenous acetate administration, and the administration of SCFA mixtures in animal models of obesity and T2DM demonstrated a decreased lipid accumulation in the liver and improved glucose homeostasis (Kondo et al., 2009; Sakakibara et al., 2006; Yamashita et al., 2009; den Besten et al., 2015). Meanwhile, the treatment of murine adipocyte with acetate and propionate reduced intracellular lipolytic activity by 50% (Ge et al., 2008). Another study examined the effect of SCFAs on lipolysis. Using a mixture of butyrate and propionate, the study found that glycerol release was enhanced but had no significant effect on in lipolysis rate (Rumberger et al., 2014).

Taken together, SCFAs tend to increase the hepatic uptake of cholesterol and decrease plasma cholesterol in animal studies. Further controlled human studies are required to examine the role of SCFAs on lipid regulation.

Figure 1.5: SCFA-mediated effects on host metabolism.

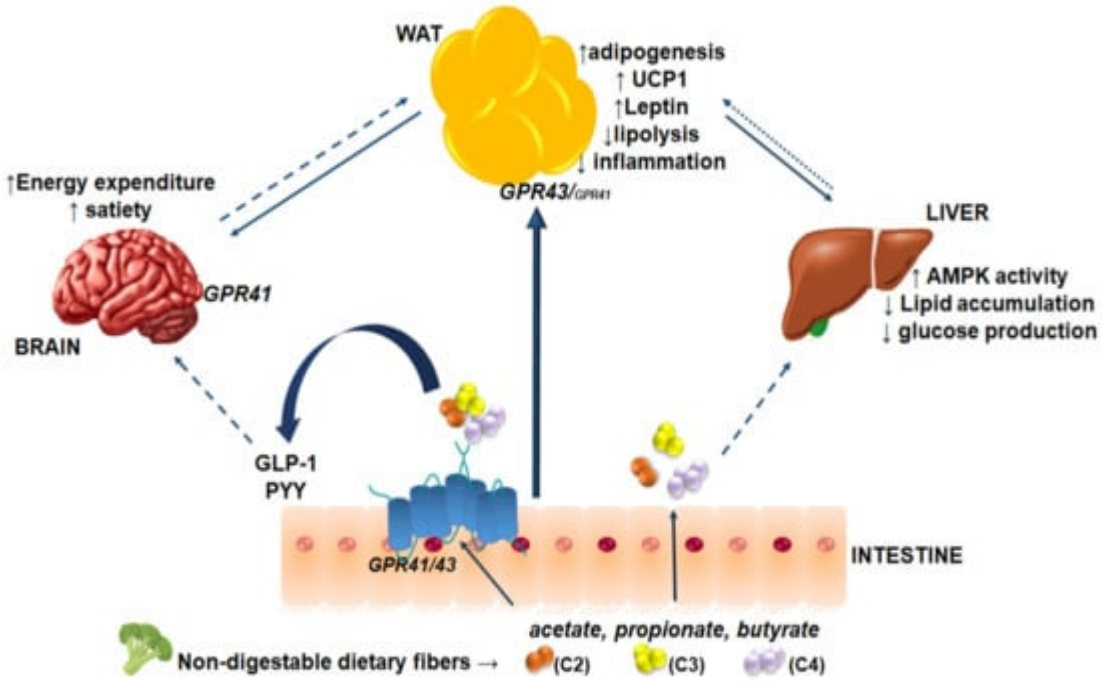


Figure 1.5. SCFA-mediated effects on host metabolism. Non-digestible dietary fibres consumed by the host are fermented by microbiota in the gut, resulting in the production of the SCFAs acetate (C2), propionate (C3) and butyrate (C4). These SCFAs are absorbed by diffusion or via GPR41 and GPR43 in the intestine. Systemic SCFAs can directly signal adipose tissue to modulate adipogenesis, lipolysis, inflammation,

browning and adipokine synthesis. SCFAs can also impact adipose tissue indirectly via their effects on gut hormones, the brain and the liver. As well as directly targeting the brain via GPR41, SCFAs can also modulate the secretion of gut hormones such as GLP-1 and PYY, which target the brain to regulate food intake. SCFAs can also target the liver to reduce hepatic glucose production and lipid accumulation, probably through the activation of AMPK. Solid lines indicate direct effects of SCFAs via adipose tissue; dashed lines indicate indirect effects of SCFAs; dotted lines indicate hypothesized effects of SCFAs. *From May & den Hartigh (2021).*

SUMMARY

Obesity is rising rapidly and has become a major global health problem associated with an increased risk of non-communicable diseases such as type 2 diabetes mellitus (T2DM), hypertension (HTN), coronary heart disease (CHD), Hypercholesterolemia, osteoarthritis (OA) and various cancers.

There is growing evidence that gut microbiota have a beneficial metabolic effect on human health through the production of short chain fatty acids (SCFAs), which are key metabolites derived from bacterial fermentation, namely acetate, propionate and butyrate. Once produced, SCFAs are absorbed locally in the colon and then circulate systemically to reach various tissues and organs.

Primary SCFAs are acetate (C2), propionate (C3) and Butyrate (C4), which are typically produced at the ratio of 60:20:20 respectively. These SCFAs act as signalling molecules, targeting metabolic organs and exerting beneficial effects by binding to free fatty acid receptor 2 and 3 (FFAR2 and FFAR3) as well as regulating energy balance and glucose and lipid metabolism.

Although the role of SCFAs in weight and energy expenditure regulation has been widely addressed, human studies investigating their impact on glucose homeostasis remain limited. Existing human tends to be short term and yields inconclusive results. Meanwhile, the physiological impact of SCFAs on glucose homeostasis in humans has been underestimated. Propionate, despite being the least-studied among SCFAs, has occasionally been implicated in promoting hyperglycaemia or exacerbating glycaemic control.

To address these knowledge gaps, we conducted the first review focusing specifically on the impact of SCFAs, both individually and collectively, on glycaemia in humans. The

second study explored the association between circulating acetate levels and glucose concentration in individuals with Type 2 diabetes. The final study investigated the impact of a chemically engineered compound – inulin propionate ester, which is designed for the targeted colonic delivery of propionate – on glucose and body weight in overweight young adults.

Overall Thesis Hypothesis:

Short chain fatty acids (SCFAs) have a beneficial effect on weight regulation and glucose homeostasis in humans.

Overall Thesis Objective:

To investigate the role of short-chain fatty acids (SCFAs) on body weight and glucose regulation in humans.

To examine this hypothesis, the following studies were conducted:

Study 1: The effect of short-chain fatty acids on glycaemic control in humans: A systematic review and meta-analysis.

Study 2: The association between plasma acetate and plasma glucose in obese diabetic subjects treated with insulin on a low-calorie diet: a double-open, randomised, parallel- group study.

Study 3: The impact of inulin propionate ester on weight and glucose regulation in overweight young adults: A multi-centre, double-blind, randomised parallel-group study.

CHAPTER 2

THE IMPACT OF SHORT CHAIN FATTY ACIDS ON GLYCAEMIC CONTROL IN HUMANS: A SYSTEMIC REVIEW AND META-ANLAYSIS.

ABSTRACT:

Introduction:

It is believed that dietary fibre exerts favourable effects on health by promoting the production of short chain fatty acids (SCFAs) through microbial fermentation in the gut. This can improve body weight and glucose metabolism and enhance insulin sensitivity.

Objective:

This systematic review and meta-analysis aims to assess the effect of SCFA intervention, individually or mixed, on glycaemic control measures in healthy and metabolically unhealthy humans.

Method:

Relevant articles published up to 7th December 2021 were extracted from Central, PubMed, Embase, Scopus and the Web of Science. Papers were included if they reported randomised controlled trials measuring glucose and/or insulin compared to a placebo in adults. Studies were categorized according to the type of SCFAs, intervention duration and health status of the participants. Random effects meta-analyses were performed for those subject categories ≥ 3 studies, or a narrative review was performed.

Results: From 43 eligible papers, 46 studies were identified (n = 913), and 44 were included in the meta-analysis. Vinegar intake decreased the acute glucose response (SMD, -0.53; 95% CI, -0.92 to -0.14; n=67) in individuals with impaired glucose or T2DM

and in healthy participants (SMD, -0.27; 95% CI, -0.54 to 0.00; n=186). However, the acute and chronic intake of acetate, propionate and butyrate had no effect on glycaemic measures.

Conclusion: Vinegar decreases the glucose response in healthy and metabolically unhealthy individuals. Significant heterogeneity, risk of bias and low to very low certainty of evidence were identified in several study categories, including acute vinegar response. Results therefore need to be interpreted with caution. Further research is required to determine the effects of SCFAs on glycaemic control.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) and its co-morbidities are increasing alarmingly worldwide. The global prevalence of diabetes was estimated at 9.3% 463 million in 2019, and is projected to increase to 10.2% (578 m) by 2030 and 10.9% (700 m) by 2045 (Saeedi et al., 2019). Around 90% of type 2 diabetic patients have a BMI greater than 23.0 kg/m^2 (Haslam, 2010). The relationship between T2DM and obesity makes it a public health issue, in which the strategies to control T2DM should focus more on obesity prevention rather than treatment.

Diet is a major modifiable risk factor for the development of obesity and its adverse effects. Recent years have seen an increase in the consumption of processed food and an inadequate consumption of high fibre food (Tristan Asensi et al., 2023). Evidence suggests that dietary fibre is consumed in lower than recommended amounts (Boyle et al., 2024). The average modern western diet contains less than 15 g/day of dietary fibre (DF), which is less than the recommended standard daily intake of ~ 25-30 g/day (Tristan Asensi et al., 2023). DF passes through the GIT unaffected by digestive enzymes, reaching the colon, where it is degraded by the colonic microorganisms, yielding gases (CO2, H2 and CH4), heat and short chain fatty acids (SCFAs) (Byrne et al., 2015).

The major SCFAs produced in human the colon are acetate, propionate and butyrate, with estimated molar ratio of 60, 20, 20 respectively (Koh et al., 2016). SCFAs activate G-protein coupled receptors known as FFAR2 and FFAR3, which are expressed in the gut and in metabolically active tissues such as the liver, adipocytes, myocytes, the pancreas and enteric neurons (He et al., 2020). It is believed that SCFAs influence glucose metabolism by stimulating the release of anorectic gut hormones such as GLP-1 and PYY. However, studies that have investigated the impact of SCFAs on glycemia in humans remain inconclusive. We therefore aim to determine the extent of the evidence regarding the impact of SCFAs on glycaemia in humans. Providing such evidence may support the consideration of SCFAs as potential preventive and therapeutic targets.

METHODOLGY

To gain an overview of the effect of SCFAs on glycaemic variables in humans, we performed a literature search in PubMed (MEDLINE), Cochrane CENTRAL, EMBASE, Web of Science and Scopus from inception to 7 December 2021. The systematic review was conducted in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (O'Dea et al., 2021). The formal screening of papers began on November 15th 2020. The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021231115) on January 14th 2021 and is available from https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=231115.

Eligibility Criteria

PICOS (patients, intervention, comparator, outcome, study design) criteria were used to establish study eligibility, and these criteria are summarised in table 2.1.

Table 2.1: PICOS criteria for study inclusion in the systematic review

	Inclusion	Exclusion
Participants	Humans who are healthy, overweight, or obese, have metabolic syndrome or type 2 diabetes.	Animals and other type of diseased humans or who undergoing clamps such as hypoglycemic or hyperinsulinemic euglycemic clamp.
Intervention	Acetate, propionate, butyrate alone or mixed and vinegar administration. Both acute (for 24h) and chronic (over 24h) administrations	SCFA conjugated with drugs or hormones.
Comparator	Placebo	Against diseased humans
Outcomes [a]	Quantifiable measures of glycaemic control as main or secondary outcomes such as fasting glucose/insulin, postprandial glucose/insulin (i.e. area under the curve), Hb1Ac, insulin sensitivity indexes (i.e. HOMA-IR, clamps)	Studies which do not include a quantifiable measure of the outcomes of interest.
Study design	Study designs that generate empirical data from interventional studies which are randomised controlled trials (RCTs). Only results analysed statistically will be included.	Reviews, conference abstracts, dissertation abstracts, lectures, information pieces, study registers and *corrigendums* were not included. Studies were limited to English language published from inception and onwards.

[a] Outcomes measured were defined according to the ADA's classification and diagnosis of diabetes: standard of care -2023, as follows:

Fasting Blood Glucose (FBG): plasma glucose concentration after an overnight fast of 8 hours.

Postprandial Glucose (PPG): plasma glucose concentration after a meal of 2 hours

Glycated Haemoglobin (Hba1c%): a surrogate marker of the average glycemia level over the past 8-12 weeks.

Fasting Insulin (FI): plasma insulin measured fasting

Postprandial Insulin (PPI): plasma insulin measured after a meal or during tests (meal tolerance and OGTT) (ElSayed et al., 2023).

Search Criteria

The search terms used during the online search for each database (PubMed, Cochrane CENTRAL, EMBASE, Web of Science and Scopus) are summarised in the Appendix (Chapter #2).

Study Selection

The study selection was performed using the online Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org. All articles identified by the search strategy were screened by title and abstract by 2 reviewers independently. Potential post-screening full texts were assessed for eligibility against the defined inclusion and exclusion criteria independently (Table 2.1). During study screening and assessment, any discrepancies in eligibility of papers were resolved by consulting a third party. Excluded studies and reasons for exclusion can be found in the Appendix (Chapter#2).

Data Extraction and Quantification

Articles considered eligible for inclusion were assigned to subject categories according to the nature of the study intervention (acute or chronic), type of SCFA (acetate, butyrate or propionate, mixed or vinegar). Study characteristics were extracted for each category including first authors, last name, publication year, study design, length of intervention (acute (<24h) or chronic (>24h)), sample size, participants' demographic characteristics (gender, age, BMI and existing health conditions), amount of SCFAs given, route of administration (oral, intravenous, gastrointestinal), time-course of measurements, energy and macronutrient matching and outcomes analysed. Some records identified contained multiple studies, which were extracted individually. Whilst all comparisons within the same record were captured in the summary of studies tables, not all comparisons were meta-analysed. Selection of the comparisons against the control were based on the highest dose or on the format used in real life (e.g. liquid vinegar over pill).

Data Synthesis and Statistical analysis

Descriptive data were reported as mean ± standard deviation (SD). Glycaemic control was defined as blood glucose and insulin (raw or change from baseline) area under the curve (AUC) or incremental AUC, fasting blood glucose or insulin, glycated haemoglobin A1c (Hb1Ac) (%), or insulin sensitivity indexes (e.g. homeostatic model assessment-insulin resistance). For subject categories that included <3 studies, a narrative review was conducted in accordance with the Cochrane handbook for systematic review of interventions (2008) (Higgins et al., 2020). For categories with ≥3 studies, a meta-analysis was performed. For this meta-analysis, raw data or changes from baseline iAUC were extracted and variance was transformed to SD. When data were available as individual time points, means and variance were extracted using the online WebPlotDigitizer 4.4 tool available in https://apps.automeris.io/. The iAUC of the mean and variance was then calculated using the trapezoidal rule (Mundt, 2007) .

Meta-analysis

A meta-analysis to estimate the pooled effect of the SCFAs on the different glycaemic outcomes was performed for each subject category that included ≥ 3 studies that reported the same glycaemic outcome. These were acute acetate, acute vinegar and acute and chronic propionate administration. For acute studies, meta-analyses of postprandial glucose and insulin iAUC were also performed. For chronic studies, meta-analyses of postprandial glucose and insulin iAUC, fasting glucose and insulin and HbA1c were performed. For these outcomes, the weighted effect for each subject category – estimated as standardised mean difference (SMD) and corresponding 95% CIs – were calculated using a Sidik-Jonkman random effects model to allow a wide 95%CI in order to reflect uncertainty in the estimation of heterogeneity between studies. For cross over studies, the SMD was calculated by assuming a parallel design for a more conservative analysis.

Heterogeneity was assessed using the I^2 statistic and visual inspection of the Gallbraith plot. I^2 values ranging from 0% to 100% with values of 25% to 49%, 50% to 74% and ≥75% were classified as low, moderate and high respectively (Higgins et al., 2003). Confidence intervals were determined using the *heterogi* command in STATA. Publication bias was assessed via funnel plots for each meta-analysis. To assess influential studies, a sensitivity (leave-one-out) analysis was performed for all categories (Supplemental

Online Material). Statistical significance was determined at a p-value ≤0.05. All reported P values are 2-sided. All statistical analyses were performed using STATA 17.0.

Risk of Bias Assessment

Studies were assessed for risk of bias by three independent reviewers using the revised Cochrane risk-of-bias tool for randomised trials (Higgins et al., 2011). The 6 methodological features assessed were randomisation, assignment of intervention, adherence to intervention, missing outcome data, measuring the outcome and selection of the reported result. Studies were classified as “high risk” if they contained methodological flaws that may have influenced the results, “low risk” if the flaw was not deemed to have affected the results and “some concerns” if insufficient information was provided to pass a judgement. Disagreements in classification were resolved by consulting a fourth researcher.

Certainty of evidence-Grading of Recommendations, Assessment, Development and Evaluation (GRADE).

Certainty of evidence was assessed using GRADE recommendations. Certainty of evidence was graded as high, moderate, low and very low (Atkins et al., 2004). A table of the findings was created using GRADEpro software (https://gradepro.org), accessed on December 10th 2021.

Assessment of Confounders of Glycaemic Control

We believe that studies that controlled for factors influencing glycaemic response may produce more accurate results. There are many confounders that might affect glycaemic control either acutely or chronically, such as physical activity, length of fasting period and fibre or alcohol intake. Others include changes in body weight and body fat percentage over the course of the study. Included studies were assessed for how they controlled for such factors, and the results are summarized in the Appendix (Chapter#2).

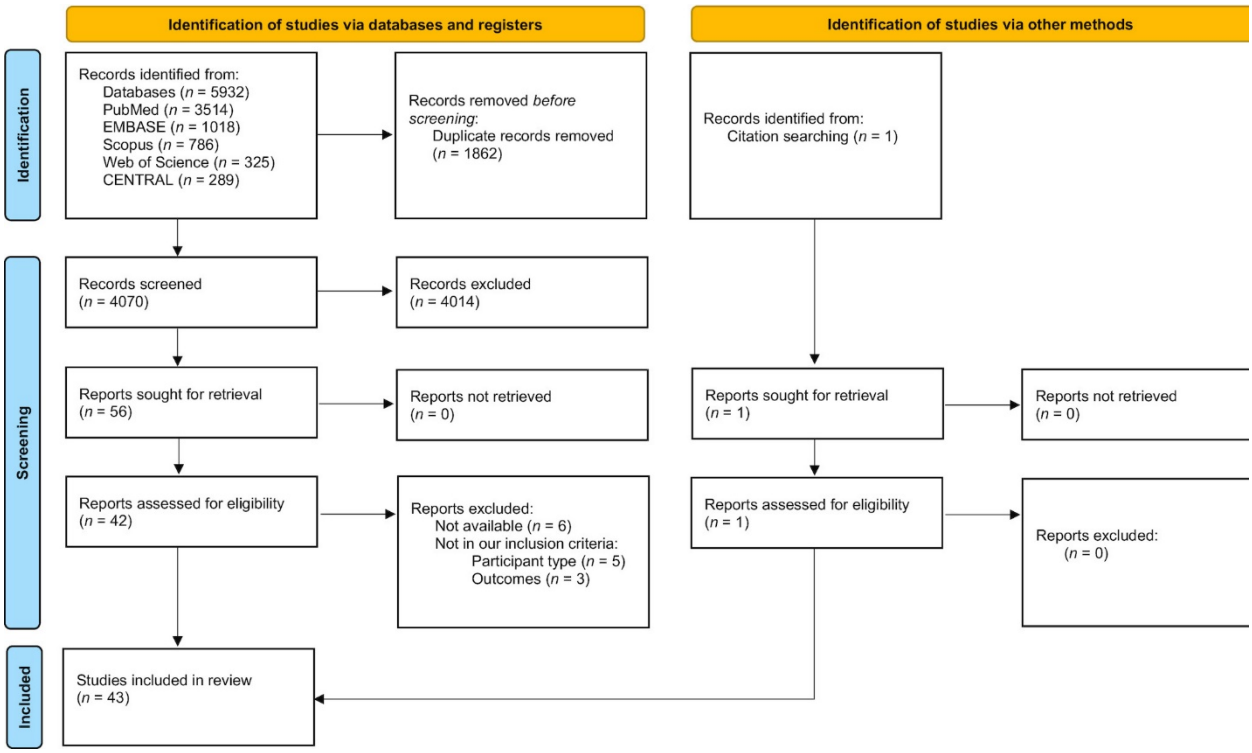
RESULTS

Study selection

The literature search identified a total of 5932 references from the database searches, specifically 3514 publications from PubMed, 1018 from EMBASE, 786 from Scopus, 325 from Web of science, 289 from Cochrane CENTRAL, and 1 from the manual search. After removal of 1862 duplicates, the remaining 4070 records were then abstract screened. A total of 56 full-text records were assessed for eligibility against the PICOS criteria (Figure 2.1). Of these, 14 records were excluded on the grounds that the studies did not match our inclusion criteria, or information related to participant, outcomes or studies were not found.

This left 43 records to be included in this review. Some records had more than 2 intervention arms/test interventions or more than 2 studies within the same record. This was the case for acetate studies such as Scheppach et al. (1988), 2 studies within the same record and vinegar studies (Brighenti et al., 1995), 2 interventions vs. the same control (Johnston et al., 2005), 2 arms with different food matrices (Johnston et al., 2010), 3 studies within the same record (Liatis et al., 2010), 2 arms with different glycaemic indexes (Darzi et al., 2014), 2 studies within the same record (Feise et al., 2020), or 3 interventions vs. the same control. Therefore, the 43 identified records included 52 actual studies, 44 of which were included in the meta-analysis. Five investigated acetate (all acute interventions), two investigated butyrate (both reporting the same chronic study), 14 investigated propionate (8 acute and 6 chronic interventions), 31 investigated vinegar (25 acute and 6 chronic interventions), and five investigated mixed SCFAs.

Figure 2.1: PRISMA flow diagram of references identified and evaluated.



PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analysis.

Study and Participants Characteristics

All the included records were parallel RCTs, published between 1988 and 2021. Overall, 90% of the studies were randomised controlled trials, cross over (RCT, XO). Most of the participants were healthy volunteers (HV), some had T2DM, and few suffered from impaired glucose tolerance (IGT). Intervention duration ranged from 60 minutes to 24 weeks, with different SCFA supplementations and different routes of administration (oral, gastric, rectal and intravenous).

Risk of Bias in Included Studies

All the studies included were assessed for risk of bias domains (D1 to D5). 47% were determined to have a high risk of bias, 44% a moderate risk of bias and 9% a low risk of bias. The domains of greatest concern arose from deviations from intended interventions (D2.2) and risk of bias in measurement of the outcome (D4) (Figure 2.2).

Study name	D1	D2.1	D2.2	D3	D4	D5	Overall
ACETATE							
Freeland and Wolever, 2010 (37)	-	+	x	+	+	+	x
Johnstona et al., 2010 (31)	+	+	+	+	-	+	-
Laurent et al., 1995 (36)	-	+	+	+	+	-	-
Scheppach et al., 1988 (28)	-	-	x	x	-	-	x
Van der Beek et al., 2016 (35)	+	+	+	+	+	+	+
BUTYRATE							
Roshanravan et al., 2017 (52)	+	-	+	+	-	-	x
Roshanravan et al., 2018 (53)	+	-	+	+	-	-	x
PROPIONATE							
Adler et al., 2021 (58)	+	+	+	-	+	-	-
Byrne et al., 2016 (57)	+	+	+	+	+	-	-
Byrne et al., 2019 (61)	+	+	+	+	+	+	+
Chambers et al., 2015 (63)	+	+	-	-	+	+	-
Chambers et al., 2018 (59)	-	+	+	+	+	+	-
Chambers et al., 2019 (62)	+	-	x	+	+	+	x
Darwiche et al., 2001 (56)	+	+	+	+	x	-	x
Darzi et al., 2012 (54)	+	-	+	+	-	+	-
Laurent et al., 1995 (36)	-	+	+	+	+	-	-
Pingitore et al., 2017 (69)	+	+	-	-	+	+	-
Tirosh et al., 2019 (60)	-	+	+	+	+	-	-
Todesco et al., 1991 (55)	-	+	+	+	x	-	x
Venter et al., 1990 (64)	+	+	-	+	+	-	-
MIXED							
Alamowitch et al., 1996 (67)	+	+	+	+	+	+	+
Canfora et al., 2017 (68)	+	+	+	+	+	+	+
Laurent et al., 1995 (36)	-	+	+	+	+	-	-
Wolever et al., 1988 (65)	-	-	+	+	+	+	-
Wolever et al., 1991 (66)	-	+	-	+	-	+	x
VINEGAR							
Ali et al., 2019 (50)	+	-	+	+	-	+	-
Brighenti et al., 1995 (29)	-	-	-	+	-	+	x
Darzi et al., 2014 (33)	+	+	+	+	x	+	x
Derakhshandeh-Rishehri et al., 2014 (48)	-	x	x	-	+	+	x
Feise and Johnston, 2020 (34)	-	-	+	+	-	+	x
Gheflati et al., 2019 (46)	-	+	+	+	-	+	-
Hlebowicz et al., 2008 (39)	-	+	+	+	x	+	x
Hosseini et al., 2011 (49)	-	-	x	+	x	+	x
Jasbi et al., 2019 (47)	-	+	-	+	-	+	x
Johnston et al., 2010 (31)	+	+	+	+	-	+	-
Johnston et al., 2004 (43)	-	-	-	+	-	-	x
Johnston and Buller, 2005 (30)	-	-	-	+	-	+	x
Leeman et al., 2005 (42)	-	+	-	+	+	+	-
Liatis et al., 2010 (32)	-	+	-	-	+	+	x
Mettler et al., 2009 (38)	+	-	+	+	-	-	x
Mitrou, 2015 (45)	-	+	+	+	+	+	-
Mitrou et al., 2015 (44)	-	+	-	+	+	+	-
Östman et al., 2005 (40)	-	+	+	+	+	+	-
White and Johnston, 2007 (51)	-	+	-	+	x	+	x
Zhao et al., 2020 (41)	+	+	+	+	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

Figure 2.2: Summary table of risk of bias of all studies by length intervention (acute or chronic) and by intervention categories (acetate, butyrate, propionate, mixed & vinegar).

Effects Of Acetate

Acute Acetate Intervention

Forest plots of the pooled effect of acute acetate intervention on postprandial blood glucose and insulin are shown in Figures 2.3 and 2.4.

Six studies were meta-analysed for blood glucose (Scheppach et al., 1988; van der Beek et al., 2014), proximal and distal (Laurent et al., 1995; Freeland et al., 2009) intravenous and rectal, (Johnston et al., 2010). The same 6 studies were also analysed for insulin.

A random effects model was used to calculate standard mean difference (SMD), and this showed that acute acetate interventions had a mean effect of 0.09 (SMD=0.09; 95% CI:-0.26-0.44; p=0.60) on postprandial blood glucose iAUC (n=44) and showed non-significant interstudy heterogeneity (I^2= 23.1%, p=0.59) (Figure2.3). For insulin, acute acetate interventions had a mean effect of 0.35 (SMD = 0.35; 95% CI: -0.07- 0.77) on postprandial blood insulin iAUC (n=35) and showed moderate interstudy heterogeneity (I^2= 30.7%, p=0.53 95% CI: 0.75) (Figure 2.4).

Homogeneity, using the Galbraith plot, and publication bias, using the funnel plot, were assessed and reported in the Appendix (Chapter#2).

Figure 2.3: Forest plots of the pooled effect of acute acetate intervention on postprandial blood glucose.

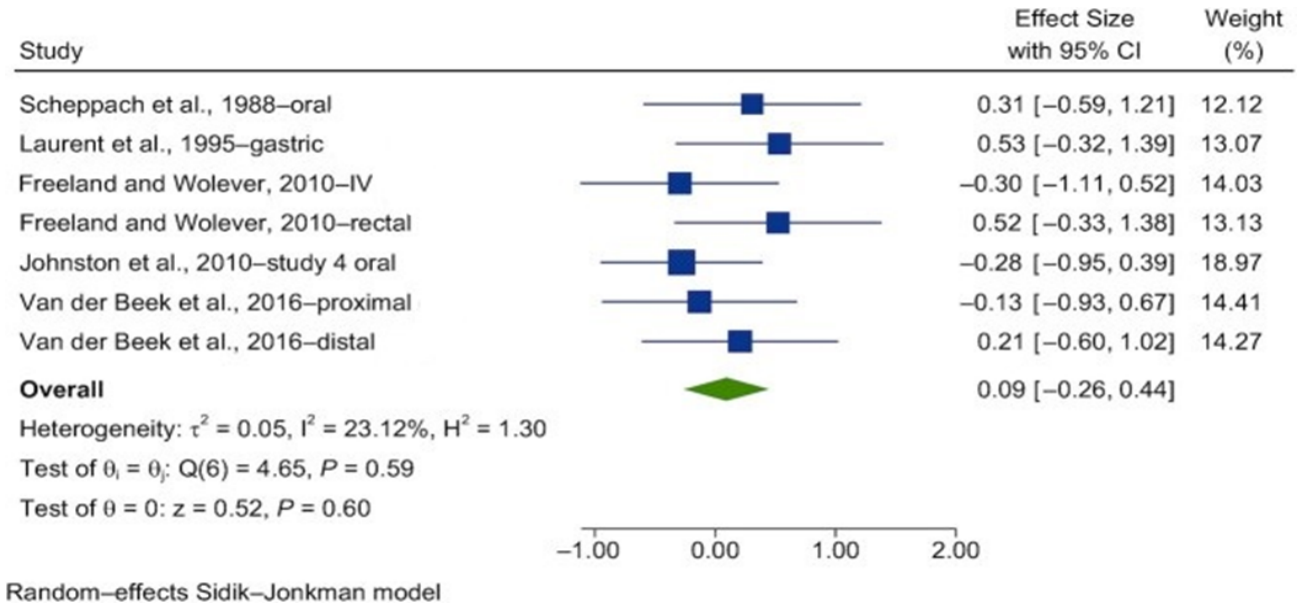


Figure 2.3: Forest plots of the pooled effect of acute acetate intervention on postprandial blood glucose. A random-effects model was used to calculate SMDs (squares), 95% CIs (horizontal lines) and summary effects (SMD; diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value ≤ 0.05 was considered statistically significant. When interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviations: iAUC, incremental area under curve; IV, intravenous; SMD, standard mean difference; T2D, type 2 diabetes.

Figure 2.4: Forest plot of the pooled effect of acute acetate intervention on postprandial insulin.

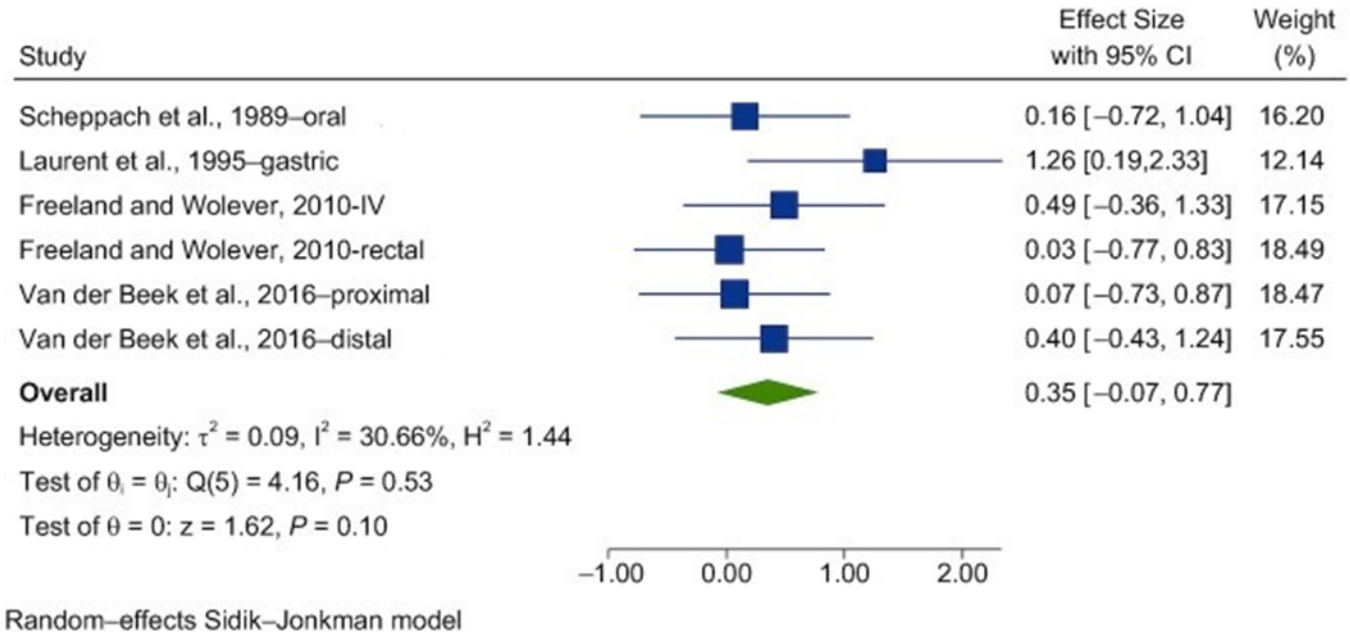


Figure 2.4: Forest plots of the pooled effect of acute acetate intervention on postprandial insulin. A random-effects model was used to calculate SMDs (squares), 95% CIs (horizontal lines), and summary effects (SMD; diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value ≤ 0.05 was considered statistically significant. When interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviations: iAUC, incremental area under curve; IV, intravenous; SMD, standard mean difference; T2D, type 2 diabetes

Chronic Acetate Intervention

No studies were found for chronic acetate administration.

		Participant characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (M/F)**	**Age, y**	**BMI, kg/m^2**	**Amount given (rate)**	**Route of administration**	**Duration**	**Control**	**Glycaemic outcome analysed**
Acute										
Scheppach et al., 1988 (study 1)	RCT, XO	HV	5	33	22.4	195 mmol (15 mmol/15 min) +50 g CHO	Oral (drink)	360 minutes	Chloride	PBG PI
Laurent et al., 1995	RCT, XO	HV	6 (3/3)	22	21.2	12 mmol/h	Intragastric (infusion)	300 minutes	Saline	PBG PI
Freeland and Wolever, 2010	RCT, XO	Hyperinsulinemic	6 (0/6)	44	31	20 mmol/L (12.5 ml/min)	Intravenous (infusion)	60 minutes	Saline IV	PBG PI
						60 mmol/L (37.5 ml/min)	Rectal (infusion)	60 minutes	Saline R	PBG PI
Van der Beek et al., 2016	RCT, XO	HV	6 (6/0)	35	31	100–180 mmol/L	Colonic (infusion)	300 min/d x 3 days	Saline	PBG PI

Table 2.2: Characteristics of acetate studies included in the meta-analysis.
Abbreviations: CHO, carbohydrate; F, female; HV, healthy volunteers; IV, intravenous; M, male; PBG, postprandial blood glucose; PI, postprandial insulin; R, rectal; RCT, randomised controlled trial; XO, crossover.

Effects Of Vinegar

The characteristics of the vinegar studies included are summarised in Table 2.3.

Acute Vinegar Intervention and post prandial glucose.

There were 15 studies with the 11 references identified that investigated the effects of acute vinegar on postprandial glucose. 15 studies in healthy individuals were meta-analysed for postprandial blood glucose and seven in metabolically compromised individuals. Five of the healthy volunteer studies and six of the non-healthy volunteer studies were also meta-analysed for post-prandial insulin levels.

Forest plots of the pooled effect of vinegar interventions on postprandial blood glucose in healthy and non-healthy are shown in Figures 2.5 and 2.6. In healthy subjects, a random effects model showed that acute vinegar administration had a significant effect on postprandial blood glucose (SMD= -0.27; 95%CI: -0.54-0.00, n=186). Inter-study heterogeneity was significant (I^2=66.2%, p=0.001; 95% CI: 48- 82). Meanwhile, acute administration of vinegar in subjects with glucose impairment (non-healthy) subjects also had a significant effect on postprandial glucose (SMD=-.053; 95% CI:-0.92 to -0.14, n= 67). Inter-study heterogeneity was not significant (I^2 = 53%, P=0.11; 95%CI: 0- 75).

Acute Vinegar intervention and post prandial insulin.

In healthy individuals, the acute administration of vinegar on postprandial insulin (PPI) showed no significant effect (SMD = -.0.29; 95% CI: -0.66- 0.08; n= 55) (Figure 2.7). The studies had non-significant heterogeneity (I^2 = 44.6%, P =0.21; 95% CI: 0- 74). In glucose impaired individuals, the acute administration of vinegar (IGT or T2DM) also had no significant effect on PPI (SMD = 0.0.16; 95%CI: -.075- 0.44, n= 58) (Figure 2.8). A high degree of heterogeneity was seen between the studies included in the meta-analysis (I^2= 77.0%, p=0.001; 95% CI: 39,88).

Homogeneity using the Galbraith plot, and publication bias using funnel plot were assessed and reported in in the Appendix (Chapter#2).

Table 2.3: Summary of the vinegar studies characteristics included in the meta-analysis.

Reference	Study design	Health status	Sample size (Male/Female)	Age, years	BMI, kg/m^2	Amount	Type of vinegar	Acetic acid, %	Duration	Co-ingested with CHO, g	Control	Outcomes analysed
Acute												
Brighenti et al., 1995	RCT, XO	HV	5 (4/1)	37	98	20 ml	White (acetic acid from vinegar)	5	95 min	50	White Bread	PBG
Johnston et al., 2004	RCT, XO	HV	8	NI	NI	20 g	Apple cider	5	60 minutes	87	Placebo drink (not specified)	PBG PI
Johnston et al., 2004-study a	RCT, XO	T2D	11	NI	NI	20 g	Apple cider	5	60 minutes	87	Placebo drink (not specified)	PBG PI
Johnston et al., 2004-study b	RCT, XO	IR	10	NI	NI	20 g	Apple cider	5	60 minutes	87	Placebo drink (not specified)	FBG PI

Johnston and Buller, 2005-study a	RCT, XO	HV	11 (1/10)	27.9	22.7	20 g	Apple cider	5	60 minutes	87	Sweetened water	PBG PI
Johnston and Buller, 2005-study b	RCT, XO	HV	11 (1/10)	27.9	22.7	20 g	Apple cider	5	60 minutes	52	Sweetened water	PBG PI
Leeman et al., 2005	RCT, XO	HV	13 (3/10)	19–32	22.5	28 g	White	6	120 minutes	50	Boiled potatoes	PBG PI
Östman et al., 2005	RCT, XO	HV	12 (10/2)	22.9	21.5	28 g	White	6	120 minutes	50	White bread	PBG PI
Hlebowicz et al., 2008	RCT, XO	HV	13 (6/7)	25	22.8	28 g	White wine	5	120 minutes	50	White bread	PBG
Mettler et al., 2009	RCT, XO	HV	27 (9/18)	26	22.5	28 g	NI	NI	120 minutes	75	Vanilla milk rice + cinnamon	PBG
Johnston et al., 2010-study 1	RCT, XO	HV	10 (4/6)	35	27.5	2–20 g	Apple cider	5	120 minutes	0	0 g acetic acid drink	PBG
Johnston et al., 2010-study 2a	RCT, XO	HV	9 (2/7)	50	33.7	20 g	Raspberry	5	120 minutes	75	Placebo (not specified)	PBG

Johnston et al., 2010-study 3	RCT, XO	HV	10 (2/8)	38	26.3	20 g	Apple cider	5	120 minutes	75	Placebo (not specified)	PBG
Johnston et al., 2010-study 4	RCT, XO	T2D	9 (4/5)	69	31.4	20 g	Apple cider	5	120 minutes	75	Placebo (not specified)	PBG
Liatis et al., 2010-study a	RCT, XO	T2D	8 (3/5)	57.4	29.8	20 g	Wine (high glycaemic index 86/100)	6	120 minutes	51	High glycaemic meal	PBG PI
Liatis et al., 2010-studyb	RCT, XO	T2D	8 (4/4)	61.4	30.1	20 g	Wine (low glycaemic index 38/100)	6	120 minutes	52	Low glycaemic meal	PBG PI
Darzi et al., 2014-study 1	RCT, XO	HV	16 (3/13)	22.2	22.1	30 g	White wine (palatable)	6	180 minutes	94.5	75 g of sugar-free squash	PBG
Darzi et al., 2014-study 2	RCT, XO	HV	14 (6/8)	27.5	22.7	30 g	White wine	6	180 minutes	60	Water	PBG
Mitrou et al., 2015-study a	RCT, XO	T2D	11 (4/7)	53.0	25.0	30 ml	NI	6	300 minutes	75	Water	PBG PI muscle glucose uptake

Mitrou et al., 2015-study b	RCT, XO	IGT	8 (4/4)	46.0	30.0	30 ml	Wine	6	300 minutes	75	Water	PBG PI Muscle glucose uptake
Feise and Johnston, 2020-study a	XO	HV	12 (5/7)	22.6	21.2	25 g	Liquid vinegar	5	60 minutes	64	Water	PBG
Feise and Johnston, 2020-study b	XO	HV	12 (5/7)	22.6	21.2	25 g	Vinegar pills	5	60 minutes	64	Water	PBG
Feise and Johnston, 2020-study c	XO	HV	12 (5/7)	22.6	21.2	25 g	Crushed vinegar pills	5	60 minutes	64	Water	PBG
Zhao et al., 2020	RCT, XO	HV	15 (0/15)	23.6	20.3	30 g	Black rice	5	200 minutes	35	White rice, dried apple	PBG
Chronic												
White and Johnston, 2007	XO	T2D	11 (4/7)	40–72	29.1	30 ml	Apple cider	0.002	2 days	Usual diet	Water	FBG

Hosseini et al., 2011	Parallel	T2D	30 (15/15)	30–60	NI	15 ml	Vinegar	NI	4 weeks	Usual diet	Water	FBG HbA1c
Derakhshandeh-Rishehri et al.,2014	RCT, parallel	HV	72 (32/40)	31.6	25.3; 22.8	21.66 g	Honey	NI	4 weeks	Usual diet	Normal diet	FBG FI HOMA-IR
Ali et al., 2019	RCT, parallel	T2D	55 (26/29)	30–60	NI	20 ml	Dates	NI	10 weeks	Usual diet	Honey in water	FBG HbA1c
Gheflati et al., 2019	RCT, parallel	T2D	62 (20/42)	49.5; 52.1	29.0; 28.9	20 ml	Apple	5	8 weeks	Usual diet	Normal diet	FBG FI HOMA-IR QUICKI
Jasbi et al., 2019	RCT, parallel	HV	45 (41/4)	29.6; 30.1	27.8; 28.5	60 ml	Red wine	6	8 weeks	Usual diet	Apple cider vinegar tablet	FBG FI HOMA-IR

Table 2.3: Abbreviations: CHO, Carbohydrate; FBG, Fasting blood glucose; FI, Fasting insulin; HbA1c, glycated haemoglobin; HV, healthy volunteer; IGT, Impaired glucose tolerance; IR, insulin resistance; NI, no information; PBG, post prandial glucose; PI, postprandial insulin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin Sensitivity Check Index; RCT, randomised controlled trial; T2DM, type 2 diabetes; XO, crossover.

Figure 2.5: Forest Plot for randomised controlled trials of acute vinegar effect on postprandial glucose in healthy subjects.

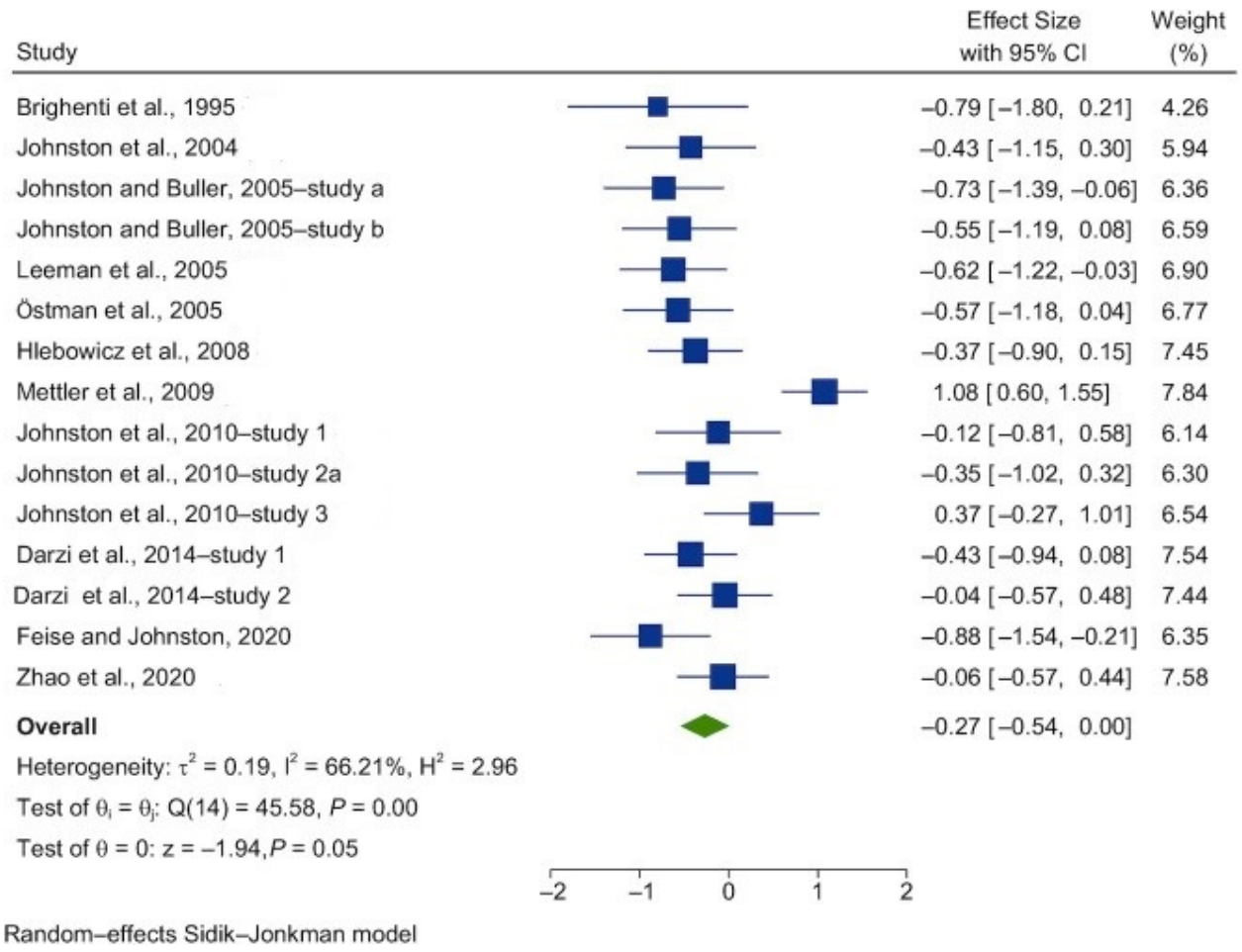


Figure 2.5. A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (SMD; diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value ≤ 0.05 was considered statistically significant. When

interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Brighenti et al. used acetic acid within vinegar (co-ingested with 50 g of CHO). Johnston and Buller used (study a) a bagel and juice meal and (study b) chicken teriyaki. Johnston et al. used (study 1) 1 g of acetic acid as vinegar consumed prior to the test meal (bagel + juice); (study 2a) 1 g of acetic acid as vinegar consumed with the test meal; and (study 3) 1 g of acetic acid as vinegar ingested immediately prior to a 75-gram dextrose load in 10 healthy adults. Darzi et al. (study 1) used both a palatable drink with 25 g vinegar vs. control, alongside a mixed breakfast. Darzi et al. (study 2) used a milkshake preload before intake of 30 g of vinegar (containing 6% acetic acid) + 150 g water or the control. Feise and Johnston used 25 g of liquid vinegar (1.25 g acetic acid). Abbreviations: CHO, carbohydrate; iAUC, incremental AUC; SMD, standard mean difference.

Figure 2.6: Forest Plot for randomised controlled studies of acute vinegar effect on postprandial glucose in non-healthy subjects.

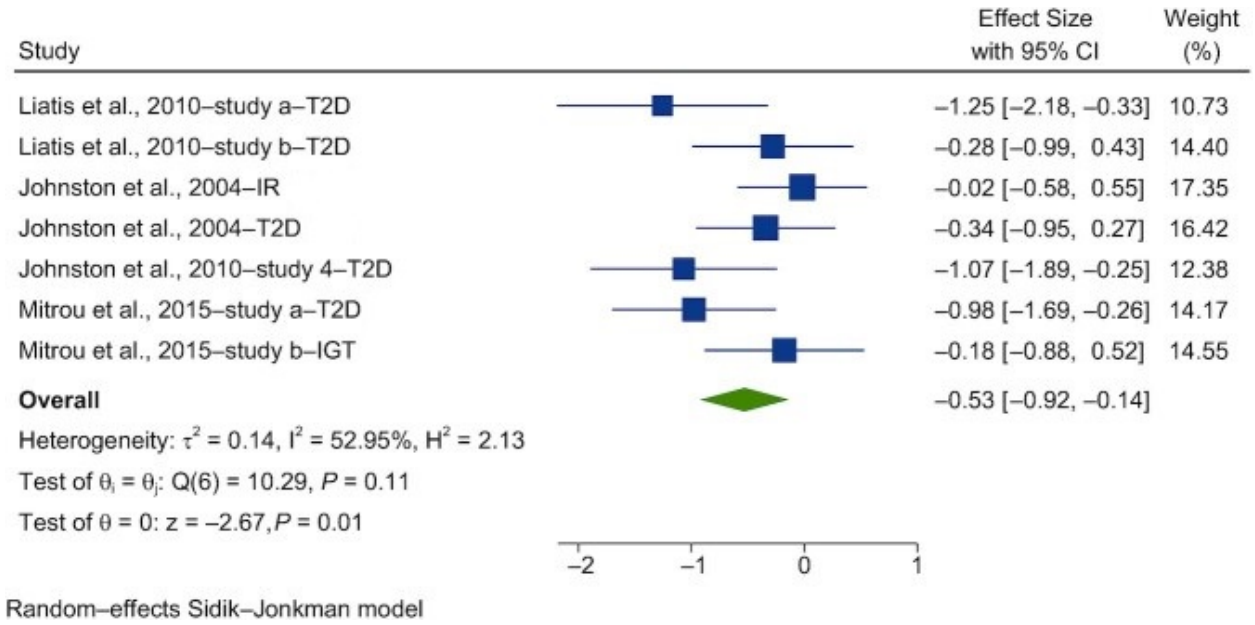


Figure 2.6. Forest plots for randomised controlled trials of acute vinegar intake on postprandial blood glucose in nonhealthy adults. Acute interventions with vinegar had a main effect of −0.53 (95% CI, −0.92 to −0.14; P = 0.01) on the postintervention postprandial blood glucose iAUC (n = 67) in nonhealthy subjects. A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines),

and summary effects (SMD; diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value ≤ 0.05 was considered statistically significant. When interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Liatis et al. used (study a) a high-GI meal and (study b) a low-GI meal. Mitrou et al. published results in the (study a) Journal of Diabetes Research (Mitrou et al.) and (study b) European Journal of Clinical Nutrition (Mitrou et al.). Abbreviations: GI, glycaemic index; iAUC, incremental AUC; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standard mean difference; T2D, type 2 diabetes.

Figure 2.7: Forest plot for randomised controlled studies of acute effect of vinegar on postprandial insulin in healthy subjects.

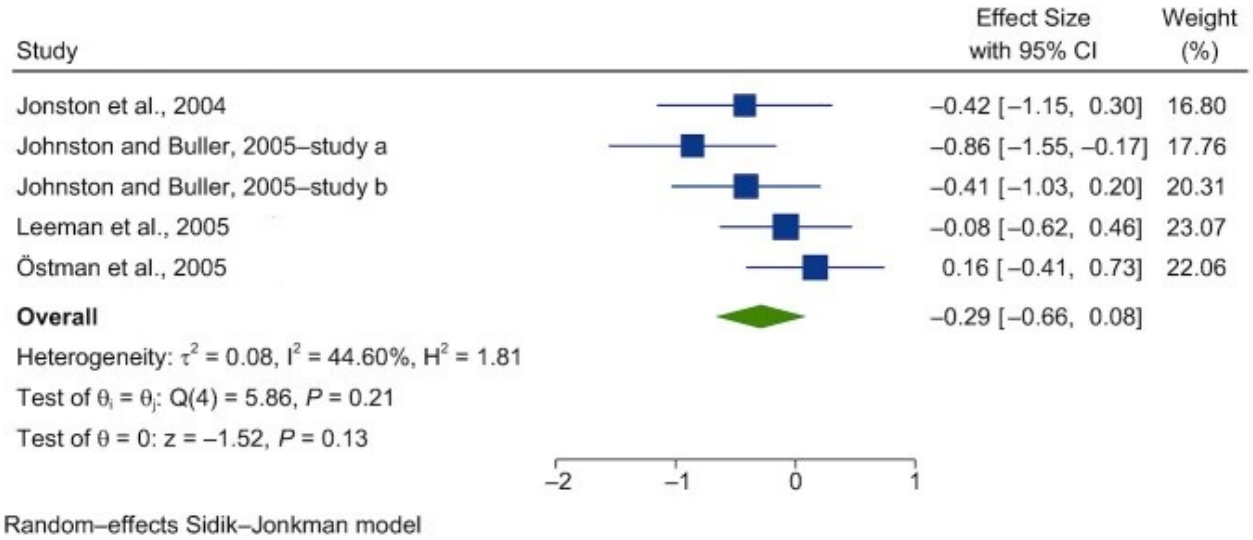


Figure 2.7. Forest plots for randomised controlled trials of acute vinegar intake on postprandial blood insulin in healthy volunteers. Acute interventions with vinegar had a main effect of −0.29 (95% CI, −0.66 to 0.08; P = 0.13) on the postintervention postprandial insulin iAUC (n = 55) in healthy subjects. A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (SMD) (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value ≤ 0.05 was considered statistically significant. When interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect

size, and values >0.70 as having a large effect size. Johnston and Buller used (study a) a bagel and juice meal and (study b) chicken teriyaki. Abbreviations: iAUC, incremental AUC; SMD, standard mean difference.

Figure 2.8: Forest Plot for randomised controlled trials of acute vinegar intake on postprandial insulin in non-healthy.

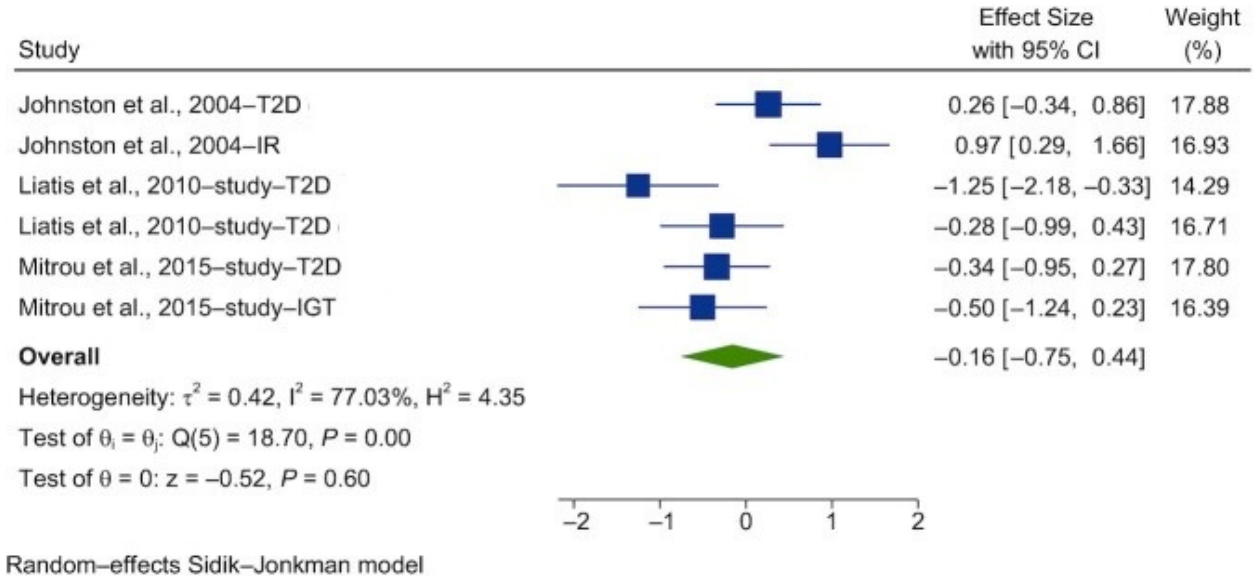


Figure 2.8. Forest plots for randomised controlled trials of acute vinegar intake on postprandial blood insulin in nonhealthy adults. Acute interventions with vinegar had a main effect of −0.16 (95% CI, −0.75 to 0.44; P = 0.60) on the postintervention postprandial blood glucose iAUC (n = 58) in nonhealthy adults. A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (SMD; diamond). The study weight (expressed as a percentage) indicates the relative contribution of individual studies to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting SMDs (or effect sizes) values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Liatis et al. 2010 used (study a) a high-GI meal and (study b) a low-GI meal. Mitrou et al. 2015 published results in the (study a) Journal of Diabetes Research (Mitrou et al. 2015) and (study b) European Journal of Clinical Nutrition (Mitrou et al. 2015. Abbreviations: GI, glycaemic index; iAUC, incremental AUC; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standard mean difference; T2D, type 2 diabetes.

Chronic Vinegar Intervention and post prandial glucose

Seven studies using chronic administration of vinegar were identified. Of these, 6 were included in the meta-analysis (Gheflati et al., 2019; Jasbi et al., 2019; Derakhshandeh-Rishehri et al., 2014; Hosseini et al., 2011; Ali et al., 2018; White & Johnston, 2007) investigating fasting blood glucose (FBG). Chronic administration with vinegar had no significant effect on FBG (SMD = -1.60; 95% CI: -4.30 -1,09; n= 143) (Figure 2.9). Interstudy heterogeneity was significant (I^2 = 99.0%, p= 0.001; 95% CI: 92-97).

Figure 2.9: Forest Plot for randomised controlled studies of chronic vinegar on fasting blood glucose.

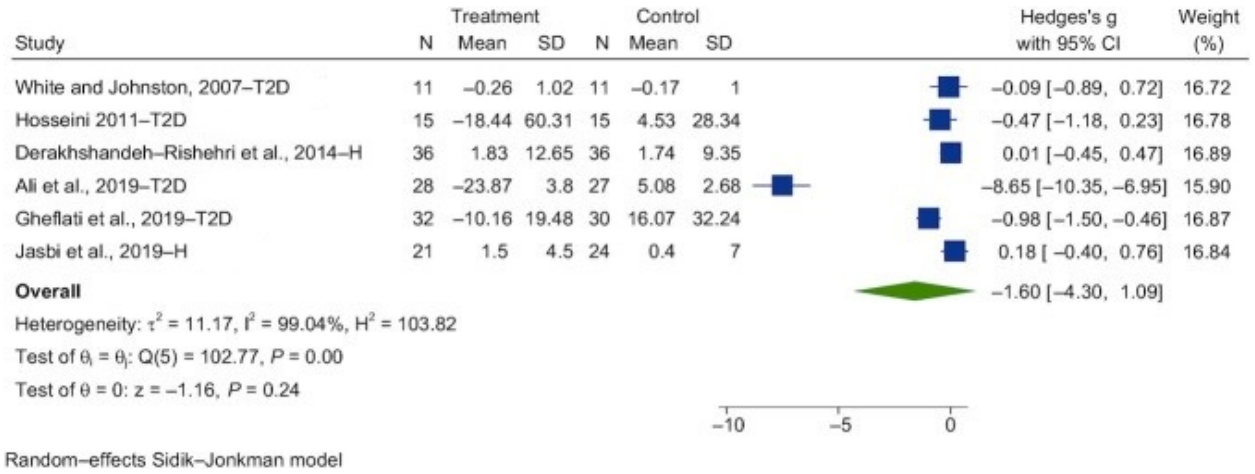


Figure 2.9. Forest plots for randomised controlled trials of chronic vinegar intake on fasting blood glucose. Chronic interventions with vinegar had a main effect of −1.60 (95% CI, −4.30 to 1.09; P = 0.24) on the postintervention fasting blood glucose iAUC (n = 143). A random-effects model was used to calculate Hedge's g (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. Abbreviations: iAUC, incremental AUC; H, healthy volunteers; T2D, type 2 diabetes.

Chronic Vinegar Intervention and post prandial insulin

Three studies were identified which investigated the effect of chronic vinegar on fasting insulin (FI) (Gheflati et al., 2019; Jasbi et al., 2019; Derakhshandeh-Rishehri et al., 2014)). Chronic administration of vinegar had a significant effect on FI (SMD =0.06; 95% CI: -0.50 - 0.62; n = 89) (Figure 2.10). Interstudy heterogeneity was highly significant (I^2= 72.2%, p= 0.03; 95% CI: 6-92).

Two further studies investigated the effect of chronic vinegar administration on HbA1C% in T2DM (Hosseini et al., 2011; Ali et al., 2018). Both studies reported a significant decrease in HbA1C of 7% and 9% with 15 ml and 20 ml of vinegar over 4 and 10 weeks respectively, compared to 1% decrease in HbA1C % and 2% increase in the placebo group.

Three more studies investigated the degree of insulin resistance using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Two of these studies investigated the chronic intake of 21g of vinegar for 4 weeks but did not show any significant effect on HOMA-IR compared to the control group (Derakhshandeh-Rishehri et al., 2014). However, a longer intake of 8 weeks led to a significant decrease of 8% in HOMA-IR compared to the control (Jasbi et al., 2019). In contrast, a study by Gheflati et al. (2019) reported no significant effect on the Quantitative Insulin-sensitivity Check Index (QUICKI), HOMA-beta, or HOMA-IR with chronic vinegar administration for 8 weeks in T2DM (Gheflati et al., 2019).

Figure 2.10: Forest Plot for randomised controlled trials of chronic vinegar intake on post prandial insulin.

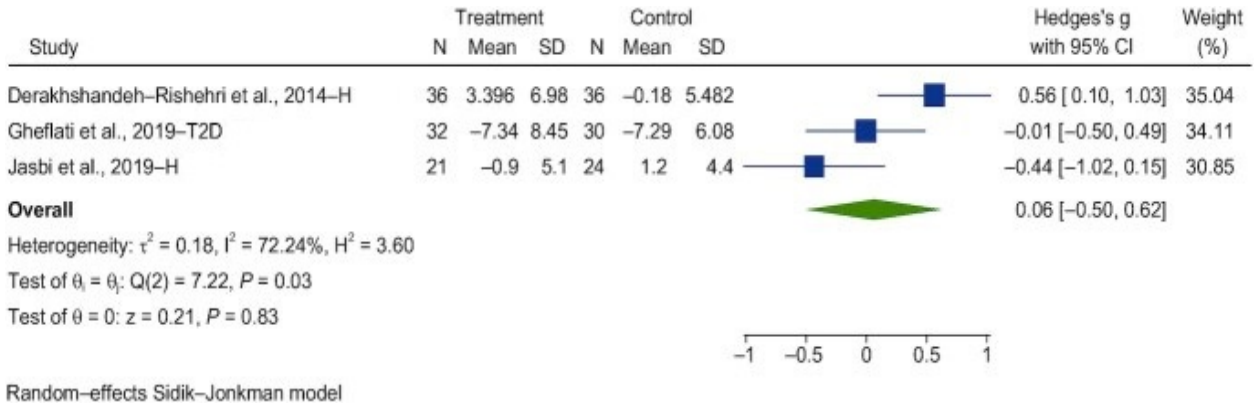


Figure 2.10: Forest plots for randomised controlled trials of chronic vinegar intake on postprandial blood insulin. Chronic interventions with vinegar had a main effect of 0.06 (95% CI, −0.50 to 0.62; P = 0.83) on the postintervention postprandial blood insulin iAUC (n = 89). A random-effects model was used to calculate Hedge's g (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviations: iAUC, incremental AUC; H, healthy volunteers; T2D, type 2 diabetes.

Effects Of Butyrate

The characteristics of the butyrate studies are summarized in Table 2.4

Acute Butyrate Intervention

No studies reported the acute effect of butyrate administration.

Chronic Butyrate Intervention

Two studies with different intervention arms reported the same effect of chronic supplementation of butyrate on glycaemic control. In both studies, the same 60 participants with T2DM were randomised in a parallel design into 4 groups (n=15 in each) in which they had to consume 6 oral capsules (100mg) and 10g of powder a day for 45 days. Two of the intervention's groups were assessed in this review. These were sodium butyrate capsules and starch powder (intervention a), and starch capsules and starch powder (control). One publication reported no significant difference in fasting blood glucose, 2h postprandial blood glucose, fasting insulin, HbA1C and HOMA-IR post-intervention compared to the control. The same study reported an increase in GLP-1 secretion by 22.57 pg/ml (p=0.008) compared to the control (Roshanravan et al., 2017). The other reported that the QUICKI was not significantly different from the control group after 45 days of intervention (p=0.137) (Roshanravan et al., 2018).

Table 2.4: Summary of the Butyrate characteristics trials included in the systematic review

		Participant characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/Female)**	**Age, years, mean**	**BMI, kg/m^2, mean**	**Amount given**	**Route of administration**	**Duration**	**Control**	**Outcome analysed**
Chronic										
Roshanravanet al., 2017	RCT, Parallel	T2D	30 (10/20)	49	30.3	600 mg Butyrate + 10 g butyrate powder	Oral (capsule)	45 days	Starch	FBG FI PBG PI HOMA-IR HbA1c
Roshanravanet al., 2018	RCT, Parallel	T2D	30 (10/20)	49	30.3	600 mg Butyrate + 10 g starch powder	Oral (capsule)	45 days	Starch	QUICKI

Table 2.4: Abbreviations: FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated haemoglobin; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, randomised controlled trial; T2D, type 2 diabetes.

Effects Of Propionate

The characteristics of the propionate studies included in the meta-analysis are summarized in Table 2.5.

Propionate Acute Intervention

Eight studies investigated the effects of acute propionate administration on glycaemic control. All were meta-analysed for glucose (Laurent et al., 1995; Darzi et al., 2012; Todesco et al., 1991; Darwiche et al., 2001; Byrne et al., 2016; Adler et al., 2021), and the same studies looked at insulin except Todesco et al. (1991).

A forest plot of the pooled effect of propionate is shown on FBG (Figure 2.11) and Insulin (Figure 2.12). A random effects model of the acute administration with propionate showed no significant effect for glucose (SMD =0.07; 95% CI:-0.32- 0.47; n= 123) or for insulin (SMD = 0.24; 95% CI: -0.30, 0.78;n=117). Interstudy heterogeneity was non-significant for both outcomes (I^2 = 75.8%, p= 0.14; CI: 0 - 72 and I^2 = 86.7%, p =0.12; 95% CI: 0 - 75) respectively.

Homogeneity using a Galbraith plot, and publication bias using a funnel plot were assessed and reported in the Appendix (Chapter# 2).

Propionate Chronic Intervention

There were five studies investigating the chronic effect of propionate administration on glycaemic control. All were analysed for PPG (Todesco et al., 1991a; Byrne et al., 2019; Chambers et al., 2019; Chambers et al., 2015; Venter et al., 1990) and the same named studies investigated PI except Todesco et al. (1991).

Forest plots are shown in Figure 2.13 for PPG and Figure 2.14 for insulin. Overall, the chronic administration of propionate had no significant effect on PPG (SMD = -0.80; 95% CI: -0.43 - 0.27; n=73) or insulin (SMD = -0.06; 95% CI: -0.39 - 0.27; n=67). Interstudy heterogeneity was non-significant for PPG and PI (I^2=15.3%, p=0.79; CI = 0-79 and (I^2 =0.3%, p=0.97; 95% CI: 0-85) respectively.

Chronic administration with propionate also had no significant effect on FBG (SMD = -0.14;95% CI: -0- 47, 0.19; n= 67) (Figure 2.15) or insulin (SMD= -0.22; 95%CI: -0.65-0.21; n=67) (Figure 2.16). Studies also had non-significant interstudy heterogeneity for glucose and insulin (I^2= 1.3%, p= 0.93; 95% CI: 0 - 85) and (I^2 = 37.4%, p=0.26; 95% CI: 0 - 88) respectively.

Homogeneity using the Galbraith plot, and publication bias using the funnel plot were assessed and reported in the Appendix (Chapter #2).

Figure 2.11: Forest plots for randomised controlled trial of acute propionate on postprandial glucose.

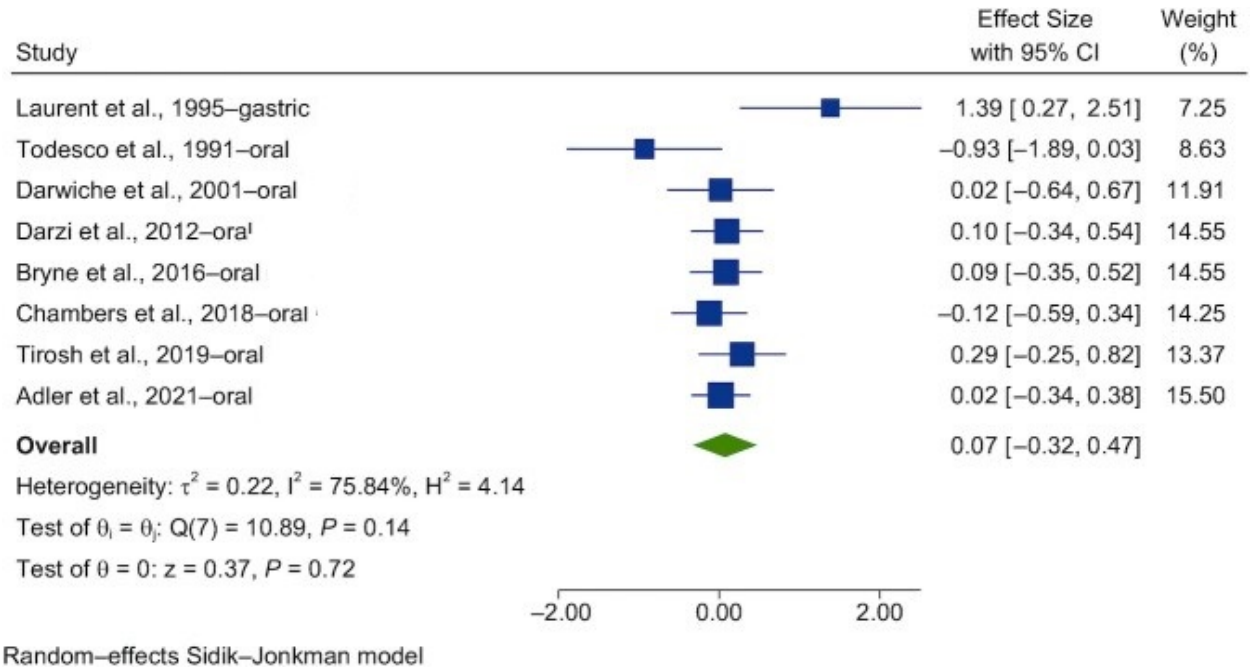


Figure 2.11. Forest plots for randomised controlled trials of acute propionate on postprandial blood glucose. Acute interventions with propionate had a main effect of 0.07 (95% CI, −0.32 to 0.47; P = 0.72) on the postintervention postprandial blood glucose iAUC (n = 123). A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviation: iAUC, incremental AUC.

Figure 2.12: Forest plots for randomised controlled trial of acute propionate on postprandial insulin.

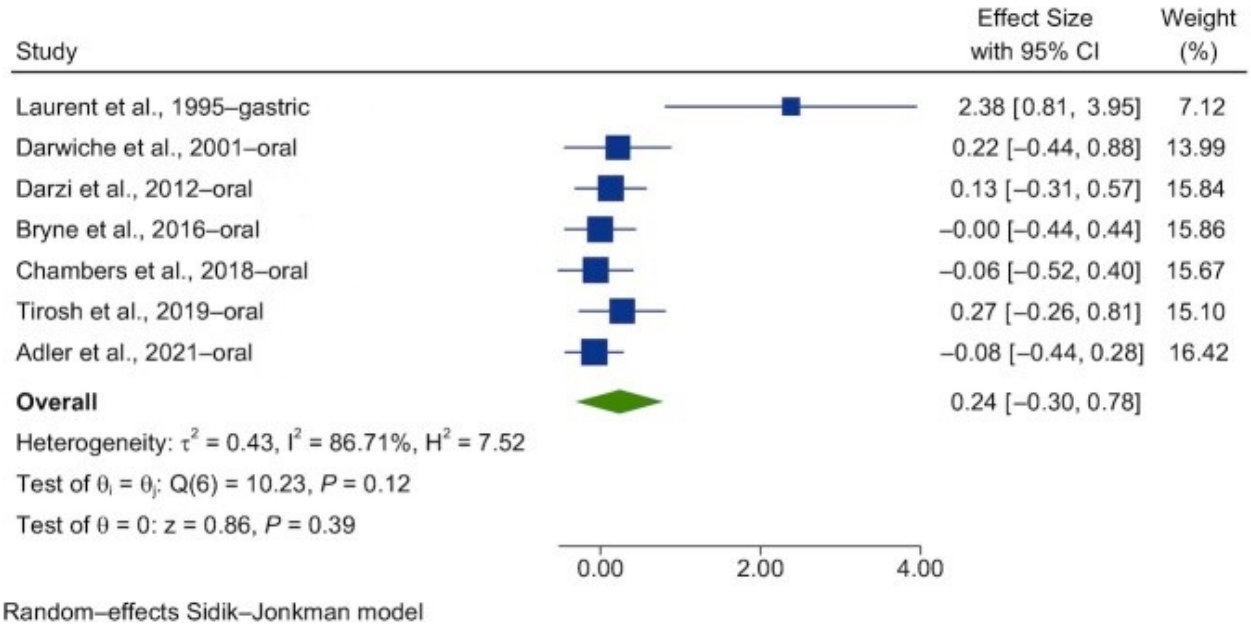


Figure 2.12. Forest plots for randomised controlled trials of acute propionate on postprandial blood insulin. Chronic interventions with propionate had a main effect of 0.24 (95% CI, −0.30 to 0.78; P = 0.39) on the postintervention intervention postprandial blood insulin iAUC. (n = 117). A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviation: iAUC, incremental AUC.

Figure 2.13: Forest plot for randomised controlled trial of chronic propionate on postprandial glucose.

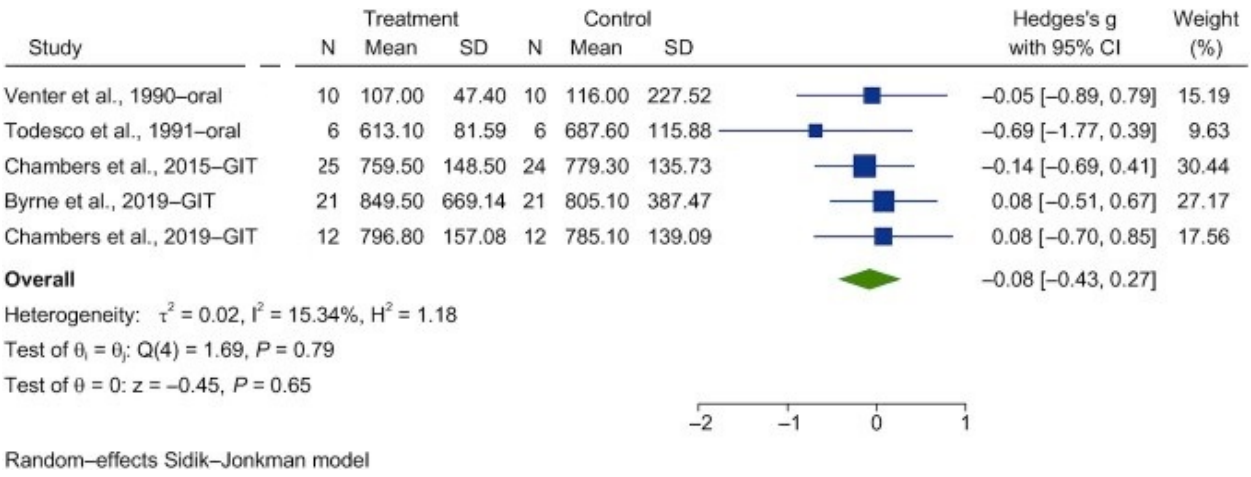


Figure 2.13. Forest plots for randomised controlled trials of chronic propionate on postprandial blood glucose. Chronic interventions with propionate had a main effect of −0.08 (95% CI, −0.43 to 0.27; P = 0.65) on the postintervention postprandial blood glucose iAUC (n = 73). Chambers et al. and Pingitore et al. reported the same study, so only Chambers et al. was reported in the meta-analysis. A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviations: GIT, gastrointestinal tract; iAUC, incremental AUC.

Figure 2.14: Forest plots for randomised controlled trial of chronic propionate on postprandial insulin.

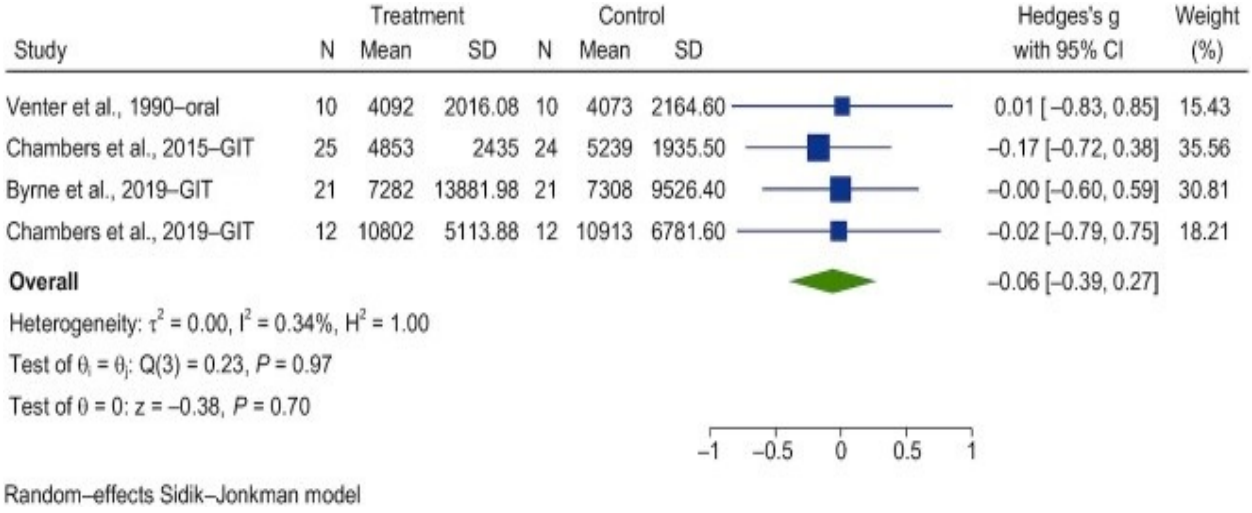


Figure 2.14. Forest plots for randomised controlled trials of chronic propionate on postprandial blood insulin. Chronic interventions with propionate had a main effect of −0.06 (95% CI, −0.39 to 0.27; P = 0.70) on the postintervention intervention postprandial blood insulin iAUC. (n = 67). A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size. Abbreviations: GIT, gastrointestinal tract; iAUC, incremental AUC.

Figure 2.15: Forest plots for randomised controlled trial of chronic propionate on fasting blood glucose.

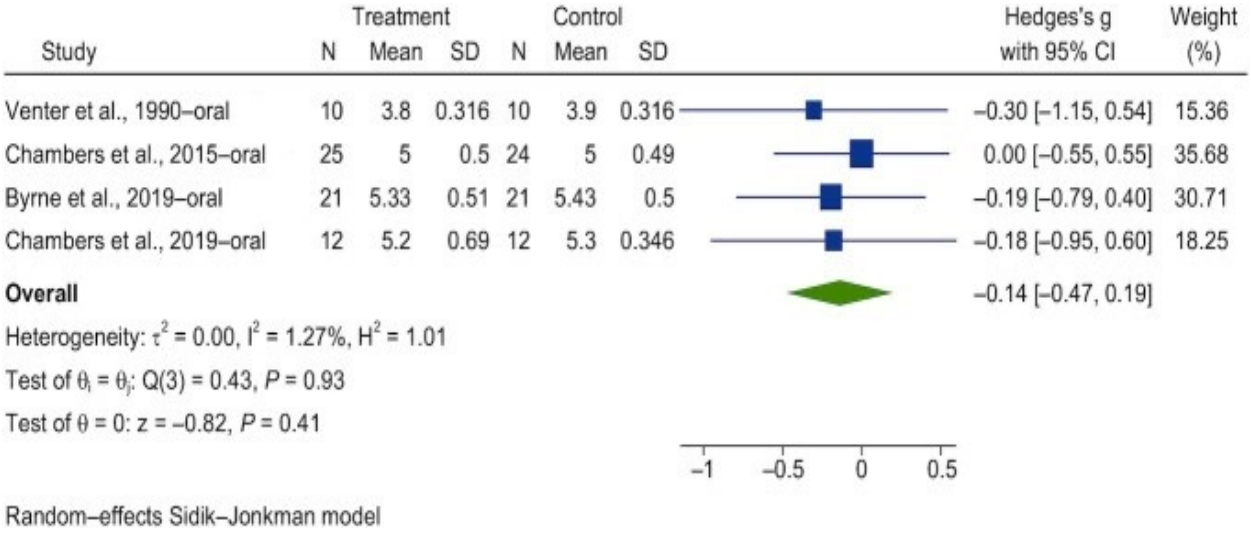


Figure 2.15. Forest plots for randomised controlled trials of chronic propionate on fasting blood glucose. Chronic interventions with propionate had a main effect of −0.14 (95% CI, −0.47 to 0.19; P = 0.41) on the postintervention fasting blood glucose (n = 67). A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size.

Figure 2.16: Forest plots for randomised controlled trial of chronic propionate on fasting insulin.

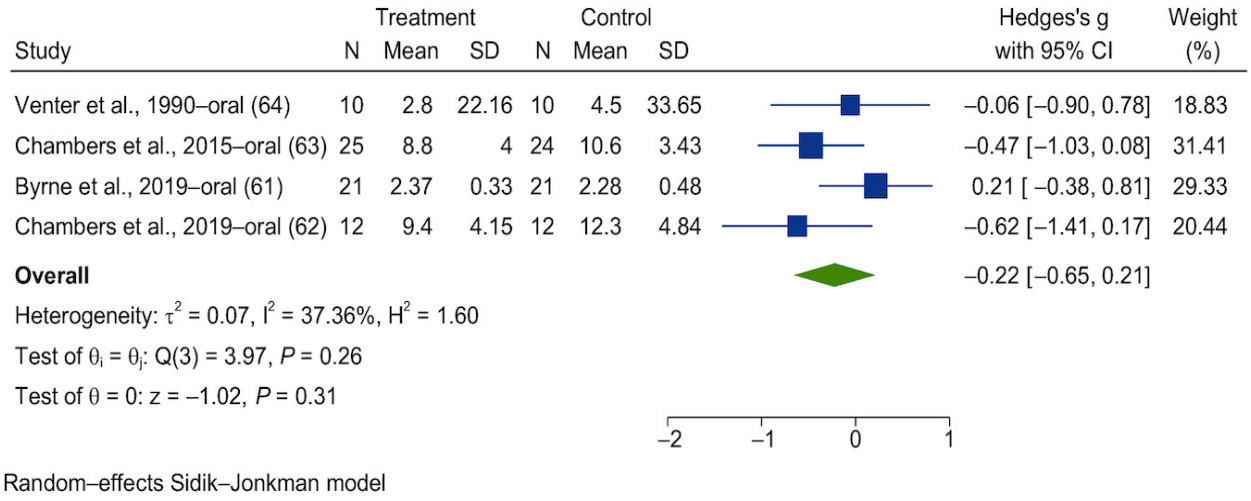


Figure 2.16. Forest plots for randomised controlled trials of chronic propionate on fasting blood insulin. Chronic interventions with propionate had a main effect of –0.22 (95% CI, –0.65 to 0.21; P = 0.31) on the postintervention intervention fasting blood insulin. (n = 67). A random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effects (diamond). The study weight (expressed as a percentage) indicates the relative contribution of an individual study to the overall pooled effect size. Between-study heterogeneity was calculated using the I^2 statistic. A P value of ≤ 0.05 was considered statistically significant. When interpreting effect sizes, values <0.40 were categorized as having a small effect size, values of 0.40 to 0.70 as having a moderate effect size, and values >0.70 as having a large effect size.

Effects of Mixed SCFAs.

The characteristics of the studies included using mixed SCFAs are summarised in Table 2.6.

Acute Mixed SCFAs Intervention

Five studies (Wolever et al., 1988; Wolever et al., 1991; Laurent et al., 1995; Alamowitch et al., 1996; Canfora et al., 2017) investigated the effects of the administration of acute mixed SCFAs on glycaemic control, all of which were given rectally or via the ileum. Wolever et al. rectally infused different ratios of SCFA mixtures: acetate 180 mmol/l, and propionate 60 mmol/l or acetate 90mmol/l and propionate 30 mmol/l, and saline solution

into 6 healthy volunteers. Neither solution induced a change in blood glucose concentration. However, the high acetate mixture led to a decrease in free fatty acids and an increase in total cholesterol and triglyceride concentrations (Wolever et al., 1988). In 1991, the same investigative team administered a combination of acetate (180 mmol/l and propionate 60 mmol/l rectally to the same population. The results showed an increase in blood glucose by +0.16 mmol/L($P \leq 0.05$) and a decrease in insulin concentration by -17 pmol/L with the mixed SCFAs compared to acetate, propionate or saline only (Wolever et al., 1991). Meanwhile, a mixture of ileal perfusion of SCFAs over 18 hours (acetate 60 mmol/L, propionate 25 mmol/L, butyrate 15 mmol/L) in healthy lean males showed no effect of on insulin sensitivity, basal hepatic glucose production, triacylglycerol concentration or total cholesterol concentration (Alamowitch et al., 1996) .

Canfora et. al. (2017) investigated a mixed rectal infusion of 200 mmol/L of high acetate, propionate, butyrate and placebo solutions over 5 hours in healthy participants, followed by a 75 g glucose lead. There were no differential effects on blood glucose or insulin levels between the three SCFA mixtures (Canfora et al., 2017) .

Chronic Mixed SCFAs Intervention.

No studies were found for chronic mixed SCFAs administration.

Table 2.6: Summary of the mixed SCFAs studies characteristics included in the meta-analysis.

		Participants characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (M/F)**	**Age, years**	**BMI, kg/m^2**	**Amount given (acetate, propionate, butyrate)**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcomes analysed**
Mixed SCFAs (acute)										
Wolever et al., 1988	RCT, XO	HV	6 (3/3)	33.0	NI	Acetate 90 mmol/L + 30 mmol/L Propionate or Acetate 180 mmol/L + 60 mmol/L Propionate	Rectal	120 minutes	Saline	PBG PI
Wolever et al., 1991	RCT, XO	HV	6	29.0	24.1	Acetate (180 mmol/L) + propionate (60 mmol/L)	Rectal	120 minutes	Saline	PBG PI
Laurent et al., 1995	RCT, XO	HV	6 (3/3)	22	21.2	Acetate 12 mmol/h Propionate 4 mmol/h	Intragastric (infusion)	300 minutes	Saline	PBG PI

	Participants characteristics					Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (M/F)**	**Age, years**	**BMI, kg/m^2**	**Amount given (acetate, propionate, butyrate)**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcomes analysed**
Alamowitch et al., 1996	RCT, XO	HV	6	26.0	20.9	90 mmol/L (Acetate: propionate: butyrate; 60,25,15 mmol/L)	Ileal	18 hours	Saline	Basal hepatic glucose production insulin sensitivity
Canfora et al., 2017	RCT, XO	HV	12 (12/0)	36.0	25–35	200 mmol/L (high acetate: 24, 8, 8 mmol/L) (high butyrate: 8, 8, 24 mmol/L) (high propionate: 8, 24, 8 mmol/L)[2]	Rectal	300 minutes	Saline	PBG PI Carbohydrate oxidation

Table 2.6. Summary of the mixed SCFs studies characteristics. Abbreviations: HV, healthy volunteer; FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated haemoglobin; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, randomised controlled trial; XO, cross over; T2D, type 2 diabetes.

Table 2.5: Summary of the propionate studies characteristics (acute & chronic) included in the meta-analysis.

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/Female)**	**Age, years**	**BMI, kg/m^2**	**Amount given**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcome analysed**
Acute										
Todesco et al., 1991	RCT, XO	HV	6 (3/3)	32	22.5	3.3. g	Oral bread	180 minutes	Bread	PBG
Laurent et al., 1995	RCT	HV	6 (3/3)	22	21.2	4 mmol/h	Intragastric (infusion)	300 minutes	Saline	PBG PI
Darwiche et al., 2001	RCT, XO	HV	9 (5/4)	32	23.6	1.85 g	Oral (bread)	125 minutes	Bread	PBG PI

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/Female)**	**Age, years**	**BMI, kg/m^2**	**Amount given**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcome analysed**
Darzi et al., 2012	RCT, XO	HV	20 (9/11)	25	23.1	43.8 g	Oral (sourdough bread)	180 minutes	Control bread	PBG PI
Byrne et al., 2016	RCT, XO	HV	20 (20/0)	52	25.2	10 g	Oral (powder)	360 minutes	Inulin	PBG PI
Chambers et al., 2018	RCT, XO	HV	18 (9/9)	50	30.5	6.8 g	Oral (tablet)	180 minutes	NaCl 4164 mg	PBG PI
Tirosh et al., 2019	RCT, XO	HV	14 (9/5)	41	23.7	1.0 g	Oral (calcium propionate)	240 minutes	Placebo (not specified)	PBG PI
Adler et al., 2021	RCT, XO	HV	27 (12/15)	30	26.7	1.5 g	Oral (calcium propionate)	240 minutes	Calcium carbonate	PBG

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/Female)**	**Age, years**	**BMI, kg/m^2**	**Amount given**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcome analysed**
Chronic										
Venter et al., 1990	Paired comparison	HV	20 (0/20)	20–21	18–22.5	7.5 g/d	Oral (capsule)	7 weeks	Calcium phosphate	PBG PI
Todesco et al., 1991	RCT, XO	HV	6 (3/3)	32	22.5	9.9 g/d	Oral (propionate bread)	1 week	Propionate free white bread	PBG
Chambers et al., 2015	RCT, Parallel	HV	49 (19/30)	54	32.5	10 g/d	GIT (powder)	24 weeks	Inulin	FI HbA1c HOMA-IR
Pingitore et a., 2017	RCT, Parallel	HV	49 (19/30)	53	32.5	10 g/d	GIT (sachet)	24 weeks	Inulin	PBG PI
Chambers et al., 2019	RCT, XO	HV	12 (3/6)	60	29.8	20 g/d	GIT (powder)	42 days	Cellulose	FBG FI HOMA-IR

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/Female)**	**Age, years**	**BMI, kg/m^2**	**Amount given**	**Route of administration**	**Time-course**	**Control**	**Glycaemic outcome analysed**
Byrne et al., 2019	RCT, XO	HV	21 (9/12)	18–65	60	10 g/d	GIT (bread roll and smoothie)	1 week	Bread	PBG PI

Table 2.5: Summary of the propionate studies characteristics (acute & chronic). Abbreviations: HV, healthy volunteer; FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated haemoglobin; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, randomised controlled trial; XO, cross over; T2D, type 2 diabetes.

CERTAINTY OF EVIDENCE

The certainty of evidence was assessed using the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) system for all glycaemic outcomes in all SCFAs investigated. Certainty of evidence of our included studies was low to very low due to the high risk of bias, small sample size and wide confidence intervals as well as differences in study methodology, dose size and study population. Moreover, glycaemic response was not the primary outcome for many studies, reducing the potential to detect an effect.

Using the GRADE system of evidence, very low certainty indicates that we have very little confidence in the effect estimate, which means that the true effect is likely to be substantially different from the estimated effect. While low certainty indicates that our confidence in the effect estimate is limited, the true effect may be substantially different from the estimated effect.

GRADE assessment of SCFAs and for each outcome are summarized in Table 2.7.

CONFOUNDERS OF GLYCAEMIC CONTROL

The studies were examined for controlling for known confounders of glycaemic control like body weight and fat change, standard evening meal, fibre intake, strenuous exercise and whether being instructed to stop alcohol and to complete an overnight fast prior to the study visit. These are summarized in in the Appendix (Chapter #4).

In acute studies, one acetate (Scheppach et al., 1988) and three Vinegar studies (Johnston & Buller, 2005; Zhao et al., 2020; Darzi et al., 2012) instructed participants to consume a standard evening meal.

In four vinegar studies (Johnston et al., 2004; Darzi et al., 2014; Johnston & Buller, 2005; Darzi et al., 2014; Feise & Johnston, 2020; Zhao et al., 2020) and two propionate studies (Darzi et al., 2012; Byrne et al., 2016), participants were instructed to avoid strenuous exercise. Moreover, in one acetate study (Scheppach et al., 1988), three vinegar studies (Jasbi et al., 2019; Darzi et al., 2014; Zhao et al., 2020) and three propionate studies (Darzi et al., 2012; Byrne et al., 2016; Chambers et al., 2018), instructed participants to stop alcohol intake the day before the study. Three acetate studies (Scheppach,,1988;

Van der Beek, 2016; Laurent, 1995), one vinegar study (Darzi et al., 2014), two propionate studies (Laurent et al., 1995; Darzi et al., 2012) and two mixed vinegar studies (Laurent et al., 1995; Laurent C, 1995; Alamowitch et al., 1996) prescribed a low fibre diet before the study visit. All acute studies included except one (Brighenti et al., 1995) instructed participants to fast for more than 6 hours before the study visit.

50% of the chronic studies accounted for changes in body weight and fat (Roshanravan et al., 2018; Roshanravan et al., 2017; Roshanravan, 2017; Roshanravan, 2018; Todesco et al., 1991; Byrne et al., 2019; T, 1991; Byrne, 2019; Chambers et al., 2019; Chambers et al., 2015; Venter et al., 1990; Pingitore et al., 2017).Three propionate studies instructed the participants to consume a standard evening meal and avoid strenuous exercise and alcohol intake before the study visit (Pingitore et al., 2017; Chambers et al., 2019; Chambers et al., 2015). Byrne instructed participants to avoid only strenuous exercise and alcohol intake (Byrne et al., 2019). One vinegar study also instructed the participants to avoid alcohol intake (Ali et al., 2018). All studies instructed participants to fast for more than 6 hours before the study visit.

ADVERSE EVENTS

The adverse events (AEs) reported for each intervention arm for each study category are shown in the Appendix (Chapter #4). AEs were not disclosed in every study included.

In acute studies, no AEs were reported for acetate or vinegar. Two studies reported AEs for propionate: one case of nausea was reported (Byrne et al., 2016) while another study documented no AEs (Darzi et al., 2012). Two studies reported AEs for mixed SCFAs administration: one had up to 6 incidences of belching in both intervention and control groups (Wolever et al., 1988), while the other reported no AEs (Canfora et al., 2017).

In chronic studies, three propionate studies reported AEs. There were 3 incidences of flatulence when consuming inulin propionate ester bread (Byrne et al., 2019). Another study reported 6 cases of nausea, 2 cases of constipation, 1 case of flatulence and 4 cases of vomiting in the intervention group, although nausea, constipation and flatulence were also reported in the control group (Venter et al., 1990). One propionate study also

assessed AEs, but no information was provided (Chambers et al., 2015). Three vinegar studies recorded AEs (Jasbi et al., 2019; Derakhshandeh-Rishehri et al., 2014; Ali et al., 2018), but only one reported one case of nausea, stomach ache and headache (Derakhshandeh-Rishehri et al., 2014).

COMPLIANCE

The adherence to the intervention for each study category was assessed and is summarized in the Appendix (Chapter #4). Withdrawal was defined as participants dropping out after randomization. One acute study reported a 40% withdrawal rate, and in another, researchers failed to clip the catheter to the colonic mucosa (van der Beek et al., 2016). Acute propionate studies reported 100% adherence, whereas chronic interventions had an average of 12% withdrawal. Chronic butyrate studies reported a 1.7% withdrawal due to lost follow-up (Roshanravan et al., 2017; Roshanravan et al., 2018). In addition, one study reported a participant withdrawal due to nausea (Venter et al., 1990).

Table 2.7: Summary of GRADE results of acetate, vinegar, propionate, and mixed SCFAs studies.						
Outcomes	**Anticipated absolute effects[2] (95% CI) Risk with placebo or treatment**	**Risk with SCFAs**	**Relative effect (95CI%)**	**Number of participants (studies)**	**Certainty of the evidence (GRADE)**	**Comments**
ACETATE						
Acute postprandial glucose	—	SMD = 0.09 (−0.26 to 0.44)	—	44 (7 RCTs)	2 Very low[1]	The evidence suggests that acetate results in little or no difference in postprandial glucose
Acute postprandial insulin	—	SMD = 0.35 (−0.07 to 0.77)	—	35 (6 RCTs)	2 Very low[1,2]	Acetate may result in little or no difference in postprandial
VINEGAR						
Acute postprandial glucose (HV)	—	SMD = −0.27 (−0.54 to 0.00)	—	186 (15 RCTs)	2 Very low[3]	Vinegar may reduce or have little or no effect on postprandial glucose in HV, but the evidence is very low
Acute postprandial glucose(T2D, IR, IGT)	—	SMD = −0.53 (−0.92 to 0.14)	—	67 (7 RCTs)	2 Very low[4–6]	Vinegar may reduce or have little or no effect on postprandial glucose in individuals with T2D, IR, or IGT, but the evidence is very uncertain
Chronic fasting blood glucose (all studies)	—	SMD = −1.60 (−4.30 to 1.09)	—	143 (6 RCTs)	2 Very low[6,7,8]	Vinegar may reduce or have little or no effect on fasting blood

						glucose, but the evidence is very uncertain
Acute postprandial insulin (HV)	—	SMD = −0.29 (−0.66 to 0.08	—	55 (5 RCTs)	2 Very low[5,6,8]	Vinegar may reduce or have little or no effect on postprandial insulin in HV, but the evidence is very uncertain
Acute postprandial insulin(T2D, IR, IGT)	—	SMD = −0.16 (−0.75 to 0.44)	—	58 (6 RCTs)	2 Very low[6,9]	Vinegar may reduce or have little or no effect on postprandial insulin in individuals with T2D, IR, or IGT, but the evidence is very uncertain
Chronic fasting insulin(all studies)	—	SMD = 0.06 (−0.50 to 0.62)	—	89 (3 RCTs)	2 Very low[6,10]	Vinegar may increase or have little or no effect on fasting insulin, but the evidence is very uncertain
PROPIONATE						
Acute postprandial blood glucose	—	SMD = 0.07 (−0.32 to 0.47)	—	123 (8 RCTs)	2 Very low[11,12]	Propionate may increase or have little or no effect on postprandial glucose (acute), but the evidence is very uncertain
Chronic postprandial blood glucose	—	SMD = −0.08 (−0.43 to 0.27)	—	73 (5 RCTs)	2 Low[12-14]	The evidence is very uncertain about the effect of propionate on postprandial glucose (chronic)
Chronic fasting glucose	—	SMD= −0.14 (−0.47 to 0.19)	—	67 (4 RCTs)	2 Very low[13–15]	The evidence suggests that propionate results in little or no

						difference in fasting glucose (chronic)
Acute postprandial insulin	—	SMD = 0.24 (−0.30 to 0.78)	—	117 (7 RCTs)	2 Very low[11,12]	The evidence is very uncertain about the effect of propionate on postprandial insulin (acute)
Chronic postprandial insulin	—	SMD = −0.06 (−0.39 to 0.27)	—	167 (4 RCTs)	2 Very low[11,14,15]	The evidence suggests that propionate results in little or no difference in postprandial insulin (chronic)
Chronic fasting insulin	—	SMD = −0.22 (−0.65 to 0.21)	—	67 (4 RCTs)	2 Very low[12–14]	The evidence is very uncertain about the effect of propionate on fasting insulin (chronic)
MIXED SCFAs						
Acute postprandial glucose	Not pooled	Not pooled	—	42(4RCTs)	2 low[16]	The evidence suggests that little or no difference in acute PPG.
Acute postprandial insulin	Not pooled	Not pooled	—	36 (3 RCTs)	2 low[16]	The evidence suggests that little or no difference in acute postprandial insulin
BUTYRATE						
Fasting glucose	—	MD= -120)-2.91 to 0.51)	—	30(1RCT)	2 very low[17]	The evidence is very uncertain as there is only one study that fits our criteria
Fasting insulin	—	MD = 0.9 (0.57 to 1.31)	—	30(1RCT)	2 very low[17]	The evidence is very uncertain as there is only one study that fits our criteria

Table 2.7. Abbreviations: GRADE, Grading of Recommendations, Assessment, Development, and Evaluation; HV, healthy volunteers; IGT, impaired glucose tolerance; PBG, postprandial blood glucose; RCT, randomised controlled trials; SMD, Standardized mean difference; T2DM, type 2 diabetes.
[1] There is a risk of bias due to a lack of blinding and selective outcome reporting. Study populations differed between studies (HV, T2D, hyperinsulinemia, and overweight or obese). The dosage of acetate varied widely, between 1 and 360 mmol. Results are not generalizable due to the small sample size. Not applicable to wider population due to administration methods for acetate. The 95% CIs are very wide.
[2] The study populations differed between studies (HV, hyperinsulinemia, and overweight or obese). The dosage of acetate varied widely, between 36 and 360 mmol. None of the studies had PI as their primary outcome. A possible publication bias was detected by the funnel plot.
[3] A risk of bias arose from a lack of allocation concealment, through the lack of blinding, and in measurement of the outcomes. Different types of vinegar were used, although similar concentrations of acetic acid were documented. There was serious inconsistency (severe heterogeneity I^2 = 65.1%; P = 0.001). The 95% CI is wide.
[4] A risk of bias arose from a lack of allocation concealment, due to the lack of blinding, measurement of outcomes and selective outcome reporting.
[5] There is inconsistency due to a very wide 95% CI
[6] There was a possible publication bias detected by funnel plot.
[7] There was serious inconsistency (severe heterogeneity I^2 = 99.0%; P = 0.001). There is a very wide 95% CI. The studies were not generalizable, as they were mostly conducted on metabolically unhealthy individuals. Some study comparators contained small amounts of vinegar or acetic acid.
[8] A risk of bias arose from a lack of allocation concealment, a lack of blinding, and in the measurement of the outcome.
[9] There was serious inconsistency (severe heterogeneity I^2 = 77.0%; P = 0.001). The 95% CI is very wide. There is a risk of bias due to a lack of allocation concealment, lack of blinding, incomplete accounting of patients and outcome events and selective outcome reporting.
[10] A risk of bias arose from a lack of allocation concealment and lack of blinding. The study populations differed between the 3 studies. Different types of vinegar were used. There was serious inconsistency (severe heterogeneity I^2 = 70.6%; P = 0.02).
[11] The risk of bias mainly arises from the lack of allocation concealment and incomplete accounting of patients and outcome events. Inconsistency may stem from differing interventions (including sodium propionate, calcium propionate, and inulin propionate-ester) and dosages (ranging from <1 g to 9.9 g of the intervention). PBG was the main outcome in 50% of studies.
[12] There is a very wide 95% CI.
[13] The risk of bias mainly arose from a lack of blinding.
[14] A possible publication bias was detected by the funnel plot.
[15] There is a wide 95% CI.
[16] It is difficult to generalize the results due to the small sample sizes. Rectal and gastric infusions mean that the interventions are not very applicable, replicable or tolerable, reducing transferability. The studies tend to have small sample sizes, increasing imprecision.
[17] The risk of bias was high due to a lack of blinding, incomplete accounting of patients and outcome events, and selective outcome reporting. Publication bias is suspected, as there is only 1 study investigating butyrate, which demonstrates butyrate has a significant effect.

Table 2.8: Summary of the results from all meta-analysis in different short chain fatty acids (SCFAs).

SCFA	Acute		Chronic
	Outcome	Summary result	
Vinegar	Glucose	-0.27 (-0.54 - 0.00), n=186, healthy -.53 (-0.92 - -0.14), n=67, T2DM,IGT	-1.6 (-4.3 – 1.09), n=143
	Insulin	-0.29 (-0.66 -0.08), n=55, healthy -0.16 (-0.75 -0.44), n=58, T2DM, IGT	0.06 (-0.50 – 0.62), n=89
Acetate	Glucose	0.09(-0.26- 0.44), n=44	No studies
	Insulin	0.35 (−0.07 to 0.77), n= 35	No studies
Propionate	Glucose	0.07 (-0.32 – 0.47), n=123	PPG, -0.08 (-0.43 – 0.27), n=73 FBG, -0.14 (-0.47 – 0.19), n=67
	Insulin	0.24 (-0.30 -0.78), n=117	PI, -0.06 (-0.39 -0.27), n=67 FI, -0.22 (-0.65 – 0.21), n= 67
Butyrate	Glucose	No studies	No meta-analysis, see narrative review
	Insulin	No studies	No meta-analysis, see narrative review
Mixed SCFAs	Glucose	No meta-analysis, see narrative review	No studies
	Insulin	No meta-analysis, see narrative review	No studies

Table 2.8. Data presented as standard mean difference SMD (CI). Abbreviation. N, number; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; FBG, fasting blood glucose; PPG, post prandial glucose; PI, post prandial insulin; FI, fasting insulin**.**

DISCUSSION

It has been shown that high DF intake improves postprandial glucose and insulin, and it is believed that DF induces favourable effects through the production of SCFAs via gut microbial fermentation. The major SCFAs produced are acetate, propionate and butyrate. In this systematic review, the effect of SCFA administration on the glycaemic control measures was evaluated in acutely and chronically, healthy and metabolically unhealthy individuals. The meta-analysis summarised the evidence from 43 publications, which included 46 studies with total of 913 participants. Our results showed that acute vinegar administration had a favourable effect on blood glucose in healthy, IGT and T2DM participants, while acute and chronic administration of acetate, vinegar, propionate, butyrate and mixed SCFAs had no effect on glycaemic measures. A summary of the results of the meta-analysis can be found in Table 2.8.

The main finding of the meta-analysis was a significant lowering effect of vinegar on post prandial glucose (PPG) in healthy (SMD = -.27(-.54 to .00), p=0.05), and impaired glucose tolerance participants (SMD= -.53(-.92 to -.14), p=0.01). However, acute vinegar administration had no significant effect on postprandial insulin (PPI) in healthy (SMD = -.29(-.66 to .08), p=0.13) or in impaired glucose participants (SMD= -.16(-.75 to.44), p=0.6). The acute vinegar intake finding is consistent with a systematic review by Shishehbor et al. (2017), which reported lower PPG and PPI areas under the curve (AUC) following vinegar intake (SMD =-0.60, (-1.08 to -0.11), p = 0.01 and -1.30(-1.98 to -0.62), p < 0.001, respectively) However, the researchers did not analyse the effect size separately for healthy and non-healthy participants. They also reported significant heterogeneity (I^2 =80.2%, p<0.001), which they attributed to differences in vinegar type (apple vs white) and participants' health status, but not to age or vinegar doses (Shishehbor et al., 2017). Moreover, several human studies reported the lower glycaemic effect of vinegar after intake of food high in starch (Johnston & Buller, 2005; Johnston et al., 2004).

Our meta-analysis found no significant effect of chronic vinegar intake on fasting glucose (SMD= -1.6, 95% CI: -4.3 to 1.09, *p*=0.24) or fasting insulin (SMD= 0.06, 95% CI: -0.50 to

0.62, *p*=0.83). Only two studies (Gheflati et al., 2019; Ali et al., 2018) reported a glucose-lowering effect under chronic vinegar intake. Notably, these studies used a higher vinegar dose (~ 20 ml/day), which may have resulted in slower gastric emptying. A higher vinegar dose may also imply a greater concentration of acetic acid, which has been shown to reduce blood glucose uptake and enhance peripheral utilization (Fushimi et al., 2002).

Some studies attributed changes in fasting glycaemia to reduced oxidative stress, which is potentially linked to acetic acid and the phenolic compounds found in vinegar (Budak & Guzel-Seydim, 2010). The differences between the acute and chronic effects of vinegar may arise from variations in metabolic rates and signalling pathways activated over different durations.

Acute vinegar consumption appears to reduce postprandial glycaemia by delaying gastric emptying and inhibiting disaccharidase enzyme activity, thereby lowering postprandial blood glucose levels (Liljeberg & Björck, 1998; Ogawa et al., 2000). However, these acute effects may not lead to lasting effects or clinically meaningful improvements in glucose control with long-term use. It is possible that chronic vinegar intake activates different or additional metabolic processes, including compensatory mechanisms that counteract the benefits observed with acute intake (Johnston & Buller, 2005). Moreover, the effect of vinegar – whether acute or chronic – may differ between healthy and metabolically compromised individuals, possibly due to differences in vinegar metabolism. Variability in outcomes related to chronic vinegar consumption may also depend on the dose used.

Vinegar studies showed no consistent impact on insulin sensitivity. For example, Gheflati et al. (2019) reported an increase in 2,2-diphenyl-1- picrylhydrazyl (DPPH), a marker of oxidative stress which was associated with reduced insulin sensitivity and worsening glycaemia (Burgos-Morón et al., 2019).

Given the inconclusive and inconsistent findings and the variation in the response between acute and chronic vinegar supplementation in both healthy and impaired individuals, further well controlled studies are warranted.

For acute acetate, a total of seven randomised controlled trials (RCTs) assessed its effect on postprandial glucose (PPG), while six examined its effects on postprandial insulin (PPI) (Scheppach et al. 1988 – oral; Laurent et al., 1995 – gastric; Freeland and Wolever,

2010 – IV & rectal; Johnston et al., 2010 – study arm 4; Van der Beek et al., 2016 – proximal & distal). The doses administered ranged from 12 to 37.5mmol per minute over durations of 60 to 300 minutes. Pooled analysis showed that acute acetate administration had no effects on either PPG (SMD =0.09(-0.26 to 0.44)) or PPI (SMD = 0.35 (-0.07 to 0.77)).

These findings are consistent with Burnier et al. (1992), who investigated the metabolic effect of infusing 0.5M sodium acetate at a rate of 2.5mmol/min over 120 minutes in nine healthy subjects. The study found no changes in plasma glucose or insulin during the acetate infusion (Burnier et al., 1992).

Interestingly, while some acute acetate studies did not observe changes in glucose or insulin, they did report reductions in circulating free fatty acids (FFAs), which are linked to improved insulin sensitivity and lower insulin resistance (Laurent et al., 1995).

At present, it remains unclear why vinegar exerts a different effect on glycaemia compared with acetate, despite the fact that vinegar is mainly composed of acetic acid (4-7%). Although they share similar chemical properties, the hypoglycaemic effect of vinegar but not acetate found in the systematic review can be explained by several factors. One key difference lies in the mode of administration: all studies included in the systematic review administered vinegar orally. This allows vinegar to interact directly with the GIT system and gut microbiota, potentially stimulating multiple mechanisms that could contribute to the lower effect of glucose such as slowing gastric emptying and the inhibition of disaccharide activity in the small intestine. In contrast, acetate was administered intravenously and or rectally in most of the studies, where it bypasses the GIT system and enters the systematic circulation. Another factor is the different concentrations of acetic acid across the included studies, which may affect the action of vinegar on glucose responses. Moreover, the taste of the vinegar may contribute to the satiety and fullness due to sensory feedback, indirectly affecting glucose, while acetate does not have this effect. Finally, differences in the test meal composition that were used in the clinical trials may have influenced the effect of vinegar on glucose (Shishehbor et al., 2017).

Studies administering acute mixtures of SCFAs found no effect on PPG or PPI, but observed significant reductions in FFAs – up to 40% in one study (Wolever et al., 1989) and 22% in another study (Wolever et al., 1991). These reductions in FFA levels were correlated with improvements in insulin resistance indices, suggesting that decreased lipolysis may contribute to enhanced insulin sensitivity (Blaak et al., 2020). The reduction in FFAs may help explain the lack of effect on PPG, despite some studies reporting a 26% increase in circulating glucagon following the administration of acetate or mixtures of SCFAs (Wolever et al., 1991). Meanwhile, the rectal infusion of SCFAs mixtures did not affect fasting or postprandial glucose following a 76 g oral glucose load, nor did it affect insulin concentrations. However, it did increase fasting PYY and reduce fasting free glycerol concentration (Canfora et al., 2017) .

In mixed SCFAs studies, different routes of administration were used. The method of delivery is important, as the production and absorption of SCFAs vary depending on the route of administration. Oral administration allows interaction with the entire GIT tract, promoting endogenous SCFA production and function. Rectal administration, while bypassing parts of the upper GIT, delivers substrates directly to the colon, enhancing the localized production of SCFAs, which is potentially useful for targeted therapeutic effects. In contrast, IV administration delivers the SCFA directly to the blood stream without stimulating endogenous SCFAs production. Variations in the route of SCFA administration is therefore likely to contribute to the differences observed in outcomes across studies (Freeland et al., 2009; Laurent et al., 1995).

The pooled analysis of acute propionate administration showed no effect in PPG (SMD = 0.07, 95%CI: -0.07 to 0.47)) and PPI (SMD = 0.24, 95%CI: -0.32 to 0.78). Similarly, chronic propionate administration showed no significant effect on either fasting glucose (SMD= -0.08, 95%CI: -0.43 to 0.27) or postprandial glucose (SMD= -0.14, 95%CI: -0.47 to 0.19) nor on fasting insulin (SMD= -0.22, 95%CI: -0.65 to 0.21) or postprandial insulin (SMD= -0.06, 95%CI: -0.39 to -0.27).

These findings contrast with previous studies in rodents, which suggest that propionate may worsen glycaemia, as it is the only SCFA that is gluconeogenic (Yoshida et al., 2019). However, in humans, chronic propionate administration appears to have more favourable

effects on glycaemic outcomes. For example, Todesco et al. (1991*)* found that supplementing white bread with 9.9g/day of sodium propionate lowered the 2-hour glucose AUC by 38% as well as the glucose peak (Todesco et al., 1991).

Other studies reported no significant changes in glucose or insulin AUC following interventions with inulin propionate ester. However, some improvements were observed in insulin sensitivity indices such as the MATSUDA index and HOMA-IR (Chambers et al., 2019; Byrne et al., 2019). Meanwhile, *in vitro* experiments showed that pancreatic beta cells incubated with insulin propionate ester showed reduced apoptosis and increased insulin release (Pingitore et al., 2017). This raises the question of whether the observed effects are due to propionate alone or other SCFAs produced during fermentation, especially given that the control group also received Inulin. Measuring circulating and faecal SCFAs concentrations would help clarify this distinction.

Two studies examined the effects of butyrate administration on fasting glucose and insulin levels in participants with T2DM. The chronic administration of sodium butyrate showed no significant impact on glycaemic measures compared to a starch-based placebo. However, an increase in GLP-1 level was observed following butyrate intervention (Roshanravan et al., 2017). This finding is supported by evidence that butyrate, along with other SCFAs, can function as signalling molecules in the colon by binding to FFAR2 and FFAR3 receptors on enteroendocrine L- cells, therapy stimulating the release of gut hormones such as GLP-1 (Yadav et al., 2013).

Roshanravan et al., (2017) also reported no significant differences in fasting blood glucose (FBG), 2-hr post prandial glucose (PPG), fasting insulin (FI), HbA1C, and HOMA-IR between the intervention and control groups. It is worth noting that individuals with T2DM often exhibit a reduced level of butyrate-producing bacteria, a factor that is believed to contribute to adverse metabolic effects including impaired glucose regulation and increased risk of obesity (Mayorga-Ramos et al., 2022).

A recent systematic review and meta-analysis evaluated the effect of SCFAs interventions on fasting glucose, fasting insulin, and HOMA-IR. Notably, the researchers found a significant reduction in fasting insulin concentrations compared to the placebo (SMD= -0.25; 95%CI: -0.43 to -0.06, p=0.008). In contrast, no significant differences were

observed for fasting glucose (SMD=-0.06; 95%CI: -0.24 to 0.13, *p*=0.55) or HOMA-IR (SMD= -0.22; 95%CI: -0.48 to 0.13, *p*=0.11). Notably, the authors stratified their analysis based on the magnitude change in post intervention SCFA concentrations (categorized as high or low). However, subgroup analysis revealed no substantial increases in SCFA levels. Consequently, no significant differences in fasting glucose, insulin and HOMA-IR were found between the intervention and control groups. A stronger correlation was found between increased circulating SCFAs concentration and a reduction in fasting insulin concentration (SMD= -0.22; 95% CI= -0.43 to -0.01; *p*= 0.04) (Pham et al., 2024)

It is also worth noting that the meta-analysed studies exhibited very low certainty of evidence for all acute outcomes, apart from the mixed SCFAs studies, so the findings should be interpreted with caution. The low certainty and lack of significant effects observed in this review could be attributed to the several factors: variability in the route of administration, dosage, participants' health status and sample size. High heterogeneity of some outcomes, such as FBG and PPG, were found in response to chronic vinegar administration. Most heterogeneity arose form a wide 95% CIs. Moreover, sensitivity analyses revealed that three studies – Brighenti et al. (1995), Johnston et al. (2010), and Mettler et al. (2009) – had a substantial influence on the outcome for acute PPG in the high vinegar group (Brighenti et al., 1995; Johnston et al., 2010; Mettler et al., 2009). Additionally, a study by Johnston & Buller et al. revealed a significant impact on the outcome of acute postprandial insulin in high vinegar group (Johnston & Buller, 2005). Publication bias was also detected across all chronic outcomes. These factors influenced the precision and directions of the results, thereby lowering the overall quality of the evidence.

A few studies reported adverse events (AEs) such as nausea and belching. However, the limited reporting of AEs hampered the ability to assess the tolerability and safety of SCFA administration. Reported reasons for withdrawals included methodological issues, non-compliance, the unpleasant taste of the intervention and participant availability.

Adverse events included nausea, flatulence, constipation, vomiting and belching. Withdrawals were primarily due to non-compliance, lost follow-up or personal reasons. In

some cases, withdrawal reasons were not reported, making it unclear whether they were related to the intervention.

STRENGTHS

The major strengths of this systematic review and meta-analysis are derived from the inclusion of randomised controlled studies that featured a wide variety of SCFA types and participant health status. Another key strength is the categorization and meta-analysis of the data based on SCFA type, participant health status and duration of SCFA administration. This approach improved homogeneity and reduced variability across studies. The use of multiple glycaemic markers – such as fasting and postprandial glucose, fasting and postprandial insulin, insulin resistance and sensitivity indices – also strengthened the validity of the measured effect sizes related to glucose regulation.

LIMITATIONS

This systematic review has several limitations. Some of the studies included did not report uniform glycaemic outcomes, which may have underestimated the overall effect of the intervention. Additionally, variations in the methodologies used to measure insulin, glucose and other glycaemic indicators were not accounted for, potentially affecting the compatibility of the results. Finally, the literature search was restricted to studies published in English.

FUTUTRE RESEARCH

Future studies are urgently needed that use standardized method-such as consistent types and site of SCFAs administration, as well as studies that account for potential confounders. Using unified methods to measure and assess the effects of SCFAs on glycaemic outcomes, along with ensuring participant compliance, would improve the reliability of the findings. Research exploring the link between SCFAs and T2DM is also essential, particularly studies that investigate the mechanisms through which the gut microbiome or microbial metabolites regulate incretin hormone expression, insulin

secretion and glucose homeostasis. Such studies would be instrumental in advancing the development of new preventive strategies and treatment for weight gain and type 2 diabetes.

CONCLUSION

In summary, this systematic review and meta-analysis found that acetate, butyrate and propionate had no effect on glycaemic measures. In contrast, vinegar was shown to acutely reduce blood glucose levels in healthy, impaired glucose tolerance (IGT) and type 2 diabetes (T2DM) participants. However, the available evidence is limited and heterogenous, with moderate to high risks of bias and low to very low certainty of evidence across all categories.

Before SCFAs can be considered as a therapeutic option, several factors need to be carefully addressed in future studies, including the specific type of SCFAs (acetate, butyrate, propionate or a mixture), the form of SCFA, the duration of administration, the route of delivery and participant compliance. These studies should also control for confounding factors that may influence glycaemic outcomes, to generate high quality, and reliable evidence.

CREDIT Statement

The work presented in this chapter was conducted in collaboration with a team of PhD students. I have primarily involved in designing the study and formulating the PICOs framework. I actively participated in the database search, as well as the screening of abstracts and full articles, and contributed to data entry. I was also involved in the extraction of data from the selected studies and interpreting the findings and outcomes. Additionally, I assisted in writing the results and discussion sections, and the overall manuscript.

CHAPTER 3:

THE RELATIONSHIP BETWEEN PLASMA ACETATE, BODY WEIGHT, AND GLUCOSE IN DIABETIC PATIENTS ON LOW CALORIC DIET

ABSTRACT

Introduction:

Dietary restriction-induced weight changes have been shown to reduce the risk of developing metabolic diseases such as type 2 diabetes mellitus (T2DM) and obesity. These changes are important to understand, as short chain fatty acids (SCFAs) play a significant role in regulating the body's metabolic status. Acetate is believed to function as a signalling molecule involved in the regulation of glucose and lipid metabolism, so changes in SCFA concentrations resulting from weight loss may carry important implications for the future management of diabetes and obesity.

Previous studies have reported impaired acetate metabolism in individuals with diabetes, with findings that showed a correlation between acetate and plasma glucose levels. Other studies suggested that weight change may induce changes in SCFA concentration, including acetate. However, knowledge about how SCFAs change during weight loss – particularly in obese individuals with T2DM – remains limited and inconclusive. Most existing studies have focused on faecal concentrations of SCFAs rather than serum levels. The present study therefore aims to examine the relationship between plasma acetate level, body weight and glycaemic variables in obese diabetic patients undergoing caloric restriction to induce weight loss.

Method:

This study is a sub-analysis of the randomised controlled trial (RCT) of the Low-Calorie Diet in Diabetes Mellitus (LCDDM) study. For this analysis, we included 51 obese patients with diabetes for whom data were available on both plasma acetate and glycaemic

variables. Pearson correlation was used to evaluate the relationship between acetate, weight change and glycaemic variables. Linear regression was also conducted to assess the strength of associations between acetate and the variables that showed significant correlations to determine how much of the variance in acetate levels could be explained by glucose concentration or changes in body weight among individuals with diabetes.

Results:

Participants in the low caloric diet (LCD) arm showed a significant mean body weight change at 12 months of -9.41 kg, compared to -4.98 kg in the control arm, resulting in a mean difference of -4.43 kg, ($p<0.05$). There was also a trend toward higher plasma acetate levels in the LCD arm compared to the control arm. However, the mean difference between arms was small and statistically non-significant (-0.0048, $p=0.293$). Overall Plasma acetate levels showed no significant association with glycaemic indices. However, in the LCD group there was a positive correlation between plasma acetate and quantitative insulin sensitivity check index (QUICKI) ($r = 0.356$, $p=0.07$). In the control group at visit 9 (24 weeks), plasma acetate was significantly and negatively correlated with body mass index (BMI) ($r = -0.502$, $p=0.001$) and showed a non-significant negative correlation with body weight (BW), $r = - 0.308$, $p=0.07$). Further analysis indicated that the changes in R^2 ($\Delta R^2 = 0.274$) was statistically significant ($P <0.01$), suggesting that BMI independently accounted for 27.4% of the variance in plasma acetate levels after adjusting for age and gender. This finding suggests that change in body weight may serve as a significant predictor of change in plasma acetate level.

Conclusion:

This study did not replicate earlier findings that reported an association between plasma acetate and glucose levels in individuals with diabetes. However, it did reveal that dynamic changes in plasma acetate levels may be linked to gradual weight loss resulting from caloric restriction. The strong association observed between plasma acetate and BMI suggests that fluctuations in body weight may influence plasma acetate concentrations. Further research is needed to better understand this relationship in obese

patients with diabetes, particularly studies that also consider the contribution of exogenous acetate sources.

INTRODUCTION

DIABETES MELLITUS (DM).

Type 2 diabetes mellitus (T2DM) is a complex and rapidly growing global health challenge. It is estimated that by 2020, approximately 336 million people worldwide will be affected with T2DM (Arathuzik & Goebel-Fabbri, 2011). DM is a leading cause of mortality and morbidity worldwide, and maintaining blood glucose within target ranges is essential for preventing acute and chronic complications. As most individuals withT2DM are obese, weight management plays a central role in diabetes care. Effective weight control helps reduce blood glucose levels, lowers the risk of complications, improves overall health and enhances quality of life. T2DM is strongly associated with chronic low-grade inflammation, insulin resistance and beta-cell dysfunction, which collectively contribute to impaired glucose homeostasis and complications over time.

Recent evidence also highlights the role of gut microbiota and metabolic intermediates, such as short chain fatty acids (SCFAs), in modulating insulin sensitivity and energy balance in diabetic patients.

NUTRITIONAL MANAGEMENT OF T2DM

Nutritional management is recommended as a key component of an integrated structural lifestyle programme for managing T2DM. This approach includes dietary education, intensive interdisciplinary therapy and regular physical exercise (Arathuzik & Goebel-Fabbri, 2011). Over the years, nutritional intervention has provided a great deal of evidence supporting its role in prevention, treatment or long-term management of obesity and diabetes. For patients with T2DM and obesity, gradual and sustained weight loss is generally recommended to improve glycaemic control, reduce complication and improve overall quality of life (Minari et al., 2023) .

Various approaches to dietary restriction have been introduced for patients with T2DM and obesity, including the use of very low-calorie diets (VLCD), defined as providing diet of fewer than 800 kcal/day. In obese diabetic and non-diabetic individuals, VLCDs have been shown to produce fast and substantial weight loss, with clinical studies reporting an average reduction weight of 20 kg over 12 weeks. However, maintaining this weigh loss over long term has proven to be challenging, particularly without behavioural therapy support (Wing et al., 1995).

Current dietary guidelines recommend a macronutrient distribution of approximately 40% carbohydrates, 20-30% protein and 30% -35% fat. While the optimal amount of carbohydrate intake for individuals with T2DM varies among studies, the majority – including recommendations from the World Health organization (WHO) – advise consuming no less than 130 g of carbohydrates per day to meet the brain's glucose requirements (Haimoto et al., 2009). Preference should be given to carbohydrates sources with a low glycaemic index (GI) and glycaemic load (GL), as these measures can influence postprandial blood glucose levels. However, some systematic reviews have reported no significant impact of GI on PPG or HbA1c, probably because of inconsistencies in GI classification across studies (Minari et al., 2023). Dietary fibre intake has been shown to improve postprandial glucose and insulin responses by slowing gastric emptying, promoting satiety and modulating gut microbiota-derived metabolites such as SCFAs. Following a high fibre diet is strongly recommended for glycaemic control and weight management.

The role of protein intake, whether low or high, remains controversial (Haimoto et al., 2009). Regarding fat intake, the ADA recommends that total fat should consist of less than 35% of total energy intake, with an emphasis on unsaturated fats.

Other dietary approaches have also been investigated, including high fibre diets, low calorie diets (LCDs), vegetarian or vegan diets, the Mediterranean diet and intermittent fasting (Arathuzik & Goebel-Fabbri, 2011). However, the question of which dietary approach is most effective for individuals with obesity and T2DM is still under extensive and active research and investigation. However, there is a strong consensus that individualised nutritional management, delivered through a multidisciplinary team, is key

to preventing and managing hyperglycaemia. An effective long-term dietary plan should be tailored to the individual's metabolic profile, cultural and socioeconomic background, medication use and personal preferences.

Role of Low Caloric Diet (LCD) in Diabetic Obese Patients.

Numerous studies have explored the role of nutritional therapy in the management of T2DM. One widely studied approach is the low caloric diet (LCD), which typically involves restriction of daily calorie intake to between 800 and 1500 calories per day, depending on individual needs and health goals. It is recognized that caloric restriction in obese individuals with diabetes is an effective strategy for improving metabolic parameters. Meanwhile, sustained caloric restriction and weight loss have been shown to reduce the risk of developing diabetes in obese individuals, highlighting its value not only in treatment but also in prevention.

In a systemic review that included 11 studies, approximately 425 participants were randomised to a VLCD and 349 participants to a behavioural weight loss programme. The VLCD group achieved an additional mean weight loss of −4.1 kg (95% CI: −7.3 to −0.8) at 12 months compared to the behavioural intervention group (Parretti et al., 2015). In a randomised controlled trial involving individuals with T2DM and obesity on insulin therapy, Brown et al. (2020) reported a mean weight loss of -9.8 kg in the LCD intervention group, compared to -5.6 kg in the control group after 12 months (Brown et al., 2020).

Benefits and Risks of Low Caloric Diets in Diabetes Patients.

LCDs promote weight loss by creating a caloric deficit, forcing the body to utilise stored fat for as an energy source. For individuals with diabetes, LCDs offer several clinical benefits, including significant weight reduction, improved insulin sensitivity, lower blood glucose levels, reduced HbA1C and a decreased need for glucose lowering medications. LCDs also contribute to improved overall metabolic health and enhanced quality of life. However, they are not without potential risk. They may lead to nutrient deficiencies, loss of muscle mass and an increased risk of hypoglycaemia, particularly individuals on insulin or certain oral hypoglycaemic agents. Furthermore, long-term caloric restriction may

elevate stress levels and negatively impact adherence to the dietary regimen. It is therefore crucial that LCDs be implemented under the supervision of health care professionals, with individualized adjustments to ensure nutritional adequacy and minimise associated risks (Henry & Gumbiner, 1991; Rondanelli et al., 2021).

CHANGES IN SCFAS IN RELATION TO WEIGHT LOSS

Research has also shown that diet can significantly alter the composition of the gut microbiota, and these alterations are associated with favourable metabolic changes, including improvements in body weight and plasma glucose levels, particularly in obese or metabolically compromised individuals. These beneficial health effects are believed to be mediated by short-chain fatty acids (SCFAs), which are the primary metabolites for the anaerobic fermentation of non-digestible carbohydrates (NDCs) by gut microbiota (Blaak et al., 2020).

These metabolites have gained significant attention for their positive impact on energy and glucose regulation (Canfora et al., 2017). Interestingly, the relationship between SCFAs and obesity appears to be bidirectional; not only can SCFAs influence metabolic health, but obesity itself may also affect the metabolism of SCFAs or alter their production. Obesity related alterations in gut microbiota may favour SCFA-producing bacteria that increase energy harvest, which could contribute to further weight gain, highlighting a feedback loop between SCFA production and body weight regulation. Some studies have reported higher concentrations of faecal SCFAs in obese compared to lean individuals (Schwiertz et al., 2010). A higher Firmicutes to Bacteroidetes ratio in obese individuals, for example, has been associated with increased SCFA production and enhanced caloric extraction from the diet. Similar findings have also been reported in animal models, with obese mice exhibiting elevated SCFA levels compared to lean mice (Gordon et al., 2006). These differences have been attributed to increased SCFA production, potentially due to variations in gut composition and function. Specifically, the obese microbiome may have an enhanced capacity for energy harvest compared to the non-obese microbiome (Schwiertz et al., 2010; Gordon et al., 2006).

Research shows that weight loss has varying effects on SCFAs production, with SCFAs potentially increasing, decreasing or remaining unchanged. Some studies have reported a reduction in the production and concentration of SCFAs following weight loss intervention. Louis et al. (2016) evaluated the impact of a VLCD on the gut microbiota composition and SCFA levels in individuals with obesity, revealing a decrease in microbial diversity and a reduction in SCFA concentrations (Louis et al., 2016). Wu et al. (2017) and Sun et al. (2017) also observed that obese individuals who underwent gastric bypass surgery experienced significant weight loss accompanied by reductions in major gut microbiota population and SCFAs production (Sun et al., 2017). Several other randomised studies have also reported a reduction in SCFA levels following weight loss intervention (Salonen et al., 2014; Tay et al., 2008; Duncan et al., 2008).

On the other hand, several investigations in animals and humans have shown that weight reduction may be associated with the increased production of SCFAs. For example, Dao et al. (2016) reported that mice fed a high fat diet and subjected to sleeve gastrostomies exhibited a remarkable increase in acetate, producing bacteria and an elevated acetate concentration compared to control mice. Interestingly, the presence of *Akkermansia muciniphila*, a gut bacterium, was noted and linked to improved glucose metabolism and leanness. However, its relevance in human remains less conclusive (Dao et al., 2016; Zhao et al., 2018).

In a more recent prospective study by Rad et al. (2023), faecal SCFA levels and serum inflammatory markers were compared between individuals following LCDs and those on a habitual diet (HD). The study found that concentration of faecal SCFAs – including acidic, propionic and butyric acid – were significantly higher in both groups at the end of the study. Notably, faecal acetic acid levels were significantly higher in the LCD compared to the HD group ($P<0.01$), suggesting that caloric restriction may enhance the production of SCFAs (Rad et al., 2023) .

However, other randomised studies have found no significant changes in faecal SCFA concentrations following weight loss interventions. For example, Benassi-Evans et al. (2010), observed no notable differences in faecal SCFAs in overweight and obese individuals who lost an average of 9.3 ± 0.7 kg over 12 weeks through either a high protein

or high carbohydrate diet, both of which had similar fibre content (Benassi-Evans et al., 2010). A study by Gratz et al. (2019) also reported no significant changes in total faecal SCFA concentrations after a 10-day intervention involving different protein-based diets in overweight participants. However, they did note a reduction in butyrate concentration levels across all diet types, particularly in participants consuming a high protein, moderate carbohydrate diet (Gratz et al., 2019).

Although weight loss is generally recommended for the prevention and management of chronic conditions such as T2DM and cardiovascular disease (CVD), it may also lead to a decline in the production of beneficial SCFAs. Given the positive metabolic effects associated with SCFAs, this raises questions about the potential unintended consequences of weight reduction on gut-derived metabolites.

Weight loss appears to exert heterogeneous effects on short chain fatty acids production, whereby SCFA concentrations may increase, decrease or remain unchanged. The underlying mechanisms responsible for these variations are not yet fully understood. However, obesity is known to be associated with gut dysbiosis and chronic low-grade inflammation, so reducing body weight could theoretically help restore gut microbial balance and potentially promote SCFA production.

Given the limited and conflicting evidence on how weight loss interventions affect SCFA levels – particularly given that most studies rely on faecal SCFA measurements – further research is warranted. Future studies should focus on explaining the effects of weight loss on the gut microbiota and clarifying the relationship between SCFA production and microbial composition. More targeted investigations are also needed in metabolically compromised populations to better understand how caloric restriction influences the gut microbiota dynamic and to identify the mechanisms underlying the observed inconsistencies in current research.

UNDERSTANDING ACETATE AND GLUCOSE METABOLISM

Glucose Metabolism Regulation

Glucose levels in the human body are maintained by the balance of gluconeogenesis and Glycolysis and controlled by bi-hormonal control, namely insulin and glucagon. (See Chapter 1).

Acetate Production and Absorption in Humans.

Acetate is the shortest chain fatty acid and represents the basic building block of all long-chain fatty acids and sterols through its basic structure of coenzyme A (CoA) (Moffett et al., 2020). Acetate is a major product of the microbiota derived fermentation of dietary fibre in the gut. Humans do not generate the same level of short-chain fatty acids from microbial fermentation as ruminants (Canfora et al., 2015), but acetate has been implicated in a range of health benefits whether derived from the diet fermentation in the colon or generated endogenously (Moffett et al., 2020).

Endogenous sources

Glycolysis contributes to the endogenous production of acetate, with pyruvate – the end product of glycolysis – serving as the primary substrate for acetate synthesis. *De novo* acetate production from glycolytic pathways has also been observed in various mammalian cells (Liu et al., 2018). Acetate is synthesized by two pathways: the decarboxylation of pyruvate, to acetyl-CoA, which is hydrolysed to acetate by acetyl-CoA hydrolase, or the reduction of carbon dioxide to carbon monoxide, which reacts with CoA and a methyl group to produce acetyl-CoA and then acetate; this pathway is the Wood-Ljungdahl pathway (Nogal et al., 2021) Endogenous acetate is required to maintain acetyl-CoA pools and lipogenesis (Liu, Xiaojing et al., 2018) (Figure 3.1).

Exogenous sources.

Acetate is mainly produced through the microbial fermentation of indigestible dietary fibres in the gut, particularly those that are classified as acetogenic fibres (Nogal et al., 2021). The microbial fermentation of other substrates, such as peptides and fats, can also generate acetate. Mice fed a high fat diet (60% fat) exhibited increased colonic and systemic acetate levels, along with a shift in gut microbiota composition marked by elevated Firmicutes/Bacteroidetes ratio (Perry et al., 2016).

Acetate is also found in various dietary compounds, where it serves as an acidity ph. regulator, preservatives or sequestrant. It is usually present in dairy products, dried pasta, bread and liquid eggs, as well as salt and coffee substitutes. Another common source of acetate is vinegar, which can significantly elevate blood acetate levels shortly after ingestion. In a study involving healthy participants, serum acetate concentrations increased from 120 µmol/L to 350 µmol/L within 15 minutes and decreased to 200 µmol/L after 30 minutes following the consumption of 100 ml of vinegar containing 0.75 g of acetic acid (Sugiyama et al., 2010).

Ethanol (Alcohol), a widely consumed substance, is also metabolized in the body as acetate or acetic acid. This metabolite enters the circulation and serves as an energy source, particularly for the brain (Jiang et al., 2013).

Figure 3.1: Summary of endogenous pathway for acetate synthesis.

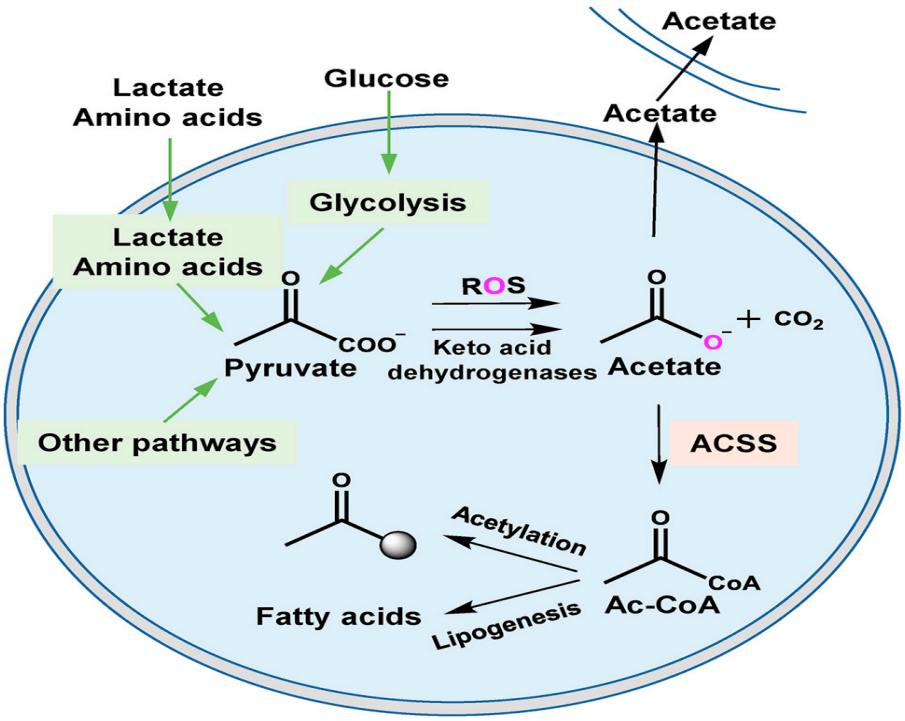


Figure 3.1. Endogenous Acetate Production. Cells produce acetate directly from pyruvate through two distinct mechanisms: (1) coupling to reactive oxygen species (ROS) and (2) enzyme activity from keto acid dehydrogenases. ACSS=Acetyl Co-A Synthetases. *From Liu et al., 2018*

Acetate concentration and absorption

The major site for acetate production and absorption is the proximal colon. There is a progressive decline in the concentration of acetate along the colon, where the cecum has the highest level of acetate (69 mmol/L), followed by the ascending colon (63mmol/L), the transverse colon (37.9 mmol/L), the descending colon (43.5 mmol/L) and the sigmoid colon (50.1 mmol/L) (Hernández et al., 2019). Following its production, acetate is rapidly absorbed by colonocytes then transported via the portal vein to the liver, from which point it is subsequently distributed into the peripheral circulation. A postmortem study estimated acetate concentration to be highest in the colon (131.32 ±13.1 μmol/L), followed by the portal vein (258 ±40.13), the hepatic vein (115 ± 28.20 μmol/L) and finally the peripheral vein (70 ±18.55 μmol/L) (Cummings et al., 1987) .

Acetate signalling and receptors.

Metabolic regulation in response to food relies on effective nutrient sensing and signalling, and the dysregulation of these processes can lead to several metabolic conditions, including obesity and diabetes. Acetate acts as a signalling molecule in the regulation of metabolism through distinct signalling transduction pathways (Moffett et al., 2020; Hernández et al., 2019).

Acetate contributes to maintaining the intracellular pool of acetyl CoA, which is subsequently incorporated into the tricarboxylic acid (TCA) cycle in various peripheral tissues. It may also affect metabolism by increasing the oxidative capacity in liver and skeletal muscle via AMP-activated protein kinase (AMPK) phosphorylation. Meanwhile, acetate promotes fatty acid synthesis via epigenetic mechanisms such as histone acetylation (Moffett et al., 2020). As well as its metabolic roles, acetate may also activate receptors involved in blood pressure regulation, including olfactory receptors 51E2 (Olfr51E2) and 78 (Olfr78) in renal tissue (Hernández et al., 2019).

Acetate is an important substrate that functions at the cellular levels. It acts by binding to GPCR41/43 which is expressed widely in human body (Moffett et al., 2020; González Hernández et al., 2020; Hernández et al., 2019). GPCR43 (FFAR2) mostly favours

acetate and propionate, whereas GPCR41(FFAR3) favours propionate, butyrate and formate (Brown et al., 2003). Both receptors are localized in pancreatic β-cells and have been linked to glucose-stimulated insulin release in mice (Priyadarshini et al., 2018).

Acetate signals through two main pathways (Figure 3.2). In the first, it acts via free fatty acids receptors – mainly FFAR2, and to a lesser extent FFAR3. The activation of FFAR2 stimulates cyclic AMP (cAMP), which subsequently activates protein kinase A and C (PKA) and (PKC). The second pathway involves the conversion of acetate to Acetyl CoA, which is a key substrate for acetylation histones, transcription enzymes and other proteins. This acetylation process plays a significant role in epigenetic regulation, influencing gene expression involved in metabolic processes and energy balance. Through these mechanisms, acetate signalling is closely linked to metabolic conditions such as obesity and diabetes (Moffett et al., 2020).

Figure 3.2: Acetate signalling pathways

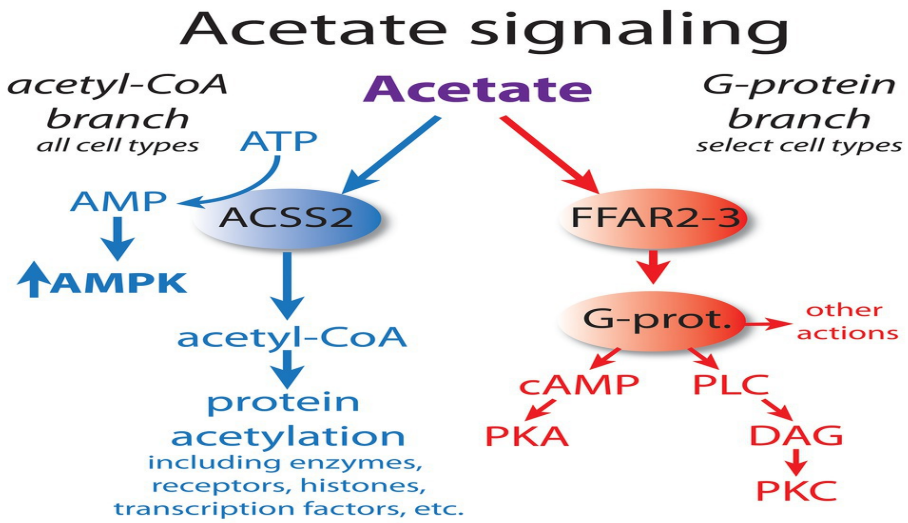


Figure 3.2. Acetate signalling pathways. Two main pathways for acetate signalling: the G-protein pathway and Acetyl-CoA. The acetylCoA branch involves regulation of protein function and gene transcription via acetylation/deacetylation cycles, as well as through AMPK kinase cascades. This signalling system operates in all cell types, but the responses are cell type specific. In contrast, the G-protein branch signals through cyclic AMP (cAMP) and phospholipase C (PLC) and is restricted to certain cell types. The G-protein branch involves metabolic regulation based on fatty acid levels. Dysregulation of both pathways occurs in cancer. AMPK, adenosine monophosphate kinase; cAMP, cyclic AMP; DAG, diacylglycerol; FFAR2 and 3,

free fatty acid receptors; G-prot., G proteins; PKA, protein kinase A; PKC, protein kinase C; and PLC, phospholipase C. *Derived from Moffett et al. (2020).*

Acetate and glucose interaction

Acetate is closely linked to glucose metabolism, although it is not a direct product of glycolysis. Instead, it is formed after a serious reaction that forms pyruvate, which is then converted to acetyl CoA, which can then be hydrolysed to acetate. It can also enter the Tricarboxylic cycle (TCA), contributing to energy production in the form of ATP (Macfarlane & Macfarlane, 2003) Acetate can also contribute to fatty acid lipogenesis through its precursor, acetyl-Co (Wolfe, 2005).

However, acetyl CoA cannot serve as a substrate for gluconeogenesis, although acetyl Co A or acetoacetyl-CoA can act as precursors for the synthesis of ketone bodies. During fasting or prolonged caloric restriction, the body shifts to fat oxidation to meet its energy needs. This process is supported by acetate, primarily through its conversion to acetyl CoA in the citric acid cycle (Kreb's cycle) to produce ATP.

As fasting continues and glycogen stores are depleted, the liver initiates glycogenolysis to maintain blood glucose levels. With prolonged fasting, hepatocytes shift to gluconeogenesis, synthesizing glucose from non-carbohydrate sources such as lactate, pyruvates, glycerol and amino acids. These metabolic processes ensure a continuous supply of glucose to vital tissues, including the brain and the red blood cells. Acetate therefore supports the overall metabolic adaptation to prolonged caloric restriction (Liu et al., 2018; Rui, 2014).

ACETATE AND GLUCOSE IN DIABETES AND NON-DIABETES.

Several investigations have identified differences in SCFA production and absorption between diabetic and non-diabetic individuals (Wolever et al., 1997; Fernandes et al., 2012). Investigations have reported increased serum acetate levels and decreased faecal acetate levels in diabetic subjects compared to non-diabetic subjects (Zhao et al., 2018; Karlsson et al., 2013). Animal research supports these findings, showing a lower faecal acetate level in diabetic rats compared to non-diabetic rats (Gao et al., 2009). These variations may be attributed to changes in gut microbiota composition as well as

differences in SCFA content and utilisation between diabetic and non-diabetic individuals (Liu et al., 2022).

To explore the relationship between acetate and glucose levels, Todesco et al. (1993) found that obese patients with T2DM showed altered patterns of acetate metabolism and higher plasma acetate concentrations compared to obese individuals with normal glucose tolerance. In contrast, no significant differences in acetate levels were observed between obese normoglycemic individuals and healthy controls. Notably, there were no differences in dietary fibre intake between the groups (Todesco et al., 1993) .

Further investigations by Akanj et al. (1989) evaluated plasma acetate level in diabetic participants assigned to three different diet groups: high-fibre (HF), high-fibre with sucrose (HFS), and a usual diet (U). The study found no significant differences in plasma acetate across all diets. However, plasma fasting glucose was positively associated with acetate levels ib all diets (rs = 0.48, P<0.005) (Akanji et al., 1989). In a subsequent study, Akanji et al. (1989) also reported that plasma acetate concentrations were significantly correlated with plasma glucose in both diabetic (r_s = 0.36, p<0.001) and non-diabetic individuals (r_s = 0.31, p< 0.001). Meanwhile, intravenous glucose administration was found to cause a rapid increase in plasma acetate (Akanji & Humphreys, 1988) .

In contrast, Scheppach et al. (1988) reported either a decrease or no change in blood acetate levels following the ingestion of 50 g of glucose by healthy individuals who were administered either oral acetate (15 mmol every 15 minutes) or chloride as control. The authors attributed their findings to individual variations in glucose tolerance and metabolism, as well as the small sample size used in the study (Scheppach et al., 1988) .

In a similar study, Muscelli et al. (2015) reported that acetate infusion enhanced whole body glucose utilisation and improved insulin responsiveness in T2DM individuals (Hernández et al., 2019). Meanwhile, Perry et al. (2016) conducted a study to examine the effects of acetate supplementation on glucose metabolism in healthy adults. Their findings showed that acetate supplementation improved insulin sensitivity and glucose tolerance, suggesting a potential role for acetate in the regulation of glucose homeostasis (Perry et al., 2016). Supporting this, De Vadder et al. (2016) found that acetate

administration in humans stimulated the release of glucagon-like peptide-1 (GLP-1), a hormone involved in glucose regulation (De Vadder et al., 2016).

These findings suggest that in individuals with T2DM, acetate metabolism and gut microbiota composition may be altered, potentially leading to changes in acetate concentration in the blood and faeces. Understanding these differences is crucial for developing targeted preventive strategies for T2DM. However, further research is needed to explain the underlying mechanisms and to clarify the role of these alterations in the pathogenesis of diabetes.

SUMMARY

To summarise, caloric restriction and weight loss appear to have paradoxical effects on gut microbiome and SCFAs production. One potentially unfavourable outcome of weight loss is the reduction in SCFA levels, which may offset some of the health benefits associated with achieving a healthy body weight, given the well-established positive effects of SCFAs on various tissues and overall metabolic health. However, existing research in this area remains controversial and inconclusive.

Studies have reported inconsistent changes in SCFA production and concentrations across different weight loss interventions and among overweight or obese individuals. To date, no definitive mechanisms have been proposed to explain these different responses. However, it has been hypothesized that caloric restriction-induced weight loss in obese individuals may reduce dysbiosis and inflammation associated with obesity, thereby improving gut health and potentially promoting SCFA production.

Despite this hypothesis, it is still unclear as to whether such changes are sustained over time or persist following the re-introduction of a normal diet (pre-caloric restriction). This is an area that has yet to be adequately explored. Moreover, data on faecal or plasma SCFAs in individuals with diabetes following weight loss interventions are scarce, and most of the current evidence is limited to surgical weight loss procedures or studies in healthy individuals.

In individuals with diabetes, existing reports suggest an impairment in acetate metabolism. However, it remains uncertain whether this impairment is driven by abnormal glucose, acetate kinetics or by a disruption in gut microbiota composition. Most studies to date have been limited by small sample sizes, lack of randomisation, reliance on faecal SCFA measurements and short study durations.

Importantly, the gut microbiota profile during diet -induced weight loss in individuals with chronic conditions such as T2DM had not been extensively characterized.

Given the current gaps in evidence regarding the effects of weight loss on SCFAs in individuals with diabetes – and whether acetate is influenced by glucose levels or calorie restriction – this study aims to investigate the relationship between plasma acetate, body weight and glucose levels in obese individuals with diabetes who are undergoing a low-calorie dietary intervention over a 12 -month period.

STUDY HYPOTHESIS.

Our hypothesis claims that plasma acetate correlates positively with plasma glucose and increases during gradual weight loss induced by low caloric diet in obese patients with diabetes.

STUDY OBJECTIVES

1. To investigate the association between plasma acetate concentrations and changes in body weight in obese individuals with diabetes following a 12-month period of caloric restriction.
2. To investigate the association between plasma acetate concentrations and changes in glycaemic outcomes in obese individuals with diabetes following a 12-month period of caloric restriction.

METHODOLGY

Study Design.

Type of the study*:* The study was a prospective, parallel group, non-blind randomised controlled trial (Brown et al., 2020). In this report, we focused on a cross-sectional sample from the LCDDM study who had plasma acetate and glucose reports available.

Study Sites: The original study was carried out in two main hospitals in London: Imperial College Healthcare National Health Service (NHS) and Guy's and St. Thomas's NHS Foundation Trust.

Duration of the study: The study was carried out over 12 months.

Study Participants.

Inclusion criteria: Male and female adults aged 18-70 years diagnosed with T2DM who have been on insulin therapy for less than 10 years - or longer if their fasting C-peptide levels exceed 600pmol/L, with BMI greater than 30 kg/m^2.

Exclusion criteria: Key exclusion criteria included being on Insulin for >10 years with a fasting circulating C-peptide of less than 600pmol/L, or type 1 diabetes or with micro or macrovascular complications, an estimated glomerular filtration rate of <30 $ml/min/1.73m^2$, pregnancy or breastfeeding, being on a weight loss programme, using weight control medications 3 months prior to the study, had eating disorders or had any chronic debilitating conditions.

Study recruitment: participants were recruited via GP clinics and DM clinics at secondary care hospitals. Eligible participants received an invitation pack that included an invitation letter, participant information sheet and reply slip directed to the research team.

Study Intervention.

Randomisation: randomisation to either the intervention or control group at a 1:1 ratio was performed using an online software tool (sealedenvelope.com). Participants were not blinded due to the nature of the intervention. Participants were randomised at baseline visit (visit 2) to receive either total dietary replacement (TDR) using the Cambridge Weight Plan, Northants, UK, or conventional/standardized dietary care. Both groups were seen

by the same specialist dietitian monthly for the first 6 months, along with telephone consultations between visits. Participants also received behavioural support to help lifestyle adherence and maintenance and were encouraged to undertake moderate exercise (Brown et al., 2020).

Dietary Intervention: Participants received a total diet replacement (TDR) using the Cambridge Weigh Plan (CWP), providing approximately 800 kcal /day during the initial 6 weeks. This was followed by additional 6 weeks of CWP in combination with gradual, structured food reintroduction to increase the total energy intake to ~1000 kcal/day. Caloric intake was then increased to 1,400-1,500 kcal/day for another 6 weeks. From week 24 until the end of the study period, participants entered a prescribed energy deficit of 600 kcal/day for weight maintenance.

During the first 12 weeks, all meals were replaced with four formula LED products per day, providing 800-820 kcal/day (57% carbohydrates, 14% fat, 26% protein and 3% fibre). Participants were also instructed to consume at least 2.25 L of energy-free beverages daily. Additional fibre supplementation was allowed if necessary to alleviate constipation, a common side effect associated with TDR (Brown et al., 2020).

Standardised Diet Care

Participants received a standardized weight management programme consisting of a 600-kcal/day energy for 12 months. The prescribed caloric intake was based on total energy expenditure which was estimated according to each participant's basal metabolic rate.

Study Visits.

After the screening visit (V1), participants attended a baseline visit (V2), which was followed by nine visits at 1 week (V3), 4 weeks (V4), 8 weeks (V 5), 12 weeks (V 6), 16 weeks (V7), 20 weeks (V8), 24 weeks (V9), 36 weeks (V10) and 48 weeks (V11). Anthropometric measurements – including body weight (BW), body mass index (BMI) and body composition – were recorded at V2, V6, V9 and V11. Blood samples were collected

at these visits to assess fasting glucose, insulin, HbA1c%, urea & electrolytes, liver function and lipid profile. Urine samples were also collected during these visits.

As well as in-person assessments, participants received regular phone calls throughout the study period to monitor their physical health, assess adherence to the dietary intervention and identify any compliance issues or adverse events (Brown et al., 2020).

Measurements and Data Collection.

Anthropometric measurements, including body weight, height, body mass index (BMI) and waist circumference (WC) were collected. Body composition was measured using Bioelectrical Impendence (BID). Participants were also asked to complete a 3-day food diary and perform a 3-day self-monitoring of blood glucose. They were also instructed to wear a 7-day accelerometer to monitor physical activity and sleep patterns at V2, V9 and V11 (Brown et al., 2020).

Method for measuring plasma glucose and insulin.

Mixed meal tolerance tests (MMTT) were conducted at baseline, 3 months and 12 months (V2, V6 and V11), following overnight fasting. Fasting and postprandial concentrations of glucose, insulin and GLP-1 were measured at V2, V9 and V11.

Method for monitoring weight loss.

Body weight and waist circumferences were measured at each study visit. Body composition was also assessed at each visit using bioelectrical impedance analysis (BIA) (Brown et al., 2020).

Method for measuring serum acetate.

After an overnight fast, blood samples were collected during baseline (V2), 12 weeks (V6), 24 weeks (V9) and 48 weeks (V11). After collection, samples were immediately centrifuged at 2500 RCF for 10-15 minutes at 4°C to separate plasma. The plasma was

then aspirated using a pipette, transferred into labelled aliquots, and stored at -80 °C until analysed.

Before the analysis, plasma samples were thawed and vortexed to remove any precipitate. Plasma samples were then centrifuged at 12,000 g for 5 minutes at 4°C. Subsequently, 300µLof serum was mixed with 300µL of 0.075M NaH_2PO_4 buffer (pH 7.4) containing 0.8mM of the internal reference standard Trimethylsilyl propionic acid (TSP) and 3.1mM NaN_3.

Serum samples were analysed using ^{1}H-NMR (Nuclear Magnetic Resonance Spectroscopy), based on the standard one-dimensional pulse sequence described above and Carr-Purcell-Meiboom-Gill (CPMG) one-dimensional pulse sequences. CPMG was used to attenuate broad interfering peaks from the lipid and proteins present in serum. The CPMG pulse sequence took the form of RD-90°-(t-180°-t) n-ACQ. Acquisition parameters were set using the same settings as the standard 1D pulse sequence, with the spin-echo delay (9t) set at 0.3ms and 128 loops (n) performed. Continuous wave irradiation was applied at the water resonance frequency during the RD. Acetate was identified and quantified based on its chemical characteristics, shift and peak area, relative to the internal standard. Spectral processing and metabolite quantification were performed using validated software (Dona et al., 2014).

Figure 3.3: Schematic diagram of LCDDM study.

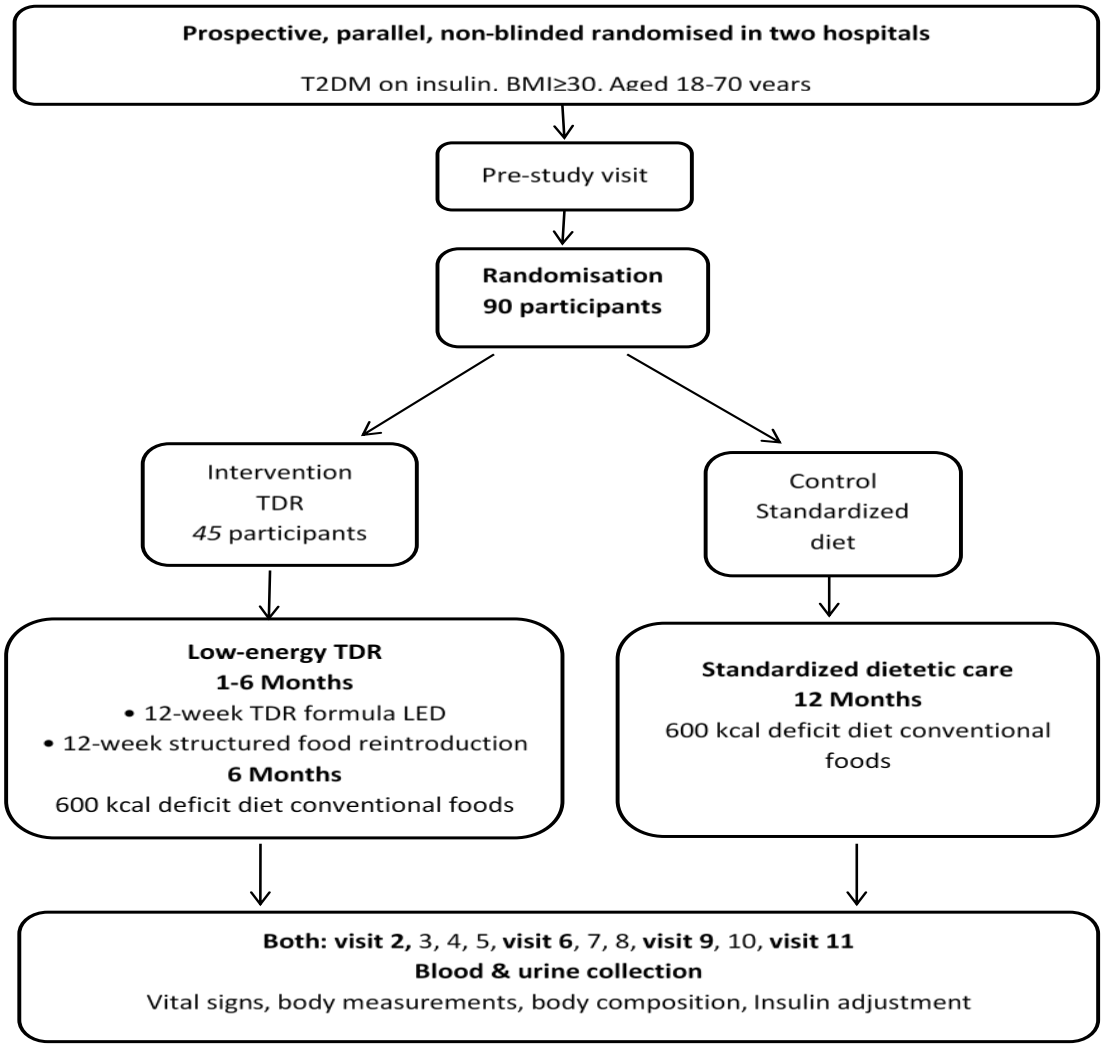


Figure 3.3: Schematic diagram of LCCDM study. Abbreviations: T2DM, type 2 diabetes mellitus; TDR, total diet replacement; LED, low energy diet; Visits 2, 6, 9, and 11 are study site visits.

Statistical Analysis.

Cross sectional data from the LCDDM study were used to examine the relationship between plasma acetate levels and body weight, body mass index (BMI), plasma glucose, insulin, HbA1C, insulin resistance and sensitivity indices. The sample size was determined according to the availability of participants with plasma acetate data, so no prior power calculation was calculated. A total of 51 participants were included in the analysis: 26 in the intervention arm (LCD) and 25 in the control arm (SD). Data distribution was assessed for normally using Q-Q plots and histograms, confirming a normal distribution. Results are presented as means ± standard deviation (SD). For each participant, mean values of variables were calculated as an average across four study visits.

Pearson correlation coefficients (r) were used to explore associations between plasma acetate and body weight and the metabolic variables of interest. Initial analysis did not adjust for potential confounding factors such as age and gender. However, for variables showing strong correlations with plasma acetate, further analyses were conducted using linear regression models adjusted for age and gender to assess the strength and proportion of variance in the dependent variables explained by acetate. Scatter plots were used to visualize the relationships between plasma acetate, glucose and body weight. Where appropriate, differences between study arms were assessed using independent sample t-tests. Statistical significance was defined as $p < 0.05$. All analyses were conducted using SPSS version 27.

RESULTS.

Baseline subjects' characteristics *.*

This sub-analysis was conducted using pre-existing data from the LCDDM study. The sample size was determined according to the availability of both plasma and acetate level results, so no power calculation was performed during the study design phase.

A total of 51 obese participants with T2DM were included and randomised into two groups: 26 in the intervention arm (receiving a low-calorie diet) and 25 in the control arm (receiving a standardized diabetic diet).

The intervention group comprised 18 women and 8 men, while the control group comprised 15 women and 10 men. The mean age of participants was 57.69 years (SD 9.6) in the intervention arm, and 58.76(SD 6.7) in the control arm. The mean body mass index (BMI) was 35.18 kg.m2 (SD 3.7) and 35.66 kg/m2 (SD 4.1) in the intervention arm and control arm respectively. The average duration of diabetes was 15.8 years (SD 7.4) in the intervention arm and 14.4 years (SD 6.0) in the control arm. Baseline fasting blood levels were 9.2 mmol/L (SD 2.7) in the intervention group and 10.06 mmol/L (SD 2.8) in the control group. Additional demographic and clinical characteristics are presented in Table 3.1.

At 12 months, participants in the intervention arm showed a greater reduction in body weight compared to those in the control arm, with a mean change of -9.41 kg versus -4.98, respectively. The between group difference was statistically significant (mean difference -4.42 kg, p=0.01). Significant differences between the groups were also observed in BMI and fasting insulin levels. A detailed comparison of pre and post - intervention measures between the two groups is shown in Table 3. 2.

Table 3.1: Baseline characteristics of the subjects at baseline visit.

	Intervention (n=26)	**Control (n=25)**
Age(year) at baseline	57.69(9.6)	58.76(6.7)
Gender (%)		
Male	11(42.3)	7(28)
Female	15(57.7)	18(72)
Weight (kg)	97.06(13.7)	96.68(12.9)
Body Mass index (kg/m^2)	35.18(3.7)	35.67(4.1)
Duration of diabetes(year)	15.8(7.4)	14.2(6.0)
Duration of insulin Rx(year)	5.08(3.4)	5.49(3.9)
Other oral Rx(n)	2.58(1.0)	2.68(0.9)
Fasting glucose (mmol/L)	9.20(2.72)	10.08(2.8)
HbA1C (mmol/mol)	70 (17.68)	75.08(17.5)
HbA1C %	8.5(1.62)	9.03(1.75)
Fasting insulin (pmol/L)	297.94(204.3)	279.02(199.4)
HOMA-IR	20.24(16.5)	19.14(13.85)
QUICKI	0.26(0.18)	0.27(0.210)

Plasma acetate(mmol/L)	0.022(0.0168)	0.0196(0.0171)

Table 3.1. Data presented as mean (SD) at baseline visit except gender as number (%), HbA1C, glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; QUICKI, Quantitative Insulin Sensitivity Index.

Table 3.2: Characteristics of participants before and after low calorie diet (LCD) intervention.

Study arm	**Intervention**			**Control**			
Variables	**Baseline** *(n=26)*	**End visit** *(n=26)*	**MC at 12 months**	**Baseline** *(n=25)*	**End visit** *(n=25)*	**MC at 12 months**	**Mean arm Diff**
P acetate (mmol/L)	0.02 (0.02)	0.04 (0.03)	0.02	0.02 (0.02)	0.03 (0.02)	0.01	0.01
Body wt. (kg)	97.06 (13.7)	87.65 (12.3)	-9.41	96.68 (12.9)	91.70 (15.7)	-4.98*	-4.42*
BMI (kg/m^2)	35.18 (3.7)	31.82 (3.7)	-3.36	35.67 (4.1)	33.89 (5.5)	-1.78	-1.58*
F glucose (mmol/L)	9.20 (2.7)	8.55 (2.92)	-0.65	10.08 (2.8)	11.12 (4.1)	1.04	-1.69
HbA1C	70.00 (17.7)	63.62 (17.7)	-6.38	75.08 (17.5)	74.83 (23.4)	-0.25	-6.13
HbA1C %	8.55 (1.6)	7.96 (1.6)	-0.59	9.03 (17.5)	9.00 (2.1)	-0.03	-0.56
F insulin (pmol/L)	297.94 (204.3)	224.39 (275.3)	-73.55	279.02 (199.4)	282.04 (311.0)	3.02	-76.57*
HOMA-IR	20.24 (16.6)	15.12 (20.5)	-5.12	20.85 (22.26)	19.14 (13.85)	-1.71	-3.41*
QUICKI	0.26 (0.02)	0.28 (0.03)	0.02	0.26 (0.02)	0.27 (0.03)	0.01	0.01

Table 3.2. Data presented as mean ± (SD). P, Plasma; F, fasting; wt., weight; HbA1C; glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin; MC, mean change; *P<0.05 (2 tailed).

Plasma acetate level at study visits.

Plasma acetate levels are presented as means ± standard deviation mmol/L (SD). At baseline (V2), the mean plasma acetate levels were comparable between the study arms: 0.0196 ± 0.017 mmol/L in the control group and 0.0215 ± 0.017 mmol/L in the intervention group (LCD).

Over the course of the study, plasma acetate levels showed a progressive increase from baseline V2 to V11 in both groups. However, greater fluctuations in plasma acetate levels were observed in the LCD group compared to the control. Notably, a drop in plasma acetate levels was observed at week 24 (V9) in both groups, with mean levels of 0.0220 ± 0.018 mmol/L in the control group and 0.02241±0.016 in the LCD group.

At 12 months, the mean change in plasma acetate was .0125 ± 0.001 mmol/L in the control group and 0.0173 ± 0.012 mmol/L in the LCD group, resulting in a non- significant mean difference of 0.0048 mmol/L (4.8 µmol/L; p=0.293). Detailed values of plasma acetate levels across all study visits and the corresponding between-group differences at 12 months are shown in Table 3.3**.**

We also explored potential effects of gender and age on plasma acetate levels. The analysis revealed no significant effect for either factor. The mean plasma acetate was 0.028 ± 0.009 mmol/L in males and 0.026 ± 0.006 mmol/L in females, with a mean difference of 0.001 mmol/L (p = 0.830).

Table 3.3: Plasma acetate values at study visits in control and intervention arms

	Control	**Intervention**	**Mean Diff.**	**P-value**
Study visits	Plasma Acetate (mmol/L)	Plasma Acetate (mmol/L)		
Visit 2	0.0196±0.017	0.0215±0.017	0.002	0.69
Visit 6	0.0268±0.0154	0.0320±0.027	0.005	0.53
Visit 9	0.0220±0.018	0.0224±0.016	0.000	0.92
Visit 11	0.0321±0.016	0.0388±0.029	0.007	0.29
MC at 12m	**.0125±0.001**	**0.0173±0.012**	**0048±.011**	**0.293**

Table 3.3. Data presented as mean ± SD. The mean change was calculated at 95% confidence interval. V2, baseline; V6, 12 weeks; V9, 24 weeks; V11, 48 weeks; MC, mean change

The relationship between plasma acetate level and glycaemic variables.

The relationship between plasma acetate levels and glycaemic variables – including fasting glucose, fasting insulin, HbA1c%, HOMA-IR and QUICKI – was assessed across both study arms and at each study visit. At baseline (V2), no significant correlations were observed between plasma acetate and any glycaemic parameter in either arm.

By the end of the study (V11), both groups demonstrated trends towards non-significant negative correlations between plasma acetate and several glycaemia markers. In the LCD arm, plasma acetate was negatively correlated with fasting plasma glucose (R = -0.215, p=0.24), fasting insulin (R = -0.244, p=0.23) and HOMA -IR (R = -0.246, p-0.23).

Similarly, in the control arm at V11, plasma acetate showed negative correlations with fasting glucose (R = -0.358, p=0.08), HBA1C (R = -0.297, p=0.24) and HBA1c% (R = -0.299, p=0.24).

Conversely, a trend toward a positive correlation was only observed in the LCD arm between plasma acetate and the insulin sensitivity index QUICKI (R = 0.365, P=0.07).

A summary of Pearson correlation coefficients between plasma acetate and glycaemic variables at each study visit in both arms is provided in Table 3.4.

The relationship between plasma acetate and body weight.

One notable finding was a significant negative correlation between plasma acetate levels and body mass index (BMI) in the control group at V9 (week 24), with a correlation coefficient of R = -.0.502, p=0.01. A weaker and non-significant correlation was also observed between plasma acetate and body weight (R = -0.308, p = 0.1). These findings were explored further through regression analysis to better understand the relationship between plasma acetate and anthropometric outcomes.

In the LCD group, a negative correlation between plasma acetate and body weight was observed at baseline (R = -0.369, p=0.04), along with a non-significant correlation with body mass index (R = -0.322, p=0.1). These results may indicate a potential inverse association between plasma acetate and measures of adiposity in response to dietary intervention.

Further correlations between plasma acetate, body weight and BMI across study visits are summarised in Table 3.4.

A correlation matrix of all variables at baseline and end of the study visits in the LCD group is summarised in Tables 3.5 and 3.6.

A correlation matrix of all variables at baseline and end of the study visits in the control group is summarised in Tables 3.7 and 3.8.

Table 3.4: The relationship between plasma acetate and variables of interest across study visits in the control and intervention arms

	Variables of interests							
	Bodyweight (kg)	**BMI (kg/m2)**	**FBG (mmol)**	**HbA1C**	**HbA1C%**	**FI (pmol/L)**	**HOMA-IR**	**QUICKI**
				INTERVENTION				
AC-V2	-.322	-.369	-.015	.035	.042	-.024	-.019	-.014
AC-V6	.164	.094	.037	-.052	-.052	-.028	-.035	-.064
AC-V9	-.064	.269	-.279	.098	.088	-.201	-.239	.121
AC-V11	-.182	-.182	-.215	-.133	-.136	-.244	-.246	.356
				CONTROL				
AC-V2	-.084	.151	.193	.257	.263	-.331	-.227	.137
AC-V6	.164	-.019	-.029	.128	.128	.055	.084	-.016
AC-V9	-.308	.502*	-.107	-.327	-.324	-.022	-.038	.045
AC-V11	.130	-.115	-.358	-.297	-.299	.069	-.040	.009

Table 3.4. Data presented as R Pearson's correlation. Abbreviations. LCD, Low Caloric Diet; SDD, standardized Diabetic Diet; AC, acetate; V, visit; BMI, Body Mass Index; FBG, Fasting Blood Glucose; FI, Fasting Insulin; HbA1C, Glycated Haemoglobin; HOMA-IR, Homeostatic model assessment; QUICKI, Quantitative insulin sensitivity check index, *P <0.05(2-tailed).

Table 3.5: Descriptive characteristics and correlation matrix of all variables at the baseline visit in the LCD arm (intervention).

Variables	Mean	StD	1	2	3	4	5	6	7	8
1. P acetate	0.02	0.02								
2. Body wt.	97.06	13.70	-0.322							
3. BMI	35.18	3.71	-0.369	.650**						
4. F glucose	9.20	2.72	-0.015	0.159	0.118					
5. HbA1C	70.00	17.68	0.035	-0.195	0.009	.540**				
6. HbA1C %	8.55	1.62	0.042	-0.196	0.004	.545**	1.000**			
7. F insulin	297.94	204.34	-0.024	-0.096	-0.163	-0.031	0.147	0.145		
8. HOMA-IR	20.24	16.56	-0.019	-0.078	-0.108	0.309	0.258	0.258	.913**	
9. QUICKI	0.26	0.02	-0.014	0.044	0.079	-.399*	-.392*	-.395*	-.812**	-.863**

Table 3.5. Abbreviations: StD, Standard Deviation; P, plasma; wt, weight; F, fasting; BMI, Body Mass Index; HbA1C, Glycated Haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin. N = 26, * P< 0.05 (2-tailed), ** P< 0.01 (2-tailed).

Table 3.6: Descriptive characteristics and correlation matrix of all variables at the end visit in the LCD arm (intervention).

Variables	Mean	StD.	1	2	3	4	5	6	7	8
1, P acetate	0.04	0.03								
2. Body wt.	87.65	12.28	-0.182							
3. BMI	31.82	3.72	-0.182	.663**						
4. F glucose	8.55	2.92	-0.215	0.232	0.320					
5. HbA1C	63.62	17.70	-0.133	0.070	0.334	.630**				
6. HbA1C %	7.96	1.62	-0.136	0.069	0.337	.630**	1.000**			
7. F insulin	224.39	275.29	-0.244	-0.197	0.010	0.159	0.371	0.370		
8. HOMA_IR	15.12	20.50	-0.246	-0.150	0.047	0.303	.416*	.416*	.982**	
9. QUICKI	0.28	0.03	0.356	-0.054	-0.342	-.578**	-.590**	-.591**	-.768**	-.806**

Table 3.6. Abbreviations: StD, Standard Deviation; P, Plasma; wt, weight in kg; F, fasting; BMI, Body Mass Index in kg/m^2; HbA1C, Glycated Haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin. N = 26* P< 0.05 (2-tailed).** P< 0.01 (2-tailed).

Table 3.7: Descriptive characteristics and correlation matrix of all variables at the baseline in the control arm.

Variables	Mean	Std. Deviation	1	2	3	4	5	6	7	8
1. P acetate	0.02	0.02								
2. Body wt.	96.68	12.94	-0.084							
3. BMI	35.67	4.13	0.151	.643**						
4. F glucose	10.08	2.83	0.193	0.216	0.153					
5. HbA1C	75.08	17.53	0.257	0.254	0.359	.443*				
6. HbA1C %	9.03	1.59	0.263	0.256	0.363	.446*	1.000**			
7. F insulin	279.02	199.41	-0.331	0.111	-0.036	0.232	0.087	0.084		
8. HOMA_IR	20.85	22.26	-0.227	0.149	-0.008	.561**	0.231	0.229	.915**	
9. QUICKI	0.26	0.02	0.137	-0.379	-0.189	-0.344	-0.152	-0.151	-.877**	-.816**

Table 3.7. Abbreviations: STD., Standard Deviation; P, Plasma; F, Fasting; wt, weight in kg; BMI, Body Mass Index in kg/m^2; HbA1C, Glycated Haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin. N = 26, * P< 0.05 (2-tailed), ** P< 0.01 (2-tailed).

Table 3.8: Descriptive characteristics and correlation matrix of all variables at the end visit in the control arm.

Variables	Mean	SD	1	2	3	4	5	6	7	8
1. P acetate	0.03	0.02								
2. Body wt.	91.70	15.65	0.130							
3. BMI	33.89	5.51	-0.115	.786**						
4. F glucose	11.12	4.08	-0.358	0.265	0.343					
5. HbA1C	74.83	23.37	-0.297	.420*	.592**	.717**				
6. HbA1C %	9.00	2.14	-0.299	.420*	.589**	.718**	1.000**			
7. F insulin	282.04	311.04	0.069	-0.048	-0.062	-0.265	-0.171	-0.175		
8. HOMA_IR	20.85	16.07	-0.040	0.244	0.166	0.250	0.168	0.164	.792**	
9. QUICKI	0.27	0.03	0.009	-.473*	-0.383	-0.315	-0.320	-0.316	-.642**	-.906**

Table 3.8. Abbreviations: SD, Standard Deviation; P, Plasma; F, Fasting; wt., weight in kg; BMI, Body Mass Index in kg/m^2; HbA1C, Glycated Haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin. N = 25, * P< 0.05 (2-tailed), ** P< 0.01 (2-tailed).

Predictors of plasma acetate change.

Following the significant correlation between plasma acetate and BMI observed in the control group at V9 (R = -0.502, p=0.01), a multiple regression analysis was conducted to further examine whether BMI or body weight could serve as predictors of plasma acetate change.

The regression was performed in two steps. In the first step, age and gender were entered as control variables. These variables collectively accounted for 9.4% of the variance in plasma acetate levels (R^2 = 0.94), although this model did not reach statistical significance. In the second step, BMI was added to the model as a predictor. The inclusion of BMI significantly improved the model, increasing the explained variance to 36.8% (R^2 = 0.368). The change in R^2 (ΔR^2 = 0.274) was statistically significant (p=0.01), indicating that BMI alone accounted for an additional 27.4% of the variance in plasma acetate levels, independent of age and gender.

The regression analysis produced a negative unstandardized Beta coefficient for BMI (B= -0.002, p= 0.01), suggesting that higher BMI is associated with lower plasma acetate levels, even after controlling for age and gender.

Details of the regression analysis are presented in Table 3.9.

Table 3.9: Results of multiple regression analysis for plasma acetate in the control arm.

Results of Multiple Regression Analysis For Plasma Acetate in the Control arm			
	Plasma Acetate at 9-months visit in Control arm		
Predictors	B	R^2	ΔR^2
Step 1			
Control variables[b]		0.094	
Step 2			
Body mass index	-.002*	0.368	0.274*

Table 3.9. Abbreviations. [a]n=25, [b] control (age & gender), B, Unstandardized Beta*
p= 0.01

Predictors of plasma acetate change.

The correlation matrix in Table 3.10 provides insights into the relationship between age, gender, plasma acetate, body wight and BMI. A weak negative correlation was observed between age and gender (r = -0.198), indicating a weak association. Plasma acetate showed weak negative correlations with age (r= -0.044) and gender (r= -0.290), although these were not statistically significant. Notably, a significant negative correlation was found between BMI and plasma acetate levels. Body weight also showed a negative correlation with plasma acetate (r = -0.303).

Table 3.10: Correlation matrix between plasma acetate and control variables at visit 9 in the control arm.

Variables	1	2	3	4
1. Age in years	1			
2. Gender	-0.198			
3. Plasma acetate	-0.044	-0.290		
4. Body weight	-.463*	-0.333	-0.303	
5. Body mass index	-.513**	0.178	-.428*	.757**

Table 3.10. Abbreviations. n= 25; *P< 0.05 level (2-tailed); **P< 0.01 level (2-tailed).

Summary of the results.

In summary, the study group showed a gradual though non-significant increase in plasma acetate concentration from baseline to the end of the study. The mean difference between arms was also non-significant (MD= 0.0048, p=0.293). A weak negative correlation was observed between plasma acetate and plasma glucose in both arms at the final visit in the intervention group (r = -0.215, p =0.2) and in the control group (r = -0.358, p=07). In

the LCD group, plasma acetate showed a positive correlation with QUICKI (r = 0.356, p=0.07).

A statistically significant negative correlation was identified between plasma acetate and BMI in the control arm at V9 (r = -0.502, p<0.01). Further analysis indicated that the change in R^2 (ΔR^2 = 0.274) was statistically significant (p = 0.01), suggesting that BMI independently accounted for 27.4% of the variance in plasma acetate levels after adjusting for age and gender. These findings suggest that BMI may serve as a significant predictor of changes in plasma acetate levels.

DISCUSSION

This sub-analysis is based on preexisting study data from the LCDDM study, which investigated the effects of dietary interventions on metabolic outcomes in obese individuals with T2DM (Brown et al., 2020). The current analysis aimed to examine the relationships between plasma acetate and plasma glucose and between plasma acetate and changes in body weight in participants undergoing a low-calorie diet compared to those following a standardized diet.

As described above, the study enrolled a total of 51 obese participants with type 2 diabetes mellitus who were randomly assigned into two groups: 26 participants in the intervention arm (receiving a low-calorie diet) and 25 in the control arm (receiving standardized diabetic diet).

Plasma Acetate and Body Weight.

At 12 months, the mean weight loss in the intervention group was -9.41 kg (SD=4.7), while the control group showed a mean loss of -4.98 (SD =5.5), yielding a statistically significant intervention effect of -4.42 kg. Accompanying this weight loss, a gradual increase in plasma acetate levels was observed in both groups by the end of the study, with a slightly larger increase in the LCD group.

These findings align with previous research Todesco et al. (1993), reported higher acetate concentrations in obese individuals with diabetes compared to normoglycemic and non-

obese participants (Todesco et al., 1993). Meanwhile, Gonzalez Herandez et al. (2020) found increased plasma acetate levels in obese but metabolically healthy participants following an LCD (González Hernández et al., 2020). Rad et al. (2023), observed significantly elevated faecal acetate in LCD versus normal groups, suggesting that diet composition influences SCFA levels ($P<0.01$) (Rad et al., 2023) .

However, our results were inconsistent with Louis et al. (2016), who reported lower SCFA concentrations and reduced gut microbiota diversity in obese individuals on a very low-calorie diet (VLCD) (Louis et al., 2016). This discrepancy may be due to differences in diet types, study population and whether SCFAs were measured in faeces or plasma. It is believed that plasma SCFAs, particularly in metabolically compromised individuals, are more closely linked to systemic metabolic outcomes (Müller et al., 2019), while faecal SCFA levels may not reflect true colonic production since the majority of SCFAs are absorbed within the colon and only 5-10% are excreted in the faeces.

A systematic review also included 9 studies to examine the effect of weight loss interventions on SCFAs concentration. Three studies out of the included studies reported a decrease in faecal SCFAs with weight loss intervention (Salonen et al., 2014; Tay et al., 2008; Duncan et al., 2008). However, most of these studies examined high or low protein dietary restriction programmes, which are less likely to be fermented in the colon to yield SCFA.

Here, we did not show faecal SCFAs data, nor did we compare it with previous reports. Additionally, our subject profile is different from the above reported studies. We studied obese and diabetic subjects who had been on insulin therapy for years. Dysbiosis and a lower abundance of gut microbiota – specifically, a reduction in the butyrate producing species and ultimately impaired SCFA production and metabolism – are reported in diabetic individuals (Crudele et al., 2023). It is also worth querying whether faecal acetate is a good representative of circulating acetate, as differences in acetate production and absorption are reported between insulin sensitive and hyper insulinemic individuals (Fernandes et al., 2014). Circulating SCFA is linked more strongly to metabolic health, which is why the measurement of circulating SCFAs is especially recommended in metabolically compromised individuals (Müller et al., 2019).

Interestingly, our analysis found a significant negative correlation between plasma acetate and BMI at V9 (24 weeks) (R = -0.502, p=0.001). Multiple regression analysis confirmed BMI as a significant predictor of plasma acetate (R^2= 0.274, p= 0.01), even after adjusting for age and gender. These results suggest that as BMI increases, plasma acetate decreases, potentially reflecting dysbiosis or reduced SCFA production capacity as individuals gain weight.

Looking more closely at our plasma acetate levels, we noticed a slight reduction in plasma acetate in both arms at V9 (week 24) compared to the baseline and V6 (W12) (~0.022 mM decrease). This may reflect decreased SCFA production during active weight loss, which is consistent with theories of reduced energy harvesting efficiency in leaner phenotypes (Rahat-Rozenbloom et al., 2014; Schwiertz et al., 2010). Towards V11 (week 48), acetate levels increased again (LCD: +0.04 mM; Control: +0.03 mM), possibly due to dietary reintroduction and a partial weight re-gain reported in the LCDDM study. These findings are consistent with Sowah et. al. (2020), who reported stable or increasing plasma acetate after an initial decline during weight loss (Sowah et al., 2020). Dao et al. (2016) also noted reduced serum acetate levels during calorie restriction but sustained high levels in individuals with abundant *Akkermansia muciniphila,* a gut microbe linked to favourable metabolic profiles (Dao et al., 2016; York, 2021).

Methodological differences may also influence the findings. Previous studies may have measured total or bound acetate, while our study focused on free plasma acetate. Additionally, age and diabetic status – many participants were on insulin therapy – might also contribute to differences in SCFA dynamics, including altered microbial composition, impaired SCFA metabolism or increased endogenous acetate production. Age-related changes in acetate turnover and the impact of insulin resistance on SCFA absorption further complicate the interpretation (Skutches et al., 1979; Fermandes et al., 2014)

Taken together, our results underscore the complexity of interpreting SCFA profiles, especially in metabolically compromised individuals. Plasma acetate appears more closely tied to metabolic health than faecal levels and may serve as a useful biomarker in dietary intervention studies targeting obesity and diabetes. Further studies mapping gut microbiota and SCFA kinetics are needed to clarify these associations (Vogt & Wolever,

2003). Meanwhile, weight reduction and prolonged periods of caloric restriction in diabetic subjects may enhance fat oxidation and ketogenesis. Increasing the Acetyl-CoA pool in the body and ultimately acetate and other markers of ketosis or ketone bodies would increase acetoacetate in the human body, as was reported in some of studies (González Hernández et al., 2020).

Weight reduction may therefore have a paradoxical effect on gut microbiota composition and SCFA production. Further long-term studies are needed to explore this effect in metabolically healthy and unhealthy individuals.

Plasma Acetate And Glycaemic Markers.

At baseline, no significant associations were observed between plasma acetate and fasting glucose, insulin, HOMA-IR or QUICKI. However, weak negative correlations were identified between changes in plasma acetate and changes in fasting glucose (R= -.279 and R-.215, p=0.2), insulin (R= -.201 and R = -.244) and HOMA-IR (R = -.239 and R= -.246) at 24 and 48 weeks, respectively.

These findings are consistent with Layden et al. (2012), who also reported a weak negative correlation between plasma acetate and fasting glucose (R= -0.21) but found no such relationship with propionate (R =0.004) or butyrate (R=0 .22). Meanwhile, Lyden et al. (2012) observed stronger statistically significant negative correlations between serum acetate and fasting insulin (R = -.56, p=0.02), as well as 2-hour OGTT glucose levels (R =-0.43) (Layden et al., 2012). These stronger associations were not replicated in our study.

Gonzalez Hernandez et al. (2020) reported a reduction in fasting insulin and HOMA -IR index following a low-calorie diet (LCD), particularly among female participants (stdβ= 0.130, p=0.033 and stdβ= 0.119, p=0.051, respectively), while no significant changes were observed in men (stdβ= -0.072, p=0.310 and stdβ= -0.066, p=0.359). These results indicate potential gender-specific differences in acetate metabolism – a trend that was not replicated in our analysis. Gonzalez Hernanez et al. (2020) found a slight positive association between changes in acetate and changes in HOMA-IR in women, but not in men (González Hernández et al., 2020). In contrast, our study consistently showed a

negative correlation between plasma acetate and metabolic variables without stratification by gender.

Our regression analysis indicated that age and gender together accounted for 9.4% of the variance in plasma acetate levels. The correlation between acetate and gender (R= -0.29) was notably stronger than that between acetate and age (R -0.044), which suggests that gender may play a larger role in influencing plasma acetate levels. Further investigation is needed to explore potential gender-specific effects in acetate metabolism.

In the original LCDDM study, the observed weight loss was predominantly due to fat mass reduction. Participants in the LCD group lost an average of 7.0 kg (SD 4.3), compared to 4.3 kg (SD 4.4) in the control group (Brown et al., 2020). This reduction improved lipolysis and hepatic ketogenesis, leading to increased fat oxidation and overproduction of acetyl-Co A – a precursor of acetate synthesis (Liu et al., 2018). This mechanism may help to explain the elevated plasma acetate levels observed in the LCD group. Our findings align with those of Layden et. al. (2012), who reported an inverse association between plasma acetate and visceral adipose tissue (VAT) in obese women, as measured by a computerized tomography scan and dual x-ray absorptiometry scans (Layden et al., 2012).

It is important to consider that acetate kinetics may be altered in individuals with hyper insulinemia. Previous studies have shown that hyperinsulinemia can impair acetate absorption, hepatic uptake and clearance, potentially altering endogenous and exogenous acetate production (Fernandes et al., 2014).

Interestingly, in the LCD group, we observed a positive correlation between plasma acetate and insulin sensitivity, as measured by QUICKI ($R = 0.356$, $p = 0.07$), which aligns with the results reported by Gonzalez Hernandez et al. (2020) using the Matsuda index (González Hernández et al., 2020). In contrast, Muller et al. (2019) reported a weak negative association between circulating acetate and peripheral insulin sensitivity in a subgroup of prediabetic individuals. However, in their overall cohort, neither circulating nor faecal acetate levels were significantly associated with insulin sensitivity or HOMA-IR (Müller et al., 2019).

Our cross-sectional analysis at the study endpoint revealed weak and non-significant negative correlations between plasma acetate and glucose levels in patients with diabetes (R= -0.215 in the intervention group and R= 0-.358 in the control group). We were unable to confirm earlier findings by Akanji et al. (1988; 1989), who reported positive associations between plasma acetate and both glucose and insulin. These discrepancies may be due to differences in the study populations, clinical phenotyping or metabolic profiles (Akanji et al., 1989; Akanji & Humphreys, 1988).

The positive association between increased plasma acetate and reduced glucose levels towards the end of the study could be explained by the successful weight and fat mass loss achieved by the study participants, which probably contributed to improved glucose levels. Another possible explanation might be related to the use of the antidiabetic drug metformin among the study participants.

Recent evidence suggests that metformin can alter the gut microbiota community composition in humans, indicating that part of its glucose-lowering effect may be mediated through modulation of the gut microbiota. Several studies have supported this hypothesis. A randomised, double blinded, placebo-controlled trial for T2DM participants who had received metformin treatment for two and four months, showed significant alterations in the relative abundance of bacterial species, mostly *Escherichia coli* and *Firmicutes gut microbiome* (Wu et al., 2017). In a subgroup analysis, metformin use for four months was also associated with an increased abundance of *Akkermansia muciniphila*, although this change was not significantly correlated with Hba1c levels. In contrast, other studies have shown that *A.Muciniphila* is positively associated with improved metabolic status in both mice (Plovier et al., 2017) and humans (Dao et al., 2016).

To further explore the functional relevance of these microbial shifts, Wu et al. (2017) transferred faecal microbiota from metformin-treated participants to germ free mice, which resulted in improved glucose tolerance compared to transfers from non-metformin users (Wu et al., 2017). Other studies have also reported that metformin takers have a higher abundance of butyrate-producing bacteria, which may contribute to favourable metabolic outcomes (Umirah et al., 2021).

Although our study did not include whole genome sequencing, it would be of great interest to investigate the faecal bacterial composition – particularly at the phylum level – and examine its association with glycaemia indices, given that most of our participants were metformin users (37 from the LCD group and 42 from the control group).

Summary

This study investigated the relationship between changes in circulating acetate, gradual weight loss and metabolic markers in obese adults with T2DM. Following a low-carbohydrate diet (LCD), a modest increase in plasma acetate levels was observed, but unlike previous studies, we did not find a positive association between plasma acetate and glucose concentrations in this population. Instead, a positive correlation emerged only with the insulin sensitivity index QUICKI. Notably, the most significant finding was a negative association between BMI and plasma acetate levels, indicating that BMI or changes in body weight may serve as a reliable predictor of changes in circulating acetate.

Future research should explore how weight loss – achieved through dietary interventions or bariatric surgery – affects gut microbiota composition and the production of short chain fatty acids in individuals with obesity and T2DM. Additional studies are also warranted to evaluate the associations between circulating SCFAs and a broader range of metabolic markers beyond glucose and insulin.

STRENGTHS

A major strength of our analysis is its randomised controlled trial (RCT) design, which allowed for a direct comparison of the effects of a low-carbohydrate diet (LCD) on body weight and metabolic markers relative to a standardized diabetic diet, despite relying only on cross-sectional plasma samples. Another strength is the use of nuclear magnetic resonance (NMR) spectroscopy to measure circulating plasma acetate, a method that has demonstrated strong correlations with metabolite concentrations obtained through liquid chromatography-mass spectrometry (LC-MS) (Lanza et al., 2010). The inclusion of

key metabolic markers – such as fasting glucose, fasting insulin, HbA1C, HOMA-IR and QUICKI – at each study visit also improves the robustness and interpretability of the study's findings.

LIMITATIONS

Our study has several limitations that should be acknowledged. Firstly, the lack of blinding represents a methodological constraint common in dietary intervention studies. Although double blinding would be ideal, it is difficult to implement in this type of behavioural and nutritional research. Secondly, the use of serum acetate as a representation of total circulating acetate remains debated, with uncertainty over whether peripheral or portal measurements more accurately reflect systemic acetate availability. Additionally, in individuals with diabetes, acetate may be produced endogenously from glucose and fatty acids. We did not measure blood ketone bodies, which could have served as an indirect marker of endogenous acetate production through alternative metabolic pathways.

A further limitation was the absence of data on acetate kinetics, particularly the extent to which colonic-derived acetate contributes to circulating plasma acetate levels. We also did not assess inflammatory biomarkers such as C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α), which are known to be associated with diabetes-related dysbiosis and could have provided additional insights into host-microbiota interactions. Meanwhile, we did not quantify the composition of gut microbiota in participants, nor did we examine its relationship with plasma acetate levels, despite existing evidence that diabetes is associated with alterations or shifts in gut microbial communities. Lastly, it is important to note that this analysis was conducted within the context of a larger study that was not specifically designed or powered to evaluate the relationships examined here, which may limit the generalizability and causal interpretation of our findings.

FUTURE RESEARCH

Understanding the relationship between gut microbiota-derived acetate, weight loss and metabolic regulation in diabetes remains an important yet underexplored area. Future studies should aim to include larger and more ethnically diverse populations in order to

improve the generalizability of findings. Investigating acetate kinetics – including its production, absorption, and utilisation – is essential to delineate the relative contributions of endogenous and exogenous sources to acetate metabolism in individuals with diabetes. In particular, the measurement of hepatic or portal vein acetate levels would provide more precise insights into circulating acetate dynamics. However, these methods are invasive and pose significant practical challenges. As a less invasive alternative, the concurrent analysis of faecal and plasma acetate levels may help to clarify the balance between colonic production and systemic absorption. Moreover, the detailed profiling of gut microbial communities, including the quantification and identification in these populations of specific bacterial taxa, is also crucial. Such analysis would advance our understanding of the complex interactions between gut microbiota, weight reduction and glucose homeostasis, and help us to explain the mechanisms driving observed metabolic changes.

CONCLUSION

This cross-sectional analysis did not identify a significant association between circulating acetate and glycaemic markers. However, a strong negative association was observed between plasma acetate and BMI, suggesting that BMI may be a reliable predictor of plasma acetate levels in obese individuals with T2DM. These findings underscore the need for further investigation into the complex relationship between obesity and SCFAs, which maybe bi-directional. Future work should also consider examining other SCFAs such as butyrate and propionate to gain a more comprehensive understanding of their associations with metabolic outcomes and their potential use as therapeutic targets in diabetes.

CREDIT Statement.

The data used in this chapter were taken from the LCDDM study, and the plasma sample analysis for acetate was carried out during the Covid 19 lockdown period by another colleague. I primarily conducted the statistical analysis used in this chapter. I extracted the data from the original LCDDM study data. I also undertook the data entry, cleaning

and statistical analysis of the study outcomes, as well as the interpretation of the results. I also prepared the tables and figures presented in this chapter.

CHAPTER 4:

IMPACT OF INULIN PROPIONATE ESTER ON WEIGHT GAIN PREVENTION AND GLYCAEMIA IN OVERWEIGHT YOUNG INDIVIDUALS: A RANDOMISED DOUBLE BLINDED CONTROLLED STUDY.

ABSTRACT

Introduction

Recent research evidence indicates that higher dietary fibre intake, which enhances colonic microbial fermentation and short-chain fatty acid (SCFA) production, is associated with lower body weight. These effects are thought to arise from SCFA's roles in regulating energy balance, glucose homeostasis and overall metabolic health through various physiological pathways. Despite national food policy recommendations to consume 30 g of dietary fibre per day, only 9% of adults meet this target. A first in-human study showed that delivering propionate (C3) directly to the colon using Inulin Propionate Ester (IPE) over six months prevented weight gain and reduced intra-abdominal fat in obese middle-aged adults; however, no significant differences were observed in postprandial glucose or insulin responses between the study groups. IPE is a novel food ingredient that can be incorporated into commonly consumed foods and may confer metabolic health benefits. This 12-month study aimed to determine whether increasing colonic propionate production via IPE supplementation prevents further weight gain and supports blood glucose regulation in overweight young adults.

Method

This multicentre, randomised, double-blind controlled trial recruited 270 overweight young adults (aged 20-40 years; BMI 25-30 kg/m^2) from two UK sites – London and Glasgow – between July 2019 and October 2021. Participants were randomly assigned to receive either 10 g of inulin propionate ester (IPE) as the intervention or inulin (IN) as the control for a duration of 12 months. Following randomisation, participants attended study visits at 2, 6 and 12 months. At each visit, anthropometric measurements (body weight and height), body composition, physical activity and lifestyle questionnaire data were collected. Fasting blood samples were also obtained to assess glucose, insulin and lipid profiles.

Results

Of the 270 participants, 226 completed the 12-month trial; 112 in the IPE group and 114 in the IN-control group. There were 42 withdrawals, and 61 participants discontinued the supplementation but remained in the study. By the end of the 12-month intervention there was no significant difference in baseline adjusted mean weight gain between the IPE and IN groups (mean difference:1.02 kg; 95% CI: -0.37 to 2.41; p=0.15; n=226). Fasting blood glucose increased by 0.11 mmol/L (95%CI: 0.01 to 0.21) in the IPE group. Overall, 63% of participants in the IPE group were compliant on more than 50% of the intervention days. No significant differences were observed between the groups in insulin resistance and sensitivity indices.

Conclusion

Inulin propionate ester had no significant effect on weight gain prevention or glycaemic indices in a young adult population, suggesting that young individuals may respond differently to such interventions compared to middle aged adults. These findings highlight the need for further research studies to explore age-related differences in metabolic responses and to guide the development of targeted obesity prevention strategies.

INTRODUCTION

Obesity is increasing at an alarming rate worldwide. It is becoming a determinant of quality of life due to its association with a wide range of chronic health problems such as type 2 diabetes mellitus (T2DM), hypertension, dyslipidaemia, rheumatoid arthritis, cancers and others. Obesity causes chronic low-grade inflammation, which is implicated in the pathogenesis of insulin resistance in key insulin sensitive tissues such as liver, skeletal muscle and adipose tissue. Obesity also causes the dysfunction of pancreatic β-cells which can contribute to the development of hyperglycaemia (Klein et al., 2022).

Gut Microbiota (GM) play a role in the modulation of glucose homeostasis and the pathogenesis of metabolic disease. Studies have shown that GM composition shifts away from butyrate producing profiles in individuals with prediabetes or T2DM compared with control participants (Tang & Li, 2021). Meanwhile, transferring GM from lean to obese individuals might improve insulin sensitivity (Canfora et al., 2015).

Clinical trials show that an increased intake of non-digestible carbohydrates (NDC) or dietary fibre is a potential nutritional strategy to modulate GM. The fermentation of NDC by GM produces short chain fatty acids (SCFAs). It is believed that these SCFAs have important metabolic functions that are crucial for gut health and host metabolism, and they may also improve energy and glucose homeostasis through several mechanisms (See Chapter 1).

Obesity/Overweight and insulin resistance risk in young adults

Changes in body weight and body fat distribution are associated with the risk of diabetes, as they are associated with insulin resistance (IR). In a US adult survey, the risk of diabetes increased 4.5% for every kilogram increase in body weight, and this association was consistent across age, sex and ethnicity (Ford et al. 1997). Meanwhile, The Health Professional Follow up Study examined the relationship between changes in body weight and body fat distribution (1986-1996) and the subsequent risk of diabetes (1996-2000) in 22,171 men. They found an increase of 7.3% in risk of diabetes with every kilogram increase in body weight, specifically abdominal fat, independent of weight gain (Koh-Banerjee et al., 2004). The study related 56% of the new diabetes cases in this cohort to

weight gains of 7 kg or more and 20% of the cases to waist circumference gain of more than 2.5 cm.

A review of 216 cohort studies showed a positive linear association between BMI and the risk of developing T2DM. They reported that a 1.72-fold increase in the relative risk of developing T2DM for every 5-unit increase in BMI, a 1.61-fold increase for every 10 cm increase in waist circumference and 1.63-fold increase for every 0.1 unit increase in waist-hip ratio (Jayedi et al., 2022).

Propionate Metabolism

Propionate is found naturally in food products like yogurt, milk, cheese and sodium/calcium propionate salt. It is also available as a food preservative (E280) because of its antifungal and antibacterial properties, but these propionic food-sources do not result in a significant amount of propionate in the human circulation in comparison to the primary natural source of propionate in humans. In the colon, propionate is produced mainly through the fermentation of polysaccharides, oligosaccharides, proteins and other substrates. However, undigested dietary fibre or complex carbohydrates also represent a major source of propionate production. Pyruvate is the principal metabolite of these fermentation reactions, and propionate is produced from pyruvate through two main pathways. Firstly, the succinate decarboxylation pathway, in which CO_2 is fixed to pyruvate from succinate, which is subsequently decarboxylated to propionate, and secondly, the Acrylate pathway, in which it is produced from acrylate with lactate as a precursor (Oliphant & Allen-Vercoe, 2019). Cummings et al. (1987) studied postpartum blood and intestinal samples, and reported that the highest concentration of propionate was in the right colon (25.3 mmol/kg) and that it decreases as it passes through out the colon (Cummings et al., 1987). Studies have also reported that propionate quantity may vary depending on the type of microbiota, the balance between production and absorption, the substrates available in the colon (quantity and type) and individual gut transient time. The majority of propionate produced is absorbed to the portal vein and taken from there to the hepatic vein, where 90% of propionate is metabolized in the liver and the rest transported into the peripheral circulation (den Besten et al., 2013).

Figure 4.1: Propionate metabolism in humans.

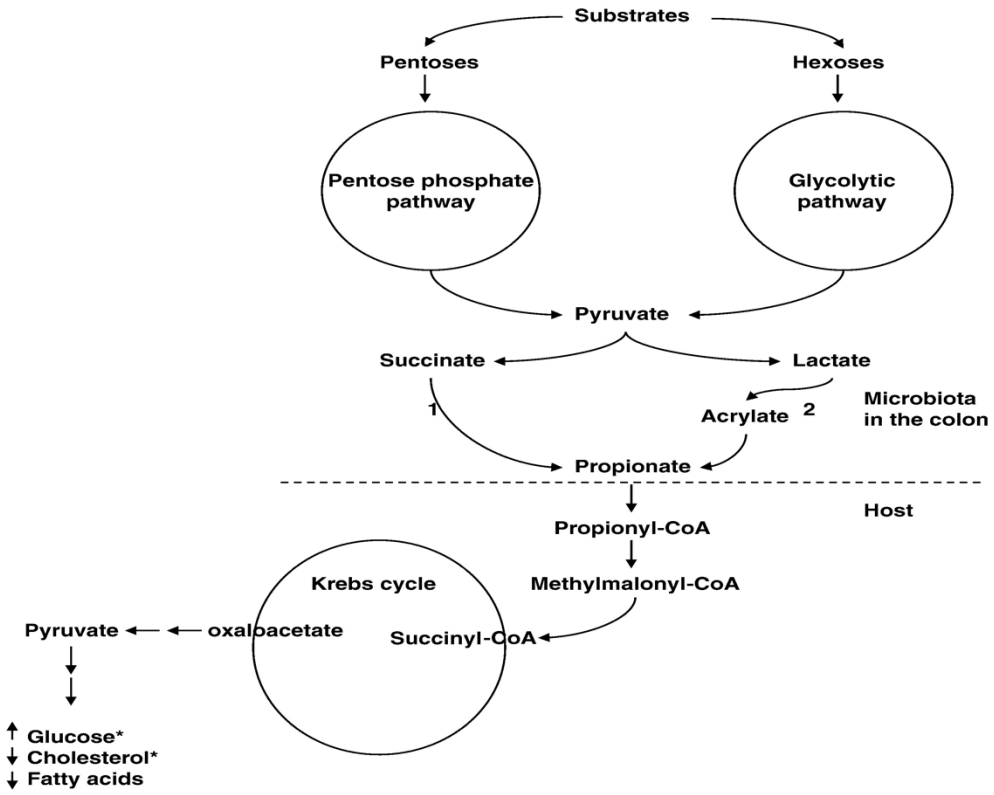


Figure 4.1: Propionate metabolism. Undigested food is broken down into hexoses & pentoses, which are further processed into pyruvate. Then, pyruvate is converted into propionate by two pathways: (1) Succinate decarboxylation or (2) Acrylate pathway. *Propionate is absorbed by the host and contributes to an increase of glucose and reduction of cholesterol synthesis. (*) controversial reports.
From Al-Lahham, Sa'ad H et al., (2010).

Propionate and Glucose Metabolism.

Among short chain fatty acids, propionate is the only one that has a different metabolism pathway in the human body. This is mainly related to the fact that roughly 30% of propionate is taken up by the liver and contributes to gluconeogenesis. Propionate is converted to propionyl-CoA and enters the Krebs cycle at the level of succinyl-CoA,

elevating oxaloacetate levels which are then converted to glucose in the liver (Al-Lahham et al., 2010) (Figure 4.1). Vitamin B12 is an essential cofactor for the enzymatic conversion of methylmalonyl CoA to succinyl CoA (Frobish & Davis, 1977). This suggests that reducing vitamin B12 increases propionate quantity, as under these conditions it is no longer converted to glucose, so deficiency in this pathway may lead to reduced energy uptake in the host organism. In patients with a congenital deficiency of methylmalonyl CoA mutase, an oral load of propionate caused hypoglycaemia (Williams et al., 1971). Meanwhile, a severe B12 deficiency in sheep due to a reduction in feed intake and an elevation of the propionate quantity in the blood was found (Macfarlane & Macfarlane, 2003). Much less is known about the effect of propionate on glucose production in humans, and existing reports are inconclusive.

Propionate, hepatic Metabolic Function and Adipose Tissue

There is accumulating evidence that propionate lowers lipids in humans. It seems that propionate reduces fatty acids that accumulate in the liver and plasma, and it is believed that the propionate inhibition of fatty acids production is derived from lowering lipolysis and enhancing adipogenesis *in vitro* (Ge et al., 2008; Hong et al., 2005) and *in vivo* adipose cell tissues (Samuel et al., 2008). It is believed that propionate sustains hepatic metabolic functions and insulin sensitivity mainly via the AMPK (AMP-activated protein kinase) dependent pathway. AMPK is a necessary regulator for maintaining the homeostasis of energy, glucose and lipid metabolism in the liver (He et al., 2017; Tang & Li, 2021). Propionate administration to human HepG2 hepatocytes resulted in the downregulation in the expression of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) through the activation of AMPK. Data suggest that the binding of propionate to hepatic GPR43 elicits the Calmodulin-dependent protein kinase β (CaMKKβ)-dependent activation of AMPK through an intracellular Ca^{2+} increase that leads to the suppression of gluconeogenesis (Yoshida et al., 2019). Meanwhile, AMPK leads to the activation of GPR43, which promotes glucose uptake and glycogen metabolism in the liver, as evidenced by the reduction of GPR43 expression,

preventing the propionate-induced phosphorylation of AMPK (Yoshida et al., 2019; Tang & Li, 2021).

SCFAs can also promote adipogenesis in 3T3-L1 adipocytes *in vitro*, although clinical studies suggest that SCFAs are not correlated with adipocyte differentiation. However, propionate has been found to inhibit the adipocyte differentiation of human chorion-derived mesenchymal stem cells (cMSCs) (Iván et al., 2017; Yoshida et al., 2019). A recent report also supports the lipid lowering effect of propionate (Haghikia et al., 2022). The report found that propionate exerts immune-regulatory effects in the gut to control intestinal cholesterol homeostasis and reduce aortic atherosclerosis lesions in hypercholesteraemic and atherosclerosis-prone mice fed a high fat diet (HFD). The same researchers later randomized 62 patients with high LDL (>115 mg/dl) in a double placebo-controlled study, and reported a reduction in LDL of -15.9 mg/dl (-8.1%) after an intake of 500 mg of propionate daily for 8 weeks (Haghikia et al., 2022). This was explained by the mechanism of increased regulatory T cells numbers which in turn increased Interleukin (IL-10) release, contributing to the suppression of the expression of Niemann-Pick C1-like 1 (NPCL1); a major transmembrane transporter that is involved in cholesterol absorption (Haghikia et al., 2022).

Taken together, strong evidence exists to support the idea that propionate may be a new personalised lipid lowering treatment. However, more evidence of a mechanistic nature is needed to prove the effects of propionate on hepatic and adipose tissues in humans.

Propionate and Insulin Sensitivity

Since high FAs contribute to inflammation and insulin resistance, and given the established role of propionate in reducing the synthesis of fatty acids, inflammation, and increased lipolysis, as discussed above, propionate therefore has the potential to improve insulin sensitivity. *In vitro* studies reported that propionate and butyrate inhibited the apoptosis of pancreatic B-cells and promoted their proliferation, leading to an increase in pancreatic B-cell mass and improved glucose homeostasis (Tang & Li, 2021).

Propionate may therefore form a link between prebiotic supplementation, the gut and its beneficial metabolic effects on obesity-related diseases.

Impact of propionate and IPE on body weight regulation in non-humans.

Dietary supplements with dietary fibre or SCFAs have been shown to reduce diet-induced obesity and its associated metabolic effects, as they play an important role in the regulation of body weight and glucose homeostasis. Since IPE is a newly synthesized molecule, there are limited studies in either animals or humans that examine its effect on weight and glucose regulation in humans and non-humans. De Vadder et al. (2014) reported a significant reduction of body weight gain of ~5 ($P<0.01$, n=6) in male rats aged 6-8 weeks fed a standard diet and butyrate or propionate orally compared to standard diet alone over 10-14 days (De Vadder et al., 2014). Den Besten et al. (2015) found that 10 weeks of the incorporation of sodium propionate in HFD fed mice led to an increase in energy expenditure and lipid oxidation by promoting changes in hepatic and adipose tissue, leading to a switch from lipid synthesis to oxidation (den Besten et al., 2015). Meanwhile, Kimura et al. 2011 demonstrated that intraperitoneal injections of propionate (1g/kg) increased oxygen consumption after 40 minutes, compared to phosphate buffered saline in C57BL/6 male mice (n=8) (Kimura et al., 2011). However, in this study the control and intervention were not energy matched.

A single study investigated the effect of inulin, propionate and IPE on body weight and appetite on HF diet induced obese mice. There were 7 group comparisons: normal control NC, Low/high inulin (LI/HI), low/high propionate (LP/HP), and low/high IPE (LIPE/HIPE). After 4 weeks, the study showed that the body weight of the animals in LP, HP and LIPE had decreased significantly ($P<0.05$), whereas the decrease in body weight was not significant in LI, HI and HIPE mice (Zhu et al., 2020).

Subsequent research looked at how SCFAs influence weight regulation. The investigation of GPR41 knockout in mice fed butyrate or propionate led to reduced body weight gain (Lin et al., 2012). In contrast, Bellahcene et al. (2013) reported that GPR41 knockout had no impact on body weight or food intake but did reduce energy expenditure (EE) (Bellahcene et al., 2013). Meanwhile, the investigation of GPR43 knockout in mice fed a

high-fat diet led to increased energy intake and EE (Bjursell et al., 2011), while another study noted a reduction in EE (Kimura et al., 2013). The increase in EE that was shown in some of the studies may be associated with increased whole body lipid oxidation, which in turn could be attributable to sympathetic nervous ganglion stimulation (Kimura et al., 2011). So far, the animal studies that have examined the propionate have shown promising results, although some are contradictory.

Impact of propionate and IPE on weight body regulation in humans

Few human studies have examined the impact of propionate on weight regulation. Darzi et al. (2012) incorporated propionate into sourdough bread and found no difference in energy intake at *ad libitum* test meals. This was attributed to the fact that propionate would have been absorbed in the upper GIT, and little would reach the colon (Darzi et al., 2012). In an acute crossover study of healthy volunteers (14 men and women), an intake of 10 g of IPE increased satiety and reduced appetite compared to inulin alone (Alhabeeb et al., 2014). Meanwhile, Polyviou et al. (2016) tested a dose of 10g of propionate over 7 days in 9 overweight healthy males aged 38 years, with BMIs of 29.8+-1.5 kg/m^2, and reported a significant reduction in energy intake during *ad libitum* test meals compared to inulin (P=0.025) (Polyviou et al., 2016).

IPE was also tested for a longer period of 24 weeks and was compared to inulin in a blind randomised controlled study in overweight men and women. IPE prevented weight gain. 4% of IPE participants gained ≥3% of their baseline body weight compared to 25% in the inulin group, and those who gained ≥ 5% of their baseline body weight were higher in the inulin group (17%), compared to (0%) in IPE group (Chambers et al., 2015). The effect of colonic IPE on reward-based eating behaviour was also investigated by the assessing brain's anticipatory reward responses during a food picture evaluation task following the consumption of IPE (2.6 g). Investigators reported reduced activation in brain regions associated with reward-processing during a food picture evaluation task and a reduced appeal of high energy foods and *ad libitum* energy intake (Byrne et al., 2016). Meanwhile, when examining the whole-body resting energy expenditure and substrate oxidation, Chambers et al. (2018) showed that acute oral sodium propionate ingestion (6.8 g)

increased resting energy expenditure and lipid oxidation in 18 healthy male (n = 9) and female (n = 9) volunteers compared to a sodium chloride control (Chambers et al., 2018).

The proposed mechanism was the stimulation of secretion of gut-derived satiety hormones such as peptide YY(PYY) and glucagon-like peptide 1(GLP-2). Several studies *in vitro* have suggested that SCFAs can stimulate anorectic hormones via GPR43, and SCFA could modulate energy intake via the brain's neural circuits (Psichas et al., 2015; Tolhurst et al., 2012). De Vadder et al. (2014) reported that elevated colon propionate production could induce vagal signalling in the gut or portal vein via GPR41 (De Vadder et al., 2014).

Taken together, few studies have examined the effect of IPE on body weight in humans, but current evidence suggests that IPE administration has a positive effect on body weight control by regulating energy intake and energy expenditure. However, long- term studies are still needed.

Impact of Propionate and IPE on Glucose and Insulin in Non-humans.

Twelve male Baboons were fed western diets with or without Kongac-Gulomannan K-GM (5%) or propionate (2%) over 9 weeks in crossover randomised study. The results reported a smaller area under the curve for the glucose with K-GM and propionate diet ($P<0.05$), but the propionate diet showed an increase in serum glucose (~6% from the baseline) (Venter et al., 1990). It was also found that CrProp supplementation (chromium III propionate complex) improved carbohydrate metabolism indices, decreasing serum insulin levels and insulin resistance indices HOMA-IR. This experiment was carried out on 32 nine-week old male rats that were divided into 4 groups of 8 rats each. The control diet, or high-fructose (HF) diet, was supplemented with various concentrations of CrProp of 1 mg or 10 mg CrProp, or 50 mg CrProp for 8 weeks (Król & Krejpcio, 2010). The only animal study that investigated IPE was reported by Zhu et al. (2020), in which the fasting blood glucose (FBG) and fasting insulin (FI) only increased in the MC group, while FBG and FI decreased in both the HI (31.64%) and the LIPE (22.14%) groups significantly ($P<0.05$). However, there were no significant decreases in the LI, LPr and HPr and HIPE treatment groups ($p>0.05$). Notably, GLP-1 and PYY levels decreased in the MC group,

whereas they increased in Inulin, Pr, and IPE groups. Only the IPE (LIPE and HIPE) groups showed a significant increase ($P<0.05$) (Zhu et al., 2020).

Inconsistencies in the experiments do not allow us to draw a definite conclusion about the impact of Propionate on Glucose and insulin in non-humans.

Impact of Propionate on Glucose and Insulin in Humans.

Among short chain fatty acids, propionate is the only one that has a different metabolism pathway in the human body. This is mainly related to the fact that approximately 30% of propionate is taken up by the liver and contributes to the gluconeogenesis process. In 1989, studies demonstrated the acute impact of propionate on glucose homeostasis in humans. For example, Wolever et al. (1989) reported that the rectal administration of propionate (180 mmol) alone increased glucose by ~ 8% ($P<0.05$) and glucagon level by ~13%, but there was no effect on insulin concentration (Wolever et al., 1989; Wolever et al., 1991). In contrast, the incorporation of sodium propionate (9.9 g per day) in white bread in a crossover design for 1 week in healthy adults showed a 38% reduction in glucose area under the curve values and a lower glucose peak compared to propionate free bread. The researchers related the glucose lowering effects to lower digestion and delayed gastric emptying of the bread-derived starch (Todesco et al., 1991). Venter et al. (1990) reported a daily sodium propionate administration of 7.5 g in healthy female adults for 7 weeks, which resulted in reduced fasting glucose and insulin response (Venter et al., 1990). However, a study by Chambers et al. (2018) showed no effect from an acute supplementation with 71 mmol of sodium propionate on fasting and insulin concentrations (Chambers et al., 2018). By contrast, in a randomised cross-over study, non-obese, non-diabetic participants were given a mixed meal with and without 5.37 mmol calcium propionate following an 8-hour fast. Propionate supplementation led to a significant decrease in insulin sensitivity, as measured by the Matsuda index (Tirosh et al., 2019)

The first human study to examine IPE found no differences in postprandial glucose ($p=0.350$) and insulin ($p=0.924$) after a 24-week supplementation of IPE in overweight/obese subjects compared to an inulin group (Chambers et al., 2015). Chambers et al. (2019) also demonstrated in a study involving 12 obese non-diabetic

adults that delivering IPE (72.4 mmol) over 42 days improved insulin resistance and sensitivity compared to low fermentable cellulose, and also significantly improved adipose tissue insulin resistance (Chambers et al., 2019).

Several mechanisms have been proposed to explain how propionate could regulate glucose homeostasis. Evidence suggests that propionate exerts its positive effect on glucose and insulin metabolism through activation of free fatty acid receptors 2 and 3 (FFAR2 and FFAR3), which in turn activates a cascade of pathways that are not completely understood. In addition, researchers have suggested that these effects may occur directly via an AMP -activated protein kinase (AMPK) dependent pathway (Yoshida et al., 2019), This mechanism is further supported by Qian Li et al. (2020) who proposed that propionate reduces hepatic gluconeogenesis through upregulation of AMPK-dependent proteins (Li et al., 2020). An indirect mechanism was also proposed via the stimulation of anorectic polypeptides PYY and GLP-1. These anorectic hormones increase insulin secretion and decrease glucagon secretion, regulating blood glucose levels (Yoshida et al., 2019). Chambers et al., (2015) also showed that propionate significantly stimulates GLP-1 secretion in human colonic cells by 1.6-fold and 2.4-fold at concentrations of 200 mmol/L and 400 mmol/L, respectively (Chambers et al., 2015). Another study found that propionate enhances glucose-stimulated insulin secretion (GSIS) via FFAR-2 activation via protein kinase C signalling, and protects human β-cells from apoptosis caused by sodium palmitate and inflammatory cytokines (Pingitore et al., 2017).

Propionate and IPE studies in humans are not yet conclusive, and whether IPE induces positive effects if it were given via a different method or in different doses over a longer period is still unproven.

Impact of Propionate and IPE on Lipid and Liver Metabolism in Non-Humans.

There is accumulating evidence that SCFAs decrease circulating fatty acids and cholesterol levels. Several studies have reported that propionate and acetate

supplementation increases the expression of leptin, a potent anorectic hormone in adipocytes *in vitro*, whereas butyrate has shown no effect (Byrne et al., 2015) . An *in vivo* study with mice adipocytes demonstrated that propionate inhibits lipolysis and results in the reduction of plasma free fatty acid (FFA) levels, mediated through FFA2 (Ge et al., 2008). This finding was supported by Hong et al. 2005, who demonstrated that propionate increases the expression of FFA2 during adipocyte differentiation and causes an upregulation of Peroxisome Proliferation activated receptor gamma (PPAR γ), a pre-adipocyte differentiation marker and a known regulator of uncoupling protein 2 (UCP2) expression, fatty acid oxidation and whole-body lipid metabolism (Byrne, et al., 2015)

Meanwhile, Den Besten et al. showed that supplementing a high-fat diet with 5% propionate for 12 weeks protected mice from diet-induced obesity through the increased expression of mitochondrial UCP2 and increased the AMP:ATP ratio. This inhibited lipogenesis and induced a shift from carbohydrate to lipid oxidation, as marked by the lower resting energy expenditure (den Besten et al., 2015). Moreover, propionate was found to inhibit cholesterol synthesis in isolated rat hepatocytes. Bush and Milligan reported that the addition of propionate or propionyl-CoA to bovine liver homogenates inhibited the HMG-CoA synthetase activity (Bush & Milligan, 1971) .

Haghikia et al., (2022) explored the role of SCFAs, particularly propionate, on plasma lipids in mice. They compared conventionally raised mice to those treated with antibiotics, feeding both groups with either a standard chow diet (SCD) or high-fat diet (HFD) for six weeks. The antibiotic-raised mice showed increased levels in VLDL and LDL. Meanwhile, oral administration of propionate (200 mg/kg) prevented HFD- showed increased levels of VLDL and LDL compared to the conventionally treated mice. Researchers also reported no significant changes in LDL receptors, HMG-CoA reductase, or FXR gene expression with HFD or propionate treatment (Haghikia et al., 2022). Overall, propionate showed positive effects on lipid metabolisms in non-human models.

Impact of Propionate on Lipid and Liver Metabolism in Humans.

Human adipose tissue is considered a major energy store in the form of TG. It increases plasma TG clearance and inhibits the release of fatty acids into the circulation. If energy

intake is higher than energy expenditure, TG stores increase adipose tissue and affects the rate of lipolysis too. These actions contribute to increasing insulin resistance and lead to the development of inflammation and obesity (Tang & Li, 2021). Reducing tissue inflammation in adipose tissue can therefore enhance lipoprotein lipase expression, reducing circulating lipid plasma (Nogal et al., 2021).

As well as lipolysis, adipogenesis may be affected by SCFAs. Hong et al. (2005) demonstrated that treatment of pre adipocytes with acetate and propionate for seven days increases adipogenesis. This increase was explained by the enhanced expression of GPR43 and peroxisome proliferator-activated receptor gamma 2(PPAR γ 2), which is a transcription factor involved in early adipogenic differentiation (Hong et al., 2005). These effects were negated when GPR43 expression was blocked using siRNA, which indicates that GPR43 is a major mediator of adipogenic differentiation in SCFAs (Canfora et al., 2015).

Adipose tissue also plays a role in secreting hormones that are involved in the regulation of energy intake and expenditure and regulating body weight such as adipokines, which is involved in energy regulation. Propionate is also found to enhance the secretion of the satiety hormone Leptin, which reduces the expression of inflammatory cytokines and chemokines (Roelofsen et al., 2010). Some researchers reported a reduction in the pro-inflammatory cytokine IL-1B after administering a mixture containing high acetate (60%) and high propionate (35%) compared to a placebo (Canfora et al., 2017). Researchers also investigated the effect of propionate on human adipose tissue (AT) *in vitro*, focusing on mesenteric, omental and subcutaneous AT where samples from eight patients were treated with propionate (1mM) and NaCl (1mM) for 24 hours. Propionate was found to enhance the expression of FFAR2, C/EBP α and UCP1 in mesenteric, omental and subcutaneous adipose tissue. However, propionate significantly increased expression of PPARγ ($P < 0.01$), C/EBPα ($P < 0.01$) and UCP1($P = 0.029$) only in mesenteric adipose tissue. This effect was attributed to the fact that propionate can reduce isoproterenol-stimulated glycerol release and IL-6 in mesenteric and omental adipocytes (Lu et al., 2023). Similarly, Al-Laham et al. (2012), after treating human omental AT *in vitro*, reported a decrease in the expression of the inflammatory cytokines and chemokines (Al-Lahham et al., 2012).

Haghikia et al. (2022) randomised 62 patients with LDL cholesterol levels >115 mg/dl in a double-blind, placebo-controlled study to receive either 500 mg of propionic acid (PA) mg twice daily or a placebo. He reported that the PA treated group showed a significant reduction in LDL-Cholesterol of -8.1% (p=0.016) compared to the placebo group (0.5%). This reduction was associated with an increase in regulatory T cells (IL-10), which could ultimately reduce intestinal Niemann-Pick C1like1(NPC1L1) expression, thus reducing cholesterol synthesis (Haghikia et al., 2022) .

Altogether, the findings of clinical research studies highlight the encouraging effect of propionate on lipid regulation in humans. However, whether these effects would be sustainable over longer treatment period is yet unknown.

Inulin Propionate Ester as a Novel Compound

Significant evidence suggests that increasing fermentable colonic DFs is crucial in achieving the high amount of propionate production that is necessary to produce beneficial effects on energy and metabolic homeostasis. A large intake of DFs (>30 g/day) is recommended to encourage these beneficial effects. However, reports showed that the average UK intake of DFs is estimated to be around 15 g/day, which is well below the recommended daily intake. A daily DF intake of 30 g/day is known to be associated with poor compliance and tolerance because of high GI side effects and unpalatability. To overcome these effects, a mechanism targeting the delivery of high level of propionate in the colon is required. A novel delivery system for this has been developed by a group of investigators at Imperial and Glasgow colleges, and it has been tested in the first-in-man study, when propionate delivered directly to the colon induced appetite and weight regulation (Polyviou et al., 2016). This novel compound is called *Inulin Propionate Ester* (IPE).

Synthesis of Inulin Propionate Ester (IPE)

There are number of methods for delivering small molecules to the colon, including encapsulation, polymer coating, pH or time dependent release, and the direct introduction of SCFAs using enemas. However, these methods have some restrictions on the dosing

amounts and are not ideal for daily or population use (Polyviou et al., 2016). Scientists have discovered a novel molecule where they can deliver high amounts of SCFAs specifically, propionate to the large intestine bypassing the digestive enzymes in the stomach and small intestines. IPE is a new molecule in which propionate is chemically bound by an ester bond to inulin. Inulin is a fructan type polysaccharide with β-D fructosyl residues linked by (2→1) and typically ending with an α-D glucose moiety at the end of each fructose chain. The number of monomers in inulin ranges from 2 to 60, with an average molecular weight of approximately 5500. Due to the β-(2 → 1) chemical structure of inulin fructose monomers, digestive enzymes in the human gut that target α-glycosidic bonds cannot hydrolyse inulin (Zhu et al., 2018). As a result, inulin may pass through the upper gastrointestinal tract without being digested to undergo fermentation in the colon, making it a suitable carrier molecule to deliver high amounts of SCFAs to the colon, regulating gut microbiota composition.

The IPE was synthesized first by reacting inulin to propionic anhydride (0.8 L/kg inulin) in water, keeping the pH between 8 and 8.5 and the temperature below 20°C. Unbound/excess propionate was then removed by filtering the mixture twice through an activated carbon column while maintaining pH of 2. Finally, the product was recovered as a fine amorphous crystalline substance through spray drying. To assess the amount of free propionate in the IPE, a sample of the ester product was dissolved in water containing butyric acid and treated in a concentrated orthophosphoric acid solution and heated at >80 °C. It was then quantified using gas chromatography, and the free propionate was measured using the ratio (free/total) × 100 (%). A 0.74 ± 0.02 degree of esterification was obtained for IPE, which means that an average of 24.6 ± 0.67% of all hydroxyl groups were replaced by an ester group. Over 97% of the propionate was bound to the inulin ester, given 2.57 ± 0.26% free propionate of the total propionate available. It was found that <1% of the bound propionate was released once it was solubilized into stomach acidic media of pH 1-2. In this procedure, heating >80 is required to release the propionate from the ester (Chambers et al., 2015; Polyviou et al., 2016).

This novel molecule propionate ester is only released when the inulin polymer is fermented by the colonic microbiota, providing direct colonic delivery. The average colonic propionate production of an average UK adult intake of 15 g/day of NSP is estimated to

be a minimum of 0.4 g of propionate per day (Polyviou et al., 2016). Investigators aimed to induce a fivefold increase in daily propionate of >2 g/day, which is equivalent to fermentation of ~75 g of NSP per day. Using an *in vitro* batch of faecal fermentation in a cross-over randomised study in overweight men, propionate release was determined using ^{13}C-labeleed IPE variants. Both IPE -27 and IPE-54 resulted in a sevenfold increase in propionate production compared with a control (Polyviou et al., 2016).

The first in-human study using an IPE was conducted in obese men and women aged 40-65 years with BMI of 25-40 in a randomised, double-blind placebo-controlled trial for 24 weeks. The study reported that IPE significantly stimulated PYY & GLP-1 secretion, and IPE also reduced food intake from 1175 kcal to 1013, resulting in beneficial changes in body weight. The study also reported a lower weight gain of 4% in IPE participants compared to the control group, with 25%. None of the IPE participants had a weight gain of >5% compared with 17% in the control (Chambers et al., 2015). Regarding the glucose homeostasis, the study found no differences in postprandial glucose (p=0.350) or insulin (p=0.924) between the groups. However, some significant changes between groups in postprandial glucose AUC were reported at week 24 weeks (p=0.037), where glycaemic control significantly worsened in inulin (from 1600 to 1690, p = 0.01) while it remained unchanged following IPE (from 1630 to 1630, p=0.99).

Summary

Recent research studies, *in vitro* or *in vivo* or clinical human studies using Inulin Propionate Ester (IPE) showed a promising effect on body weight, glucose and lipid regulation in humans. Clinical studies in healthy overweight or obese participants, showed a reduction in body weight and fat mass and increased insulin sensitivity, glucose and lipid homeostasis. These effects are believed to result from several mechanisms, mainly because propionate acts as a signalling molecule for FFAR-2 and FFAR-3, which are expressed in various human tissues, including colonic L-cells, pancreatic cells and hepatic cells. Because of these encouraging findings, further long-term studies should be carried out to determine the longevity and sustainability of these metabolic effects and to understand the potential mechanisms behind them. Meanwhile, clinical studies are also

required among different age groups to understand the variability of the responses if presents. Long-term research studies would allow us to discover any adverse effects of prolonged IPE use in humans.

To explore the uncertainties, we should target young adult population that is prone to weight gain to investigate the effect of long-term supplementation of IPE compared to inulin on weight and glucose regulation.

Study Hypothesis

We hypothesized that long-term IPE supplementation prevents weight gain and helps maintain glucose levels in a younger population (20-40) who are at increased risk of significant weight gain.

Primary objective

To examine the effect of IPE on weight gain prevention, measured by the change in body weight from baseline to 12 months.

Secondary objective

To examine the effect of IPE on glycaemic markers measured by glucose and insulin and lipid profile markers, by assessing changes in fasting biochemistry from baseline to 6 and 12 months

STUDY METHODOLOGY

Ethics Approval

This study protocol was granted ethical approval from the London Hampstead Research Ethics Committee (REC) on 29th January 2019, under REC reference 19/LO/0095 and IRAS project ID 229300. All participants provided informed consent prior to recruitment. All study activities were done in accordance with local norms and with the approval of

IRB. All study data was kept in a secured electronic system called Inform, with login access requiring a specific username and password.

Study Design

Type of study

The study was a randomised controlled parallel double-blinded trial. Participants were randomised to either the IPE (Intervention) or IN (control) arm for 12 months. The double blinded randomisation was managed by a Sealed Envelope Software computer randomisation web.

Trial Intervention and Randomisation

After an initial screening visit to establish participants eligibility for the study, randomisation was carried out at the baseline/randomisation visit using Sealed Envelope Software (open-source software www.sealedenvelop.com). Randomisation was double-blinded; neither investigators nor participants were aware of the supplements assigned. Participants were identified by a unique study identification number (ID). Randomisation was balanced between the arms by centre, sex, BMI and ethnicity, and whether they took part in the mechanistic sub study to ensure that equal and representative numbers were assigned to each intervention.

The study interventions were Inulin Propionate Ester (IPE) and Inulin (IN) control supplements. Participants were given blinded, identical-looking and identically packaged study interventions. IPE or IN were in 10 g sachets and both were in the form of white powder. Participants were instructed to take one sachet per day at any time with their normal diet or mixed with a drink or water for 12 months. Each sachet was also identified with a unique treatment code that was linked to the allocation and study identification (ID). The study supply was dispensed at the randomisation visit and subsequent supplies were dispensed at 2 and 6 months. The treatment code was not broken because there were no medical emergencies or any unexpected and related Severe Adverse Events (SAE) to the REC.

The IPE and control supplies were manufactured by Moorepark in Ireland and packaged by Cambridge Weight Plan in the UK, who distributed packaged and labelled sachets to the trial sites.

Study setting

All study visits, including the screening visit, were conducted for the clinical study at two UK sites: Imperial College Research Facility (ICRF) in London Imperial College Healthcare NHS Trust and Glasgow CRF-NHS Research Scotland.

Study Population

Study recruitment

Participants were recruited via posters and flyers spread out at General Practitioner (GP) surgeries, Sexual Health Centres, Dental GPs surgeries, universities and other public places. Another source of potential participants was the healthy volunteer database at the NIHR Imperial Clinical Research Facility. Responders to posters were sent an email containing a pre-screening eligibility questionnaire and a participant information sheet (Appendix chapter#4). Potential responders were then invited to a screening visit at the NIHR Imperial Research Facility to assess them for full eligibility.

Study participants

Male or female adults aged 20-40 years who were overweight, south Asians (BMI 24-27kg/m^2) and non-South Asians (BMI 25-29.9 kg/m^2).

Inclusion criteria

Subjects were recruited if they had fulfilled the following criteria:

1. Age 20-40 years.
2. Body Mass Index (BMI) of 24 -27 kg/m^2 if South Asians ethnicity or 25-29.9 kg/m^2 if non-South Asian and at least one of the following:
    - ✓ Self-reported weight gain of 2 kg or more over the last 12 months.

- ✓ Low self-reported physical activity (low is defined as per international Physical Activity Questionnaire (IPAQ).
- ✓ Low self-reported fruit and vegetable intake (<2 servings of fruit and vegetables per day).
- ✓ Self-reported high intake of sugar sweetened beverages (>1 serving per day).

3. On stable medication (if taking any) at point of screening.
4. Written informed consent.

Exclusion criteria

Subjects were excluded if they were:

1. Diagnosed with chronic disease; Type1 and type 2 diabetes, cancer, renal failure, heart disease or organic acidaemia (propionates or methylmalonic acidaemia).
2. Diagnosed with gastrointestinal condition including coeliac disease, inflammatory bowel disease and irritable bowel syndrome.
3. Had previous bowel reconstruction surgery.
4. Pregnant or lactating.
5. Using antibiotics at any time in the past 3 months.
6. Anaemic and Vitamin B12 deficient (<160 ng/L).
7. Taking part in a weight loss program or consuming a weight loss product.
8. Had lost 3 kg or more in the last 3 months.
9. Had any other metabolic disease that caused weight gain.
10. Had any other reason in the opinion of the investigator.

Withdrawal criteria:

Subjects were considered to have withdrawn if they:

1. Decided to withdrawal their consent decision (Withdrawal of consent).
2. Were lost to follow-up.

3. Were included in the trial in violation of the inclusion and/or exclusion criteria.
4. Were pregnant or intended to become pregnant.
5. Had participated in another clinical study within one month before screening.

Figure 4.2: The study flow diagram.

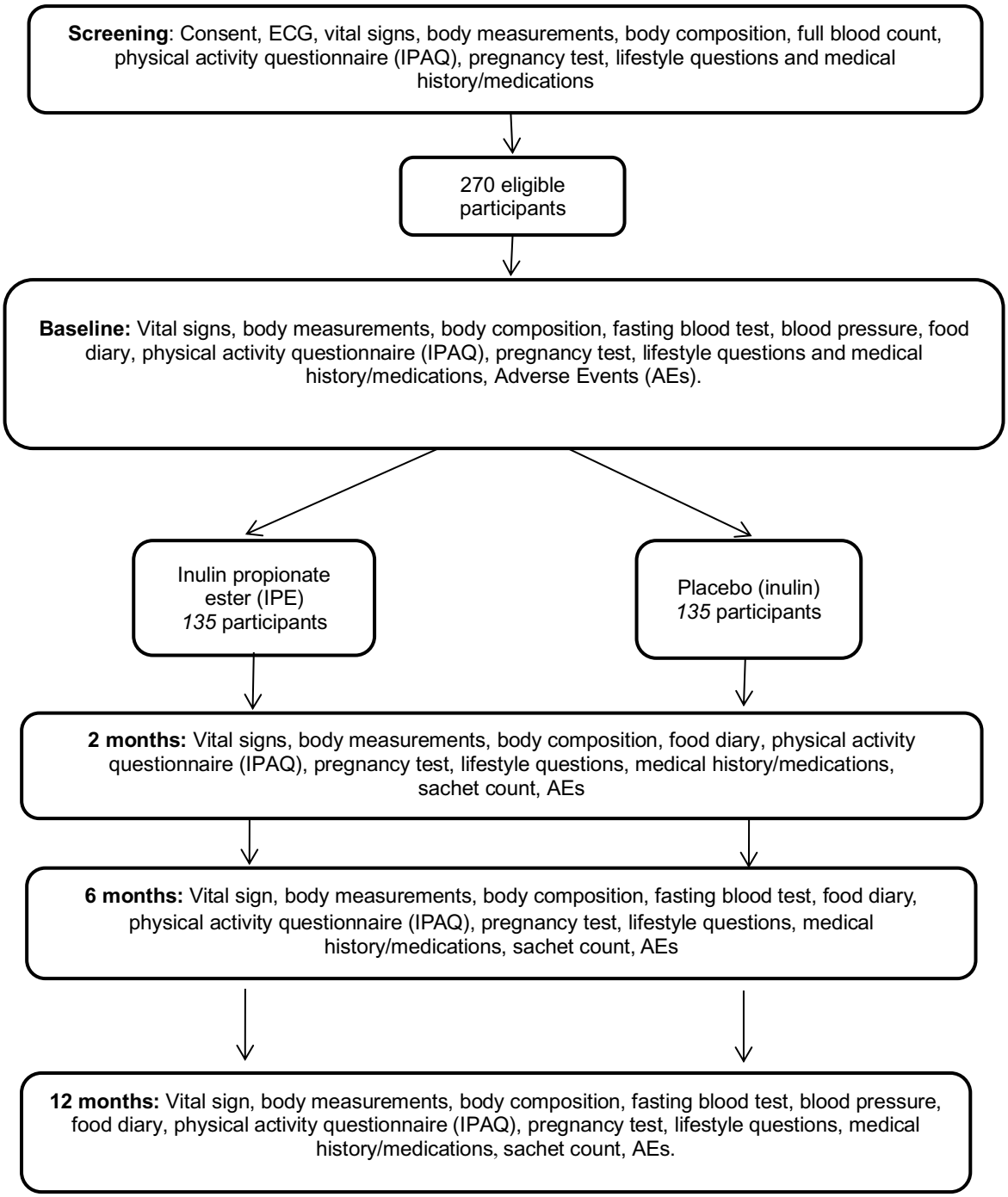


Figure 4.2: The study flow diagram: study randomisation arm, study visits and study procedures at each study visit.

STUDY ACTIVITIES

During the screening process, participants underwent an interview to assess their medical and drug history. All subjects then provided written informed consent (See Appendix). Following this, fasting blood glucose, full blood count, electrocardiogram (ECG), blood pressure, body weight and height and body composition were measured. All women of child-bearing age completed a urinary pregnancy test. Blood results were evaluated according to the inclusion criteria and approved by the study clinician before participants were formally invited to the baseline/randomisation visit.

Study Visits

Screening visit

During the screening visit, participants were assessed further for their eligibility through an interview in which medical history and concomitant drug history were discussed. All participants were then provided with the informed consent form to sign (See Appendix). Afterwards, study procedures and measurements were collected including ECG, blood pressure, height, weight, waist, hip, body composition and blood sample and all women underwent a urinary pregnancy test. Data on lifestyle factors (smoking and alcohol), and an International Physical Activity Questionnaire (IPAQ) were also collected (See Appendix).

Baseline/Randomisation visit:

Eligible participants were invited to attend the randomisation visit 1-4 weeks after the screening visit, at which all procedures of the screening visit were repeated except ECG. More blood was collected for fasting glucose, insulin, and lipid profile. Randomisation was undertaken using a web-based randomisation sealed envelope. Study data were fed and saved into the Electronic Data Capture system (EDC). Food diary was also distributed at this visit to the participants and being instructed on how to fill in the food diary.

Follow up visits

At 2 months, an update in medical, concomitant medications and adverse events was collected. Vital signs and body measurements were taken. IPAQ and lifestyle questionnaires were also completed and the food diary was collected. Trial intervention/control sachets were dispensed to the participants.

At 6 months, participants were asked to come after fasting, and another update in medical, concomitant medications and adverse events was collected. Vital signs and body measurements were taken, along with a blood sample for fasting biochemistry.

At 12 months, participants were invited to their last visit. The same study procedures and data were collected, and a fasting blood sample was collected too.

Table 4.1 summarizes all study procedures and blood collection at the scheduled study visits.

It is worth noting that participants were asked to attend the study visits in the morning, after fasting for at least 8 hours, and were asked to abstain from alcohol and intense physical activity the day before the visit. At 2-, 6- and 12-months, compliance was measured by counting used and unused inulin/IPE sachets. The occurrence of adverse events (AEs) or SAEs was also documented.

STUDY ASSESSMENT

Vital Signs

Vital signs including pulse and blood pressure (BP) were measured at screening and at subsequent visits after participants had rested for five minutes in a sitting position. Three blood pressure readings were recorded, and the average was automatically calculated by the Inform Electronic Dat Capture (EDC) System.

Body Weight and Height

Body weight was measured at screening and at each subsequent visit together with the body composition using standard Tanita electronic weighing scales. Participants were

asked to remove any heavy items of clothing, shoes and accessories. They were also asked to empty their bladder before the measurements were taken.

Body height was recorded in centimetres once at screening and used throughout the study along with weight to calculate BMI.

Waist and Hip Ratio

Waist and hip measurements were taken at every study visit, and WHR was calculated automatically by the Inform system.

Body Composition

Body composition was measured by bioelectrical impendence (BIA) at each study visit and recorded to the nearest 0.1 kg using the Tanita BC 418MA scales. These measurements included total body fat mass (FM) and body water mass (WM). Fat-free body mass (FFM) was calculated by difference in body weight (BW) and body fat mass (FFM= BW-FM). Participants asked to remove heavy items of clothing, shoes and accessories, and were also asked to empty their bladder before the measurements were taken.

Pregnancy Test

A pregnancy test was performed at all trial visits for all female participants regardless of whether they were using adequate contraception. If the participant was found to be pregnant at any point during the trial, the intervention was withdrawn for safety reasons but follow up was continued until the end of trial.

Food Diary

Participants completed a 7-day food diary at baseline, 2, 6 and 12 months to monitor changes in food intake in the home environment.

Physical Activity

All participants completed the International Physical Activity Questionnaire (IPAQ) at each study visit.

Other Lifestyle Activities

Questions relating to smoking, alcohol and recreational drugs were asked at each study visit to monitor any changes from screening over the duration of the study.

LABORATORY EVALUATIONS

Fasting Biochemistry

Full blood count

Blood samples for Full Blood Count (FBC) were collected at the screening visit. B-12 was measured at screening only for potential participants who had anaemia, and that decision was left to the study doctor. Blood samples at screening were processed at the local pathology laboratory for each study site.

Fasting glucose, fasting Insulin, and lipid profile.

Fasting biochemistry was taken at baseline, 6- and 12-month study visits, where blood samples were collected after an overnight fast to measure blood glucose, plasma insulin and HDL-cholesterol, LDL-cholesterol, total cholesterol and triglyceride concentrations. Blood samples for lipid profiling were processed immediately at the local pathology laboratory in accordance with standard practice protocols. Fasting glucose and insulin were processed immediately at the local ICRF laboratory. Plasma and serum were separated by centrifugation (2,500 RCF at 4 °C for 10 minutes), aliquoted into four labelled Eppendorf tubes, and stored at -80 °C until further analysis.

After completion of the trial, fasting glucose and insulin samples were analysed at Imperial College London lab. Fasting glucose was measured using a calorimetric enzymatic activity assay (GL354, Randox, UK) and fasting insulin was measured using a commercial

Human/Canine/Porcine Insulin DuoSet Enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc. Bio-Techne, Minneapolis, Minnesota, United States). Gut hormones were analysed at Imperial College London lab using established in-house radioimmunoassay (RIA).

Markers for Insulin Resistance and Beta-Cell Function.

The Homeostasis Model Assessment (HOMA).

This model was developed in 1985 as an interaction between insulin and glucose dynamics to predict insulin resistance. The model predicts fasting steady-state levels of plasma glucose and insulin for any given combination of pancreatic cell function (HOMA%B) and insulin sensitivity (HOMA%S). The following formulas were used to calculate HOMA:

1. **HOMA-IR:** This is defined by the product of the fasting glucose and fasting insulin, divided by a constant. The formula for the HOMA-IR model is:
   HOMA = Fasting Insulin (μIU/ml) × Fasting Glucose (mmol/l)/22.5

The denominator of 22.5 is a normalizing factor, based on the product of normal fasting plasma insulin of 5 IU/ml and normal fasting plasma glucose of 4.5 mmol/l obtained from an “ideal and normal” individual (Petrelli et al., 2024) .

2. **HOMA-B** was used to estimate pancreatic cell function, calculated using this formula:
   HOMA-beta= FPI concentration (μU/ml) × 20/FPG (mmol/L)-3.5
3. **HOMA-S** was used to estimate the insulin sensitivity of cells and tissues.

HOMA-IR, HOMA-B and HOMA-S were calculated using the validated online HOMA calculator (version 2.2.4) provided by the Oxford University Innovation department.

Quantitative insulin sensitivity check index (QUICKI).

QUICKI is an empirically derived mathematical transformation that measures fasting blood glucose and plasma insulin concentrations. QUICKI is a thoroughly evaluated and validated surrogate index of insulin sensitivity. It provides a reliable, reproducible and accurate index of insulin sensitivity with excellent positive predictive power. QUICKI is defined by this formula:

QUICKI= 1/ log (fasting insulin, µlU/ml) + log (fasting glucose, mg/dl)

Table 4.1: Summary of the procedures and investigations at study visits.

Assessment	Screening	Baseline/Randomisation (1-4 weeks after screening)	2 months (+/- 1 week)	6 months (+/- 2 weeks)	12 month (+/- 2 weeks)
Consent	X				
Demographics	X				
Randomisation		X			
Medical History	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Pregnancy test	X	X	X	X	X
Electrocardiogram (ECG)	X				
Vital signs: Diastolic Blood Pressure(DBP) Systolic Blood Pressure (SBP) Heart rate (HR)	X	X	X	X	X
Trial intervention (IPE or inulin control)		X	X	X	
Height, body weight, waist/hip measurements, BMI, body composition	X	X	X	X	X
Fasting blood test (glucose, insulin, lipid profile)		X		X	X

EXPLORATORY MECHANISTIC STUDY

52 out 270 participants consented to join the mechanistic sub-study. They attended a day-long study that lasted for 8 hours from 8:00 am to 17:00 pm at the baseline and 12 months visits.

Prior Study Visit Requirements.

On the day before the study visit, participants were asked to wear accelerometer arm band prior the study visit 24 hours a day for 7 days. The evening before the study visit, participants were instructed to drink deuterated water ($2H_2O$), and to consume an evening meal of their choice (shop-bought) before an overnight fast of at least 8 hours. They were also asked to collect a stool sample on the night before or the day of the visit, and they were asked to avoid alcohol consumption and strenuous exercise.

Study Visit Protocol.

Participants were asked to arrive at NIHR Imperial at 8:00 am. Upon arrival, compliance to prior study visit requirements was assessed by asking participants. They were asked to bring back the collected stool sample, accelerometer arm band and the nutritional packaging and receipt for the purchase of their evening meal. Participants were then asked to empty their bladder and to provide urine samples for pregnancy test where appropriate. Urine was collected after each visit for the reminder of the study for further processing to calculate the total urine output and sample it for a metabolomics profile. During the same visit, routine measurements and questionnaire were collected.

A cannula was then inserted into the participant's cubital vein by the study physician. Baseline (-30 min) 10 ml blood was collected to measure gut hormones (4ml), insulin (2ml), glucose(1ml) and extra samples (3ml), then repeated blood samples were collected at -15, +15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Participants were also asked to blow into tubes to measure breath hydrogen every 15 minutes from -30 to 420 minutes.

Energy expenditure was measured using indirect calorimetry (Gas Exchange Monitor, GEM Nutrition). Each assessment lasted approximately 20-30 minutes and measurements were recorded between -30 to 0, 30 to 60, 210-240, and 390-420 minutes. During measurements, the participant was instructed to lay fully supine or semi-supine at a 30–45-degree angle under the calorimetric hood. Participants were allowed to listen to soft music under the hood if they remained in a relaxed position without talking or moving. It is worth noting that physical activity was also restricted between energy expenditure, and throughout the study visit participants remained mainly resting on their beds, although they were allowed to read, work on laptops or listen to music.

Glucose and insulin

Glucose and insulin were measured from blood samples taken from the cannula at -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Glucose was measured using a colorimetric enzymatic activity assay (GL364, Randox, UK). Insulin was measured using a commercial sandwich ELISA (DY8056-05, Biotechne, USA).

Fasting Gut hormones

Participants arrived at the CRF after an overnight fast, and a cannula was inserted into the forearm vein for repeated blood sampling. Blood samples for PYY and GLP-1 were taken at -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Gut hormone concentrations were measured using established in-house radioimmunoassay (RIA)(Kreymann et al., 1987; Adrian et al.).

Sub study meals and Appetite measurement

Participants were given a standard breakfast as mixed meal tolerance, containing 200 mg of palmitic acid [U-^{13}C], Ensure Plus, Abbott, UK: 500 kcal, 67.3 g carbohydrate, 13.4 g fat, 20.8 g protein), together with deuterated water. Breakfast was served at 0 minutes after 2 fasting blood samples were taken, and participants asked to consume it within 15 minutes. Standard Lunch was served at 180 minutes, provided as a 550, 650 or 750 kcal portion depending on the participants' basal metabolic rate. The lunch consisted of a white

bread cheese sandwich and salted crisps (Tesco, UK: 10.0g carbohydrate, 5.1g fat, 3.1g protein, 0.5g fibre per 100kcal). Subjective appetite measurements were taken using a standardised 100 mm appetite questionnaire visual analogue scale (VAS) to assess hunger, fullness, nausea, and thirst at -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes (Flint et al., 2000). At 420 minutes, Ad Libitum meals were served, consisting of a large quantity of pasta and tomato pasta sauce (Tesco, UK: 126.5 kcal, 21.4g carbohydrate, 2.9g fat, 3.3g protein per 100g) to ensure that participants could eat until they felt comfortably full. This meal was weighed before and after food intake to calculate the total amount eaten (g). The measurement was converted into kilocalories to determine energy intake.

A summary of the mechanistic sub-study procedures is shown in Figure 4.3

Figure 4.3: The exploratory mechanistic study procedures, samples, and timing of investigations.

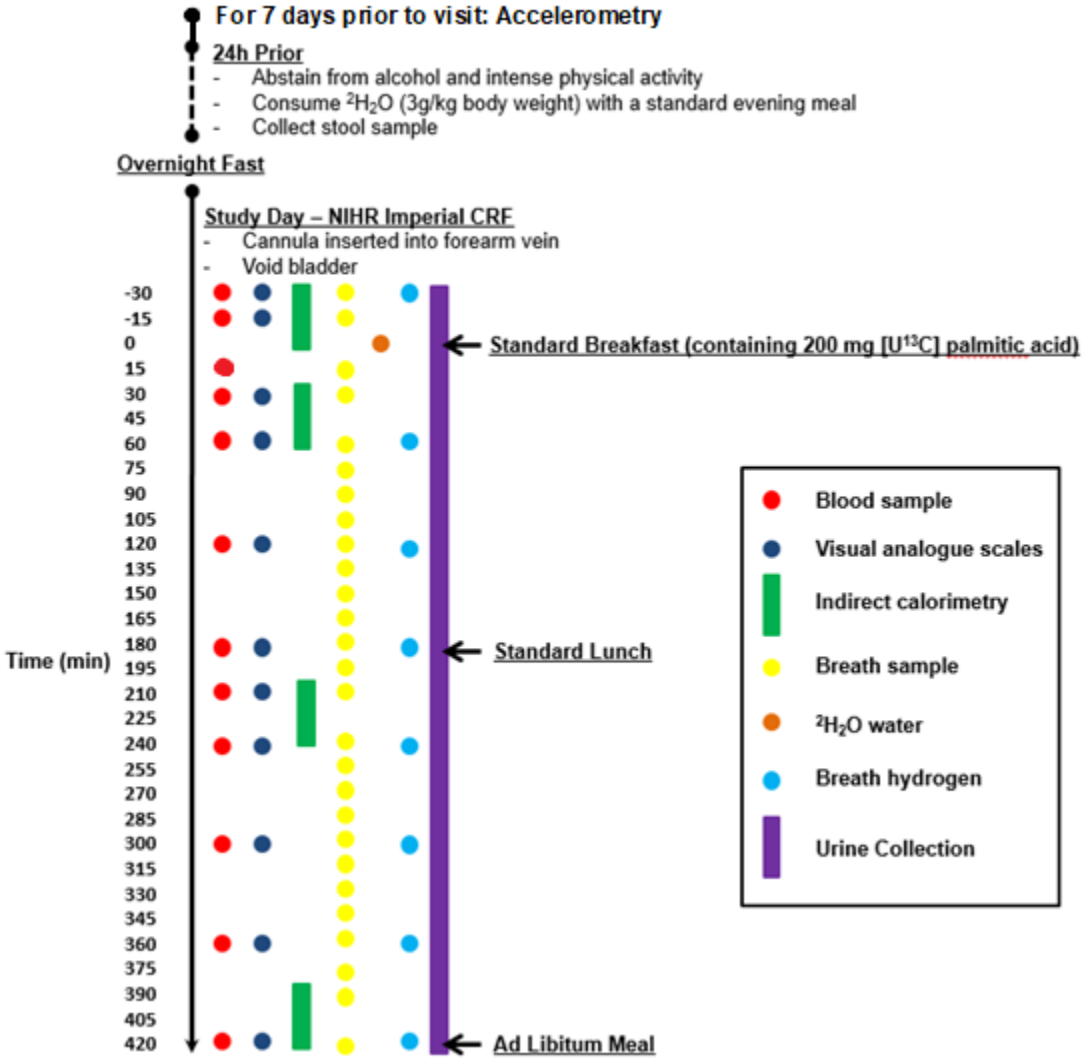


Figure 4.3. The exploratory mechanistic study procedures, samples and timing of investigations**.** NIHR-CRF, National Institute for Health and Care Research Clinical Research Facility.

TRIAL AND COVID-19

Remote visits

This study was considerably affected by the Covid-19 pandemic, which stopped the physical participants study visits for almost 6-9 months. During the period, participants had their 2-month, 6-month, and 12-month visits remotely via phone calls. A self-reported weight was collected, and other physical and lifestyle questionnaires were collected where possible.

During 6-month and 12- month remote visits, blood sample, vital signs, and weight related body measurements could not be taken, but participants were recommended to come in for clinic visit at study site once the lockdown restrictions were lifted- before 11 months for those who completed the 6-month remote visit ,and within one month after 12-month for those who completed the12-month remote visit.

For participants who had their self-reported weight collected at a remote visit, a pre-clinic self-reported weight was collected again prior to their next clinic visit, together with the clinic weight. A note was added to the Inform EDC database to specify that visits were done remotely and for self-reported weight.

During the Covid-19 period, further supply of the study supplements was posted to those who completed 2 or 6-month remote visits.

Discontinuation of the Study Intervention and Withdrawal from the Study

Discontinuation of the trial intervention

Participants were allowed to discontinue study supplements but continue with study procedures if they suffered any adverse or serious adverse events, or at the participant's request to discontinue the study supplements. However, they were invited to attend study visits if possible, to allow for the collection of key outcomes and safety data.

Withdrawal from the trial

Withdrawal from trial was referred to as the discontinuation of study supplements and procedures that had occurred from participants' decisions to withdraw their consent or lost follow up.

SAFETY OUTCOMES

Adverse Event (AE).

An AE is defined as unwanted medical occurrence in the study participants, whether or not it was considered related to the study supplements. AEs were reported by the study researcher and documented into eCRF under AE. AEs were also assessed for severity and cause to the study supplements as mild, moderate or severe, and as unrelated, unlikely, possible or probable, respectively,

Serious Adverse Event (SAE).

An SAE was defined as any event that resulted in death or life-threating conditions or required hospitalisation or the prolonging of an existing inpatient's hospitalisation, or resulted in disability or congenital abnormality.

STATISTICAL ANALYSIS

Sample size and power Calculation.

The total sample size was estimated using data from Chambers et al. (2015) This study identified that the change in body weight over 24 weeks was 1.4 g (95% CI: -0.3 to 3.1), p=0.099. By 24 weeks, there were significant reductions in the proportion of intervention participants gaining 3% or 5% of body weight from a mean baseline of 90 kg. This baseline aimed to detect a 2 kg effect and achieved 4.3 kg, with a pooled standard deviation (S) for body weight change of 4.35 kg and 81% retention (Allman-Farinelli et al., 2016). We therefore chose a 2 kg between arm 12-month effect size. On this basis, a sample size of 270 randomised participants (135 per arm) was chosen to provide 90% probability of detecting a 2 kg difference in mean body weight change between arms over

12 months, using a two -sided 5% significance test and assuming a 4.3% kg with 25% dropout allowance.

Power calculation for Mechanistic sub-study.

For the mechanistic sub-study, 34 volunteers (17 per group) would provide enough statistical power to detect a 15 pmo/L effect size in PYY and GLP-1 concentrations between groups, with 90% power. This was estimated using data from previous published findings that reported an enhanced gut hormones release following IPE supplementation (Polyviou et al., 2016; Chambers et al., 2015). A subsample of 52 volunteers (26 per group) was chosen to allow for a 70% retention rate.

Primary outcome analysis.

The analysis of the primary outcome was based on “intention to treat”, and used interim measurements of body weight in a linear mixed effects (LME) model. This was adjusted for covariates of the source of measured weight (clinic versus self-reported), baseline continuous weight, age, sex, BMI by ethnicity, whether included in sub-study and UK sites.

Participants who withdrew during the study without providing the 12-month measure were therefore able to have their previously observed data from earlier time points included (i.e. 2-month and/or 6-month measures), and the statistical model accounted for these through correlations between them and the 12-month measure. Further specification of the role of timepoint and correlation structure was detailed in the Statistical Analysis Plan (SAP) (Pugh et al., 2022). The implicit missing at random assumption (that the correlation between earlier timed measures and the 12-month measure is the same for those who withdrew as was estimated in those who continued to the 12-month measure) was challenged through a set of sensitivity analyses (Sivaprasad et al., 2017). As these sensitivity analyses involve all randomised participants, the linear mixed effects (LME) model and the sensitivity analysis taken together constitute an Intention to Treat Strategy (Bell et al., 2014) .

IBM SPSS Statistics (RRID: SCR_019096) version 28.0.1 was used for all statistical analyses.

Secondary Outcomes Analysis

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 at each outcome time point for the baseline of the measurement, age, sex, BMI within ethnicity, sub-study inclusion, UK site and any missing indicator variables (unless there were no missing data for the baseline of the outcome). Comparisons between arms for binary outcomes were summarised as differences in proportions. 95% confidence intervals were used to make inferences from estimated effect sizes.

All primary and secondary outcomes were summarised as differences in means between IPE -IN arms, 95% confidence intervals,

For the mechanistic sub-study, incremental areas under the curve (AUCs) were used to summarise responses at each of the baseline and 12-month follow-up visits. 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, 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 12-month follow-up visit, pre-baseline/baseline measurements were also imputed using the baseline measurement at the baseline visit from the same participant on two occasions.

Due to non-negligible differences between groups in the baseline visit incremental AUC in small groups, the analysis was undertaken on the change from 12-month visit to baseline visit in the incremental AUC 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 adjust for baseline further using multiple regression, as had originally been planned. Sensitivity analysis to imputations was not undertaken due to the small numbers with data at both timepoints, 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) was defined here as the first to the third quartile. The 95% confidence interval for the difference in medians between two independent samples was obtained using the Hodges-Lehmann estimator. An exception for this was accelerometer outcome data, where daily data allowed more appropriately distributed summary averages to be calculated per outcome. Here, the nonparametric Bootstrap method was used to calculate 95% confidence intervals.

This incremental AUC approach (iAUC) was used for each mechanistic outcome unless indicated otherwise in the following section (e.g. Oxidation), for reasons of clinical relevance or compatibility with previous research analysis methods.

RESULTS.

PRIMARY OUTCOMES

STUDY POPULATION.

Study recruitment

In this randomised, double-blind placebo-controlled clinical study with allocation ratio of 1:1, a total of 325 eligible individuals were screened. Most screening failures (n= 40) were due to BMI counts that were outside the eligible range. The remaining (15) were not randomised due to withdrawal of consent (n=6), lost to follow-up (n=3) or other reasons (n=6), such as job commitment or loss of interest. 270 out of 325 overweight participants were voluntarily recruited between August 2019 and October 2021. Recruitment was paused between March and September 2020 due to Covid-19. The recruitment target of 270 participants was reached in October 2021. These 270 participants were randomly assigned to either the intervention arm (IPE) or the control arm (IN), and they were followed for 12 months from the randomisation date. In this study, the minimization

procedure was implemented in order to produce a comparable distribution of the stratifiers between arms. A total of 226 out of 270 (84%) of participants were included in the final analysis, meeting the required participant follow-up for the primary outcome of 80% in the power calculation and distributed as 114 in the control arm and 112 in the intervention arm. For more details on the participants recruitment and follow-up, see participant flow diagram in Figure 4.4.

Study Withdrawals

There were 42 study withdrawals, distributed almost equally between the IPE and IN groups (22 and 20, respectively). By the end of the study, 61 participants had stopped the supplements but continued the study. There were more participants who stopped the supplements in the control arm (41), compared to 20 in the intervention arm. The difference in the withdrawal rate between arms at 6 months was 0.7% (-6.4% to 7.9%) and at 12 months 1.5% (-10.1% to 7.2%). The difference between arms in those either withdrawing or discontinuing the supplement at 6 months was -7.4% (-17.5%- 2.7%), and at 12 months -14.1% (-25.5% to -2.6%) (Table 4.2). The baseline characteristics between those who withdrew from the study and those who did not were comparable (Table 4.3).

Figure 4.4: Participant Consort flow Diagram

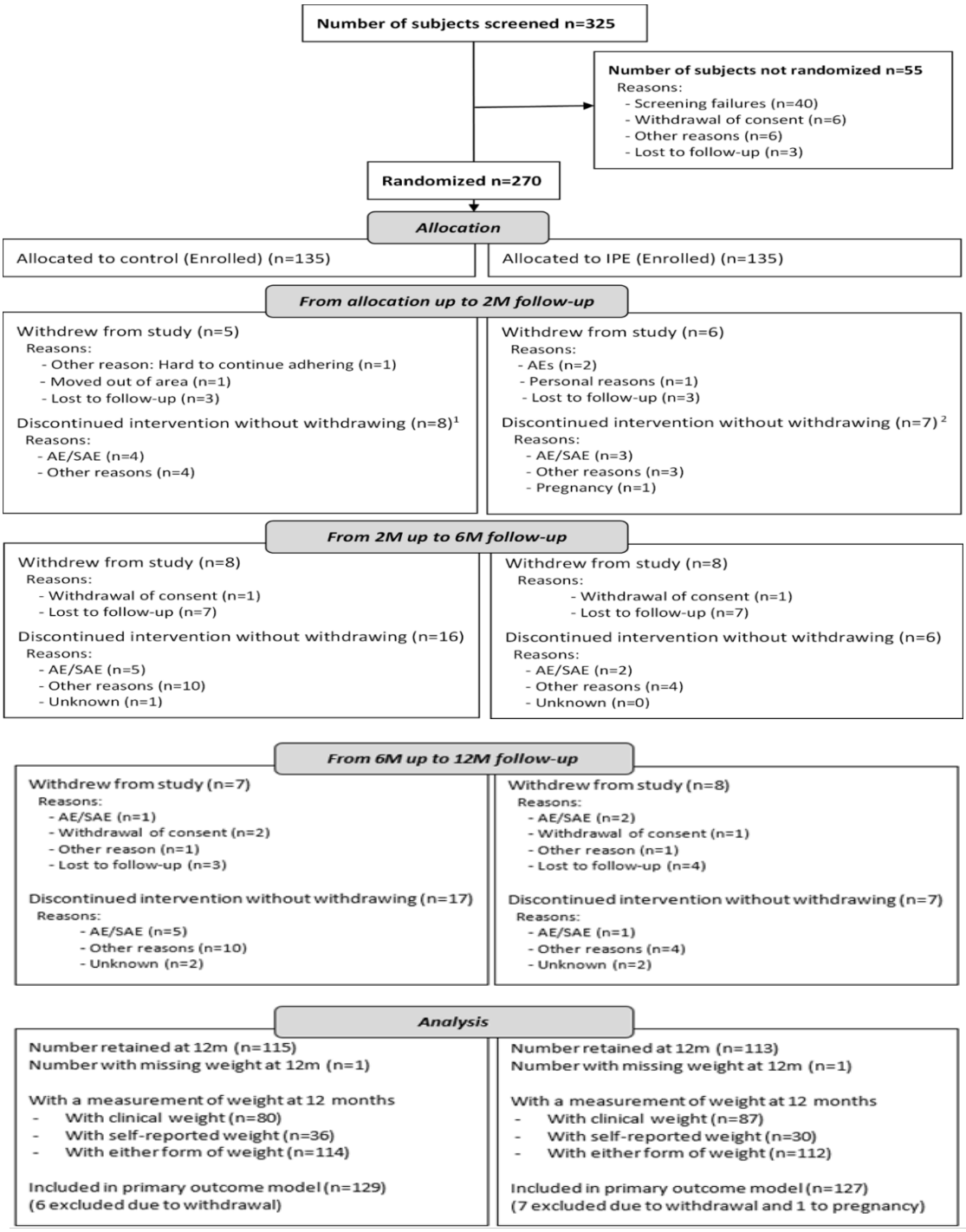


Figure 4.4. This diagram describes the flow of participants through the study stages. A total of 325 eligible participants were screened. Only 270 participants were randomised to either study arm. A total of 228 participants' data were analysed, *(IN, n=115*) and *(IPE, n=113*).
Abbreviations. IPE, Inulin Propionate Ester; M, month; AE/SAE, Adverse Event/Serious Adverse Event.
[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.

Table 4.2: Cumulative numbers of participants who withdrew from the study and discontinued the supplement at each time point by study arm.

	IN			IPE			Total
	2 M	6 M	12 M	2 M	6 M	12 M	Up to 12M
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Withdrawn	5 (4)	13 (10)	20 (15)	6 (4)	14 (10)	22 (16)	42 (16)
Discontinued	8 (6)	24 (18)	41 (30)	7 (5)	13 (10)	20 (15)	61 (23)
Total	13 (10)	37 (27)	61 (45)	13 (10)	27 (20)	42 (31)	103 (28)

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; M, month; N, number; %, Percentage

Table 4.3: Baseline characteristics of those who withdrew from the study and those who completed the study.

	Withdrawn from study		Completed study	
	IN	IPE	IN	IPE
	N=20	N=22	N=115	N=113
Age in years (SD)	29.1 (4.4)	29.0 (5.6)	30.2 (5.8)	30.5 (5.1)
Sex (Female)	50% (10)	73% (16)	66% (76)	62% (70)
Site (London)	60% (12)	50% (11)	63% (72)	65% (74)
In sub-study	20% (4)	9% (2)	20% (23)	20% (23)
BMI within study				
NSA: BMI25-27.49 kg/m^2	50% (11)	50% (10)	41% (46)	41% (47)
NSA: BMI:27.50-30.00 kg/m^2	41% (9)	40% (8)	46% (52)	45% (52)
SA: BMI:24.00-25.49 kg/m^2	5% (1)	0% (0)	4% (4)	4% (5)
SA: BMI:25.50-27.00 kg/m^2	5% (1)	10% (2)	10% (11)	10% (11)
Baseline weight (Kg)	81.6 (10.1)	77.0 (11.6)	78.7 (10.6)	80.1 (10.7)

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, number; SD, Standard deviation; BMI, Body mass index; NSA, non-South Asian; SA, South Asian.

Baseline demographic data.

Table 4.4 shows the socio-demographic and clinical characteristics of the study participants by arm at baseline presented as a mean by number and percentage. The main characteristics between arms are well balanced. The mean study age was 30.1 and 30.3 in the control and IPE arms, respectively. The average body weight at baseline was 79.1 kg in the control arm, compared to 79.6 in the IPE arm. The average BMI was almost the same in both arms; 27.4 and 27.5 in IN and IPE arms respectively. The baseline participant characteristics also showed that white was the majority participant ethnicity at the start of the study, forming 95% of the IN arm and 90% of the IPE arm. Asian ethnicity came second at both arms with 15% (IN), and 10% (IPE).

Table 4.4: Participants' socio-demographic and clinical characteristics by arm at baseline.

	IN **N= 135** **N (% total)**	**IPE** **N= 135** **N (% total)**	**Total** **N= 270** **N (% total)**
Age (years) (Mean (SD))	30.1 (5.7)	30.3 (5.2)	**30.2 (5.4)**
Sex			
Female	86 (64%)	86 (64%)	172 (64%)
Male	49 (36%)	49 (36%)	98 (36%)
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%)
Others	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 (63%)	244 (90%)
Drinking status			
Current	110 (81%)	106 (79%)	216 (80%)
Ex-drinker	9 (7%)	12 (9%)	21 (8%)
Never	16 (12%)	16 (12%)	32 (12%)
Recreational drug use	6 (4%)	7 (5%)	13 (5%)
Weight(kg)	79.1 (10.6)	79.6 (10.9)	79.3 (10.7)
(Mean (SD))			
BMI(kg/m^2)(Mean(SD))	27.4 (1.5)	27.5 (1.6)	27.5 (1.5)
Height(cm)(Mean(SD))	169.5 (9.3)	169.6 (9.5)	169.6 (9.3)
Waist(cm)(Mean(SD))	93.2 (8.2)	94.0 (8.7)	93.6 (8.5)

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, Number; %, Percentage; BMI, Body mass index; SD, Standard deviation.

WEIGHT CHANGES OVER 12 MONTHS.

Data for Principal Weight by Visit and Study Arm.

"Clinic weight" refers to the weight of the participants at study site while "self-reported weight" refers to the weight of the participants collected at home due to the Covid-19 pandemic. The "principal weight" was the weight used in the primary analysis. This was the clinic measured weight. Where this was missing, the self-reported measured weight was used. The number of self-reported weights was higher at 2- and 6-months visits, which was consistent with the fall of clinic visits during the pandemic period.

By end of 12 months, we had a total of 84% (226/270) of participants who provided the principal weight, meeting the required participants follow-up to the primary outcome of 80% in the power calculation. Of these, 62% (167/270) provided a clinic weight at 12 months and 22% (59/270) provided a self-reported weight. The distribution of measured weights, whether clinic or self-reported, was comparable between arms (See Appendix), and the baseline characteristics comparison between those who gave a clinic weight or a self-reported weight was also comparable (See Appendix).

Weight Changes Between Arms from Baseline to 12-months.

Unadjusted weigh change from the baseline

The unadjusted mean of weight changes from baseline to 12-months in the IPE arm showed a weight change of 0.4, 0.53 and 1.22 at 2, 6 and 12 months, respectively. In contrast, the IN arm mean change in body weight was 0.16, -0.07 and 0.07 at 2, 6 and 12 months, respectively (Figure 4.5; Table 4.5).

Figure 4.5: Unadjusted mean changes from baseline per arm across the study timepoints.

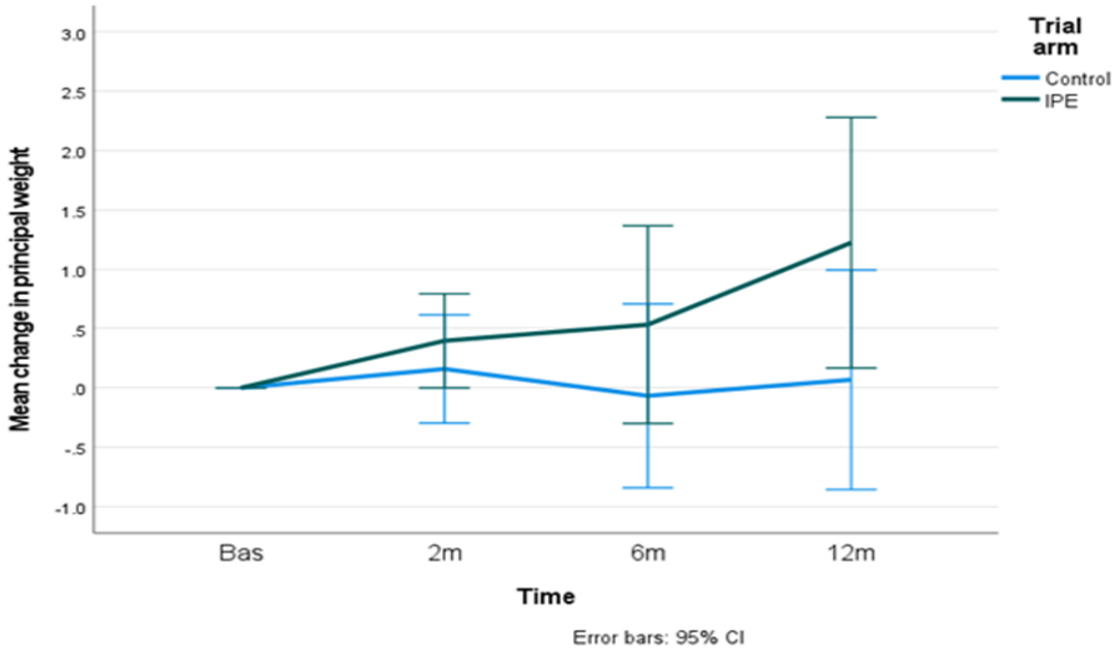


Figure 4.5. The IPE arm showed a mean change in body weight of (+1.22) kg at 12-months. Control Inulin; IPE, Inulin propionate ester; CI, Confidence interval.

Table 4.5: Unadjusted mean changes across the study visit within each trial arm.

		IN		IPE
Body weight(kg)	**N**	**Mean (SE)**	**N**	**Mean (SE**
Baseline principal weight	135	79.1(0.9)	135	79.6 (0.9)
Change from BL to 2M	121	0.16 (0.23)	125	0.40 (0.2)
Change from BL to 6M	106	-0.07(0.39)	108	0.53(0.42)
Change from BL to 12M	114	0.07 (0.47)	112	1.22 (0.53)

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, number; BL, baseline; M, month; SE, Standard error

Adjusted weight changes from the baseline.

Adjusted differences here refer to the differences between arms (IPE and IN) that were calculated after controlling for the effects of the variables or covariates such as baseline weight, age, sex, BMI and ethnicity to improve the precision of estimated intervention effects.

The weight was adjusted at each timepoint for the covariates of the source of the measured weight (clinic versus self-reported, baseline weight, age, sex, BMI by ethnicity, whether included in the sub-study and UK sites) using a linear mixed effects model. The adjustment model was run on 95% of randomised participants (n=256; 129 in the control arm and 127 in the IPE arm), 200 participants contributed at the three follow-up time points, 30 contributed with two and 26 with one. There was no significant arm effect between weight measurements measured within the principal weight – i.e., whether clinical or self-reported (three-way interaction *p*-value = 0.23 at 12 months). The treatment arm effect of clinic weight measurements at 12 months was +0.77 (-0.78 to 2.32), which gives consistent conclusions.

The adjusted difference in weight changes between the IPE and IN arms was +.2, +0.19 and +1.02 at 2, 6 and 12 months, respectively. In summary, there was non-significant an intervention arm effect of the principal weight of +1.02 (-0.37 to +2.41, p=0.15) at 12 months. (Table 4.6).

Table 4.6: Adjusted differences in mean weight between arms.

Body weight (Kg)	IN		IPE		Adjusted difference in means
	N	Mean (SD)	N	Mean (SD)	(IPE – IN) (95% CI)
At baseline:					
Clinical weight	135	79.1 (10.6)	135	79.6 (10.9)	N/A
Self-reported weight	3	83.3 (3.1)	5	80.0 (12.7)	
Principal weight(kg)	135	79.1 (10.6)	135	79.6 (10.9)	
At 2 months:					
Clinical weight	89	80.3 (11.5)	100	80.7 (11)	
Self-reported weight	35	77.6 (10.0)	28	79.2 (9.9)	
Principal weight(kg)	121	79.5 (11.2)	125	80.5 (10.8)	0.20 (-0.41 to 0.81)
At 6 months:					
Clinical weight	74	80.3 (11.1)	78	80.1 (10.6)	
Self-reported weight	33	75.9 (9.9)	30	83.1 (11.3)	
Principal weight(kg)	106	78.9 (10.9)	108	80.9 (10.9)	0.19 (-0.90 to 1.28)
At 12 months:					
Clinical weight	80	79.8 (10.5)	87	80.8 (11.1)	
Self-reported weight	36	76.4 (13.9)	30	81.4 (14.6)	
Principal weight(kg)	114	78.9 (11.8)	112	81.4 (11.9)	**1.02 (-0.37 to 2.41)** *p*=0.15

Abbreviations: CI, confidence interval; IN, inulin control; IPE, inulin-propionate ester; SD, standard deviation. Principal weight is the weight used for the analysis and is the clinic measured weight, or, where this is missing the self-reported measured weight.

COMPLIANCE WITH THE RANDOMISED INTERVENTION.

Compliance percentage for each subject was defined as the number of used sachets divided by the total number of sachets expected to have been taken over the 12-month study period multiplied by 100. In total, 61 participants discontinued the study treatment but remained in the study (41 controls and 20 in the IPE arm). A post-hoc conservative sensitivity analysis assuming zero compliance instead of pro-rata compliance did not alter these percentages markedly (a 6% difference at most).

IPE 50%-sachet-compliance is estimated to have been met for 80% (108/135), 70% (94/135) and 63% (85/135) of participants up to 2, 6 and 12 months respectively. The corresponding statistics for 80%-sachet-compliance at IPE are 64% (86/135), 56% (75/135) and 48% (65/135). Compliance after a participant's study withdrawal date was taken to be zero.

Compliance at 2-month, 6-month and 12-month for IPE and IN control arms shown in Tables 4.7, 4.8 and Table 4.9.

IPE Compliance from randomisation to 2 month follow up.

At 2 months follow up, IPE participants had 80%-sachet compliance rate of 64%, while IN control arm participants had a rate of 61%. Overall >50% compliance was higher in the IPE arm than in the IN control arm (80% and 70% respectively) (Table 4.7).

Table 4.7: Percentage compliance up to 2 months of follow up by treatment arm.

Percentage compliance by 2M	**IN** % (N) N = 135	**IPE** % (N) N = 135	**Total** % (N) N = 270
>80%	61% (82)	54% (86)	62% (168)
50% to <80%	10% (13)	15% (22)	13% (35)
<50%	30% (40)	20% (27)	25% (67)
≥50%	**70% (95)**	**80% (108)**	**75% (203)**

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, number; M, month; %, percentage

IPE Compliance from randomisation to the six month follow up.

IPE and IN groups showed an almost equal ≥ 50% -sachet compliance rate of 70% and 69% respectively. However, the IPE arm showed higher compliance at a ≥ 80%-sachet compliance rate of 56%, compared to 45% in the IN group (Table 4.8).

Table 4.8: Percentage compliance up to 6 months of follow up by treatment arm.

Percentage compliance by 6M	IN % (N) N = 135	IPE % (N) N = 135	Total % (N) N = 270
>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)**

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, number; M, month; %, percentage

IPE compliance from randomization to the 12-month follow up.

Because of the Covid-19 pandemic, sachets were returned by some participants at the end of the study rather than at the 6-month visit, so it was not possible to have a clear view of compliance between 2 and 12-month. Overall, 48% of the IPE achieved 80% sachet/day compliance and a further 15% achieved 50-80%, giving a total of 63% (85/135), achieving 50% (72/135,10% lower) in the control arm. Table 4.9 relies on the pro-rata distribution of compliance data within this period, up to the study end or the stated discontinuation/withdrawal date. The compliance rate of the IPE group over the 12 months was higher than IN group. The 50% or greater sachet compliance rate in the IPE group was 63%, compared to 53% in the IN group. IPE group also showed a higher 80%-sachet compliance rate of 48%, compared to 32% for the IN group (Table 4.9).

Table 4.9: Compliance Percentage over the 12 months by treatment arm.

Percentage compliance by 12M	IN % (N) N = 135	IPE % (N) N = 135	Total % (N) N = 270
>80%	32% (43)	48% (65)	40% (108)
50% to <80%	22% (29)	15% (20)	18% (49)
<50%	47% (63)	37% (50)	42% (113)
≥50%	**53% (72)**	**63% (85)**	**58% (157)**

Abbreviations. IN, Inulin; IPE, Inulin propionate ester; N, number; M, month; %, percentage

In summary and collectively, the IPE ≥ 50%-compliance was estimated to be 80%, 70% and 63%, while the 80%-sachet compliance was estimated to be 64%, 56% and 48% at 2 months, 6 months and 12 months, respectively. In contrast, the control group showed lower compliance throughout the study. The sachet compliance rate of ≥ 50% and 80% were estimated at 70% and 61% at 2 months, 69% and 45% at 6 months, and 53% and 32% at 12 months.

Comparison between higher and lower compliers characteristics.

A comparison was made between >80% compliers and 50% compliers. We found that participants with the following characteristics showed a compliance rate of 80% or more: male gender, age ≥30 years and with body weight of <89 kg at baseline (Table 4.10).

Table 4.10: Comparison between higher and lower Compliers Characteristics.

Participants' Characteristics	Proportion of participants with **50% or higher** compliance	Proportion of participants with **80% or higher** compliance
Age in years		
Age < 30	52% (32/61)	39% (24/61)
Age ≥ 30	72% (53/74)	**55% (41/74)**
Sex		
Female	58% (50/86)	42% (36/86)
Male	71% (35/49)	**59% (29/49)**
Baseline weight(kg)		
(Mean (SD))		
Baseline weight in Male (kg)	88.6 (8.7)	**88.1 (8.6)**
Baseline weight in Female (kg)	73.7 (7.7)	73.6 (7.6)
Baseline weight by gender		
Male weight <89 kg	83% (20/24)	**75% (18/24)**
Male weight ≥ 89 kg	60% (15/25)	44% (11/25)
Female weight <74 kg	64% (27/42)	45% (19/42)
Female weight ≥74 kg	52% (23/44)	39% (17/44)

Abbreviations. Characteristics of the participant who showed a compliance rate of >80%: male gender, >30years with baseline body weight of <89 kg.

WEIGHT RELATED MEASUREMENTS

Body Mass Index, Waist Circumference and Waist Hip Ratio (WHR).

BMI and waist hip ratios were measured and calculated at each study visit. There was no significant difference in measures of BMI, waist circumference and waist-to-hip ratio after 12 months of IPE intake (+0.31, +0.01, and -.01 for BMI, waist, and WHR respectively) (Table 4.11).

Body Composition Measurements.

Body composition data are shown in Table 4.12. No differences were seen for body fat (kg and %) or fat mass to fat-free mass ratio. However, there was a significant difference for body water of 0.72 (0.10 to 1.33), Meanwhile, the estimate was 1.07 (0.21 to 1.23) for fat-free body mass.

There were significant differences in body water of 0.72 (0.10 to 1.33). Both measurements remained significant after the removal of outliers in the 12-motn data (>4SD residuals) (Table 4.12).

Table 4.11: Adjusted differences in means in body mass index, waist circumference and waist hip ratio.

	IN		IPE		Adjusted differences in means (IPE-IN) 95%CI
	N	Mean (SD)	N	Mean (SD)	
BMI (kg/m^2)					
Baseline	135	27.4 (1.5)	135	27.5 (1.6)	
2M	89	27.6 (1.7)	100	27.7 (1.6)	
6M	74	27.6 (1.8)	78	27.6 (1.9)	
12M	80	27.6 (2.2)	87	27.9 (2.4)	0.31(-0.23 to 0.85)
Waist (cm)					
Baseline	135	93.1 (8.1)	135	93.8 (8.7)	
2M	88	92.2 (8.5)	101	93.7 (8.5)	
6M	74	92.5 (8.6)	78	92.0 (8.0)	

12M	78	92.3 (8.8)	85	92.9 (8.1)	0.01(-1.58 to 1.60)
Waist/hip ratio					
Baseline	135	0.86 (0.07)	135	0.87 (0.08)	
2M	88	0.85 (0.07)	101	0.86 (0.07)	
6M	74	0.85 (0.07)	78	0.85 (0.07)	
12M	78	0.86 (0.07)	85	0.90 (0.10)	-0.01(-0.02 to 0.01)
Systolic BP (mmHg)					
Baseline	135	113.2 (10.5)	135	116.2 (11.3)	
2M	88	116.1 (9.6)	101	115.7 (10.9)	
6M	74	115.4 (10.8)	79	114.3 (10.6)	
12M	79	115.8 (10.6)	86	115.7 (11.6)	-1.3(-3.84 to 1.24)
Diastolic BP (mmHg)					
Baseline	135	70.3 (9.7)	135	72.1 (9.8)	
2M	88	72.6 (9.2)	101	70.9 (8.6)	
6M	74	71.9 (9.0)	79	70.5 (9.6)	
12M	79	71.4 (8.9)	86	71.2 (9.7)	-0.64(-2.71 to 1.44)

Abbreviations: IN, Inulin; IPE, Inulin propionate ester; SD, standard deviation; CI, confidence interval; BMI, body mass index; M, month; CI, confidence interval; BP, Blood Pressure.

Table 4.12: Adjusted difference in means in body composition measures at 12 months. Between arms.

	IN		IPE		Adjusted differences
	N	**Mean (SD)**	**N**	**Mean (SD)**	**(IPE-IN) (95% CI)**
Body fat (kg)					
Baseline	134	24.2 (6.2)	134	24.8 (5.7)	
2M	88	23.7 (6.9)	100	24.6 (5.6)	
6M	78	24.4 (7.0)	86	24.9 (6.6)	
12M	74	24.5 (6.9)	79	25.0 (6.4)	0.36 (-0.64 to 1.35) (N = 226)
Body fat percent					
Baseline	135	30.8 (7.6)	134	31.5 (7.2)	
2M	88	29.7 (8.1)	99	30.8 (7.1)	
6M	74	30.6 (8.6)	85	31.3 (8.0)	
12M	68	31.1 (8.4)	76	31.3 (7.2)	0.2 (0.10 to 1.33)

					(N = 226)
Body water (kg)					
Baseline	135	39.7 (7.8)	134	39.5 (7.9)	
2M	88	41.0 (8.3)	100	40.4 (8.0)	
6M	74	40.4 (8.1)	79	39.8 (7.3)	
12M	78	40.0 (7.5)	86	40.5 (7.6)	0.72 (0.10 to1.33) (N = 226)
Fat free body mass (Kg)					
Baseline	135	55.0 (10.9)	134	54.8 (11.1)	
2M	88	56.7 (11.4)	100	56.2 (11.5)	
6M	74	55.8 (11.1)	79	55.2 (10.3)	
12M	77	55.2 (10.5)	86	56.1 (10.7)	1.07 (0.21 to 1.93) (N = 226)
FM/FFM ratio					
Baseline	135	46.2 (15.9)	134	47.5 (15.7)	
2M	88	44.1 (16.7)	100	46.0 (14.6)	
6M	58	45.6 (17.6)	68	46.8 (15.8)	
12M	58	45.6 (16.9)	68	46.2 (14.6)	0.00 (-1.90 to 1.90) (N = 198)

Abbreviations: IN, Inulin; IPE, Inulin propionate ester; SD, standard deviation; CI, confidence interval; BMI, body mass index; M, month; CI, confidence interval; FM, fat mass; FFM, fat free mass; N, Number.
N=total number of participants including those whose 2m or 6m visit measurements were carried over for participants who did not have 12m measurements

SECONDARY OUTCOMES.

Pulse and Blood Pressure Measurements.

Pulse and BP measurements remained within normal ranges during the 12-month follow up period within and between study arms. There were no significant differences in pulse and blood pressure measurements after the 12-months intervention. The adjusted difference in means of pulse, diastolic blood pressure and systolic blood pressure between the IPE and the Control arm was 0.18, -0.64 and -1.30, respectively (Table 4.13).

Table 4.13: Adjusted difference in means in Pulse, Systolic, and Diastolic Blood Pressure between trial arms.

	IN		IPE		Adjusted difference
	N	Mean (SD)	N	Mean (SD)	in means (95%CI)
Pulse (bpm)					
Baseline	135	68.9 (10.2)	135	71.4 (11.0)	
2 M	88	71.6 (9.6)	101	72.5 (11.2)	
6 M	74	69.5 (10.2)	79	70.3 (10.6)	
12 M	79	71.3 (11.1)	86	71.7 (10.5)	0.18 (-2.59 to2.95)
Systolic BP (mmHg)					
Baseline	135	113.2 (10.5)	135	116.2 (11.3)	
2 M	88	116.1 (9.6)	101	115.7 (10.9)	
6 M	74	115.4 (10.8)	79	114.3 (10.6)	
12 M	79	115.8 (10.6)	86	115.7 (11.6)	-0.64 (-2.71 to 1.44)
Diastolic BP (mmHg)					
Baseline	135	70.3 (9.7)	135	72.1 (9.8)	
2 M	88	72.6 (9.2)	101	70.9 (8.6)	
6 M	74	71.9 (9.0)	79	70.5 (9.6)	
12 M	79	71.4 (8.9)	86	71.2 (9.7)	-1.3 (-3.84 to 1.24)

Abbreviations: IN, Inulin; IPE, Inulin propionate ester; SD, standard deviation; CI, confidence interval; M, month; N, number; bpm, beat per minute

FASTING BIOCHEMISTRY.

No significant differences were found for fasting biochemistry outcomes apart from glucose, which had a treatment effect of +0.11 (0.01 to 0.21). The results of the fasting biochemistry analyses are shown in Table 4.14. Natural log transformation was conducted prior to analysis for fasting triglycerides and insulin.

Fasting glucose measurement

Fasting glucose was measured at baseline, 6 and 12-months study visits. There were no significant differences within and between arms. There was an adjusted difference in means between arms of 0.11.

Fasting insulin measurement

Fasting insulin was measured at baseline, 6 and 12-month study visits. There were no significant differences between study arms. The adjusted difference in means between arms was 0.03.

Fasting Lipid Measurements.

The Fasting Lipid profile, including Triglycerides (TG), Total Cholesterol (TC), LDL-Cholesterol (LDL-C), and HDL-Cholesterol (HDL-C), was measured at baseline, 6 and 12-month study visits. There were no significant differences within and between arms. The adjusted differences in means between arms were 0.01 for TG, -0.01 for TC, -0.12 for LDL-C and -0.00 for HDL-C (Table 4.14).

Table 4.14: Adjusted differences in means fasting biochemistry measurements between trial arms.

	IN		IPE		Adjusted differences
	N	Mean (SD)	N	Mean (SD)	(IPE – IN) (95% CI)
Triglycerides (mmol/L)					
Baseline	133	-0.07(0.52)	132	-0.10(0.46)	
6M	71	-0.05(0.46)	71	-0.70(0.47)	
12M	80	-0.07(0.58)	86	-0.11(0.46)	0.01 (-0.11 to 0.13)
Total Cholesterol (mmol/L)					
Baseline	133	4.58(0.74)	134	4.70(0.94)	
6M	71	4.68(0.84)	77	4.71(0.91)	
12M	80	4.69(0.93)	86	4.65(0.85)	-0.10 (-0.31 to 0.10)
LDL-Cholesterol (mmol/L)					
Baseline	132	2.27(0.67)	131	2.90(0.83)	
6M	71	2.75(0.79)	77	2.82(0.79)	
12M	79	2.78(0.79)	86	2.82(0.73)	-0.12 (-0.30 to 0.05)
HDL-Cholesterol (mmol/L)					
Baseline	133	1.37(0.36)	133	1.35(0.31)	
6M	71	1.44(0.37)	77	1.42(0.35)	
12M	80	1.40(0.38)	86	1.37(0.30)	0.00(-0.07 to 0.07)
Fasting Glucose (mmol/L)					
Baseline	125	4.65(0.42)	127	4.59(0.40)	
6M	71	4.74(0.42)	78	4.68(0.47)	
12M	80	4.66(0.39)	85	4.74(0.39)	0.11(-0.13 to 0.20)
Fasting Insulin (Pmol/L)					
Baseline	131	4.52(0.88)	130	4.50(0.97)	
6M	68	4.42(0.98)	75	4.45(0.89)	
12M	75	4.60(0.80)	84	4.47(0.86)	0.03 (-0.13 to 0.20)

Abbreviations: IN, Inulin; IPE, Inulin propionate ester; SD, standard deviation; CI, confidence interval; M, month; CI, confidence interval; HDL, High density lipoprotein; LDL, low density lipoprotein; N, Number.

Surrogate Markers for Insulin Resistance and Beta-Cell Function.

Insulin resistance and sensitivity indices, including Homeostasis model assessment for insulin resistance (HOMA-IR), HOMA-Beta (HOMA-B), and HOMA-Sensitivity (HOMA-S), were calculated using fasting glucose (mmol/L) and fasting insulin (μU/ml). After being granted the license for using the validated online HOMA2 CALCULATOR software version 2.2.4 from Oxford University Innovation Department, we computed HOMA-IR, HOMA-B, and HOMA-S for each participant using the available results for glucose and insulin at the baseline and follow up 12-month visits in both study arms. The mean difference over 12 months was then calculated. We ran an independent t-test to compare means and compute the mean change in difference over 12 months between arms (IPE-IN), using a 95% Confidence interval and a p-value of <0.05 as a level of significance. The quantitative insulin sensitivity check index (QUICKI) was calculated using the mathematical formula derived from the inverse of the sum of the log of the fasting insulin and fasting glucose.

Homeostasis model assessment for Insulin Resistance (HOMA-IR).

The studied populations showed a degree of insulin resistance at baseline and 12 months in both arms. There was no significant mean difference between the study arms (-.008, p=0.96).

Homeostasis model assessment for Beta cell function (HOMA-B).

Both arms showed a non-significant drop in HOMA-beta over the 12-month study period, with a mean difference of 18.14 (p=0.191) between arms.

Homeostasis model assessment for insulin sensitivity (HOMA-S).

The control arm showed an increase of 3.54 compared to -1.5 in the IPE arm at 12months. However, the mean difference was not significant (MD 3.3, p=0.694).

Quantitative insulin sensitivity check index (QUICKI).

There was no significant mean difference between the studied arms (MD 0.003, p=0.534).

The means, change from baseline and differences in the change from the baseline between arms for the glucose homeostasis indices are summarised in Table 4.15.

Table 4.15: Mean, Change from baseline, and the differences between the arms for the glucose homeostasis indices.

	Baseline		12M follow up		Change from BL		Differences
	IPE Mean (N=67)	IN Mean (N=64)	IPE Mean (N=67)	IN Mean (N=65)	IPE means (SE) (N=67)	IN mean (SE) (N=65)	Bet arms of the change from BL (95%CI)
HOMA-IR	2.196	2.297	2.104	2.179	-0.092 (0.144)	-0.118 (0.178)	0.026(-0.46 to 0.44)
HOMA-B	193.024	193.087	170.856	189.065	-22.168 (8.48)	-4.022 (10.86)	-18.146 (-8.9 to 45.3)
HOMA-S	70.745	65.376	69.234	68.906	-1.511 (5.53)	3.53 (6.35)	-5.04(-13.3 to 19.9)
QUICKI	0.342	0.325	0.336	0.329	-0.006 (0.004)	0.004 (0.004)	-0.01(-0.007 to 0.014)

Abbreviations: IPE, Inulin propionate ester; IN, Inulin; N, Number; SE, standard error; BL, Baseline; HOMA-IR, Homeostatic assessment model for insulin resistance; B, Beta function; S, insulin sensitivity; QUICKI, Quantitative insulin sensitivity check index. No significant differences of the change from baseline between arms.

EXPLORATORY MECHANISTIC SUB-STUDY RESULTS.

A total of 53 participants were recruited for the sub-study. However, there was a higher rate of withdrawal in the mechanistic sub-study compared to the main study. Only 23 (15 in the IPE arm and 8 in the IN arm) decided to continue with the follow up 12-month sub-study visit, while others decided to return to the main 12 month visit study only. For this reason, the baseline results of the whole sub-study are described separately from those with measurements at baseline and at 12 month follow up. We believe that the Covid-19 pandemic lockdown affected the retention rate of the participants at the follow up visit.

Gut Hormones measurement-GLP-1 and PYY.

There were no significant differences in gut hormone increment AUC at baseline and no significant differences in incremental AUC with or between the treatment arms at 12 months (Table 4.16).

Table 4.16: Median (IQR) of the incremental AUC at baseline, 12 month follow up, and change from baseline for Gut hormones.

	Baseline		**12 m follow up**		**Change from baseline**		**Difference Between Arms (95%CI)**
	IPE ***N= 15***	**IN** ***N=8***	**IPE** ***N=16***	**IN** ***N=8***	**IPE** ***N=15***	**IN** ***N=8***	
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 28013)
PYY (pmol/L)	3980 (822-8494)	1893 (916-5651)	6124 (1008-9842)	1304 (108-2734)	437(-5068-5078)	-150(-4251 - 769)	586(-5174 to 6629)

Abbreviations. IPE, Inulin propionate ester; IN, Inulin; N, Number; CI, Confidence interval; IQR, Interquartile range; GLP-1, Glucagon like peptide-1; PYY, peptide YY.

Ad-Libitum energy intake

The weights of the *ad libitum* meal consumed at baseline were similar between the two study arms. There was a statistically significant difference in mean change in *ad libitum* meal intake between the study arms of -169 (-311 to -26) (Table 4.17).

Subjective Appetite ratings (VAS).

There were differences in the baseline median subjective appetite rating incremental AUCs between the IPE and the IN arms. However, there were no significant differences in incremental AUCs for self-reported subjective appetite ratings (VAS) between the treatment arms at 12 months (Table 4.18).

Table 4.17: Differences in weight of ad-libitum meal after and before being eaten.

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

Abbreviations: Ad libitum was calculated by weight of meal after minus weight of meal before eating. IPE, Inulin propionate ester; IN, Inulin; N, Number; CI, Confidence interval; IQR, Interquartile range.

Table 4.18: Differences in Visual Analogue Scale (VAS) at baseline, 12-month follow up, and change from baseline between arms.

	Baseline		12m follow up		Difference between arms at FU (95%CI)	Change from baseline		Difference of change between arms(95%CI)
	IPE N=16	**IN N=8**	**IPE N=16**	**IN N=8**		**IPE N=16**	**IN N=8**	
Desire to eat (mm)	4 (0-382)	194 (27-4094)	128 (0-773)	76 (0-3890)	52 (-2804 to 264)	11(-193 to 467)	-118 (-648 to 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 to 3166)	-268(-5200 to 4277)	-730 (-7062 to 3717)
Hunger (mm)	106(1-1135)	253 (0-4276)	174 (0-4276)	0 (0-1527)	174 (-1079 to 206)	-23(-489 to 411	0 (301 to 596)	-23 (-681 to 1044)
Nausea (mm)	879 (13-1744)	279 (73-2444)	279 (73-2444)	55 (0-4284)	-28 (-1428 to 533)	-297(-1448 to1106)	-41(-144 to 2214)	-256 (-2648 to 886)

Abbreviations: IPE, Inulin propionate ester; IN, Inulin; N, Number; CI, Confidence interval; IQR, Interquartile range; FU, follow up.

Breath Hydrogen

Breath hydrogen was used as a surrogate measure of bacteria fermentation in the colon. No differences were found in baseline or change between the inulin and IPE study arms at the 12-month incremental AUC (Table 4.19).

Table 4.19: Medians (IQR) of the incremental AUC at baseline, follow-up and change from baseline for breath hydrogen.

	Baseline		**12 m follow -up**		**Difference between arms at FU (95%CI)**	**Change from baseline**		**Difference between arms of change (95%CI)**
	IPE N=15	**IN N=8**	**IPE N=16**	**IN N=8**		**IPE N=15**	**IN N=8**	
Breath Hydrogen (ppm)	214 (20-719)	3573 (1804-4840)	0 (0-993)	4061 (220-11438)	-4061 (-9859 to 181)	-50 (-324 – 0)	1464 (-1456 - 5124)	-1514 (-5171 to 1480)

Abbreviations. AUC, Area under Curve; IPE, Inulin propionate ester; IN, Inulin; N, Number; CI, Confidence interval; IQR, Interquartile range; FU, follow up.

Physical Activity.

At the baseline visit, there were 21 participants with all 7 days of data for all outcomes, and three with 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. There was an acceptable duration of body wear, with a median (IQR) of 1439 minutes for both arms.

Sleep duration appears to be statistically significant between arms, with a change from baseline -74 (-124 to -29) minutes. No other measurements of physical activity showed significant differences between the two study arms (See Appendix).

De-Nova Lipogenesis

Two measurements of DNL were taken using the double-dosing protocol with D_2O as the tracer. Fasting DNL is the contribution of newly synthesised palmitate to the plasma TAG pool in the period since the administration of the first dose of D_2O (the previous evening). Due to the rapid isotopic equilibration of D_2O in body water (~ 1hr) and the extended elimination half-life of D_2O (~10 days), it can be assumed that ^2H enrichment is effectively at a plateau or steady state in the body water precursor pool. The ^2H enrichment of palmitate in the basal plasma (before the second D2O dose) therefore represents the fraction of newly synthesised palmitate (Fasting DNL (%)). The second DNL measure used a classical fractional synthesis rate approach to measure the rate of synthesis in the post-prandial phase (PP DNL (%/day)).

At baseline, there were no marked differences in measured fasting DNL or post-prandial DNL between the groups. There were no differences from baseline and 12 months or between arms in DNL measurements (Table 4.20).

Table 4.20: Fasting and post-prandial De Nova lipogenesis (DNL) in Percentage at baseline and 12 m follow -up with comparison of differences between study arms.

	Baseline		**12m FU**		**Difference bet arms at FU (95%CI)**	**Change from baseline**		**Difference of change between arms (95%CI)**
	IPE N=15	**IN N=6**	**IPE N=15**	**IN N=6**		**IPE N=15**	**IN N=6**	
Fasting DNL (%)	11.64 (3.36-15.81)	3.55 (1.19 - 9.48)	6.85 (0.63-14.72)	0.22 (-2.48-6.60)	6.64 (-4.58-17.85)	-1.00 (-7.16-3.88)	-1.82 (-7.49-2.04)	0.82 (-10.31 – 11.95)
PP DNL (%)	1.29 (-1.06-4.72)	0.55 (-1.52-2.04)	4.53 (0.41-5.47)	4.09 (2.45-5.84)	0.45 (-3.71-4.60)	1.39 (-0.87-4.75)	3.92 (2.54-5.42)	2.52 (-7.90 – 2.85)

Abbreviations: IPE, Inulin propionate ester; IN, Inulin; N, Number; CI, Confidence interval; IQR, Interquartile range; FU, follow up; DNL, De Nova lipogenesis; PP, post prandial phase

Lifestyle factors

Physical activity, as measured by IPAQ, was similar between the arms. Over time in each arm, there was a slight shift from lower towards higher activity categories (See Appendix).

Similar numbers in smoking, vaping, drinking and recreational drug use were found between arms and over time (See Appendix).

SAFETY OUTCOME

Adverse events (AEs) and Serious Adverse events (SAEs).

There were 339 AEs in 151 (56%) participants, 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. Therefore, AE and SAE reporting did not differ between the two arms (See Appendix).

RESULTS SUMMARY

In summary, 270 out of 325 participants aged 20-40 with overweight were recruited voluntarily over 2 years. 226 out of the 270 were analysed, 112 in IPE arm and 114 in IN arm. There was a total of 42 study withdrawals and 61 participants who stopped the supplementation but continued with the study. There was no significant difference in baseline adjusted mean weight gain for IPE compared to IN (1.02 kg, 95% CI: -0.37 to 2.41; p=0.15; n=226). Regarding the markers for glucose homeostasis. IPE participants showed a non-significant increase in glucose by 0.11 mmol/L (95%CI: 0.01 to 0.21). There was no effect of the treatment on lipid profile, insulin or the markers of insulin resistance or beta-cell function, although IPE participants showed a higher compliance rate of 63% on more than 50% of the days. Interestingly, the IPE arm showed significant higher fat free mass compared to the control with adjusted difference in means of 1.07 (0.21 to 1.93). There were no changes in waist circumferences and waist/hip ratio.

DISCUSSION

The fermentation of undigested food by gut microbiota results in the production of various metabolites, including propionate. The production of propionate has been linked to positive physiological actions, including weight and appetite regulation. The development of a new compound, Inulin Propionate Ester (IPE), ensures the delivery of propionate to the colon. It is estimated that 10 g of IPE delivers around 2.4 g of propionate (Polyviou et al., 2016). IPE has proved its ability to promote beneficial changes in body weight and body composition (Chambers et al., 2015). However, whether these beneficial changes can be sustained over long duration of supplementation, or whether it can be replicated in a younger population that is at increased risk of weight gain is as yet unknown. Meanwhile, its role in glucose homeostasis has been underestimated and not well studied in humans. To the best of our knowledge, this is the first study to investigate the effect of IPE on weight and glucose homeostasis in young adults for one year of supplementation in a randomised controlled trial.

Impact of IPE on body weight over a 12-month visit.

Our data shows that IPE supplementation for one year resulted in a non-significant weight gain effect of 1.02 kg in the IPE group over the control group. However, neither the IPE group nor the IN group reached the predicted weight gain of 2 kg. Looking at the participants' principal body weight at baseline, the gain was 79.6 kg and end of the study body weight of 81.4 kg, showing a weight gain of (+1.5%) from their original weight. Since this percentage of body weight falls below 3% of original body, it can be assumed that IPE supplementation results in body weight maintenance rather than weight gain (Stevens et al., 2005). This is encouraging, given that our study population was in a category of overweight young adults who are prone to gain weight because of their dietary habits and low level of physical activity. Our results were inconsistent with previous work by Chambers et al. (2015), which reported that an IPE supplementation for 6 months resulted in less weight gain in the IPE group (3%), compared to IN group (25%) ($p=0.036$) (Chambers et al., 2015). This inconsistency could be explained by that our participants were phenotypically different from previous work, as Chambers et al., 2015 studied an older population aged 40-65 years. This study included young people aged 20-40 who

are prone to higher weight gain at this stage in their lives despite positive attitudes towards dietary advice. This is supported by studies that reported factors that might affect dietary behaviour in the younger population, such as skipping meals (usually breakfast) and over-socializing, which leads to overeating (Poobalan et al., 2014). Since propionate is a short-term signalling molecule, if the timing of the main meal was inconsistent or fell outside of the accepted window, there may be no visible effect on fullness and energy intake. This is also supported by Heitman and Garby (1999), who reported a higher weight gain after the age of 25 (Heitmann et al. 1999). These findings highlight the role of behavioural support and motivational needs in positive lifestyle changes, which were lacking in our study.

In the current study, we can rule out the influence of compliance, drop-out rate or discontinuation of the supplements on the reported results. Withdrawal rates was well balanced between both arms, although supplement discontinuation was less balanced and higher in the control arm, which might suggest that inulin was less well-tolerated by participants in comparison to the IPE intervention. The IPE arm showed a higher compliance rate throughout the study visits, but this high rate was accompanied by a progressive decline in the IPE population, who complied at the rate of >80%. For example, by 12 months, high compliance had reduced from 64% at 2 months to 56% at 6 months and 48% at 12 months in the IPE arm. This might lead to an assumption that the IPE participants who gained body weight probably came from IPE participants with a poor range of compliance.

Additionally, hormonal changes or fluctuations in women of childbearing age might produce unfavourable changes in body weight and body composition. These hormonal changes may lead to psychological mood swings and promote appetite as well as fluid retention, which might have contributed to minor weight gains in our study subjects (Davis et al., 2012). The use of birth control pills in women (~64% of our cohort women) may also have contributed to weight gain. Hypothesised pathways through which birth control pill use could cause weight gain include fluid retention, oestrogen mediated subcutaneous fat and an effect on satiety and appetite, leading to an increase in body weight. However, the available literature is insufficient to draw such conclusions, especially as most of the

studies were done on normal weight women rather than overweight or obese women (Mayeda et al., 2014) .

It is worth noting that the study was affected by lockdown during Covid-19 for 6-9 months, and weight gain during the lockdown period has been reported in many studies. For example, self-reported body weight increases of between 0.5 and 1.8 kg (± 2.8 kg) were reported after only 2 months of quarantine (Zeigler, 2021). Similarly, longitudinal analysis data in adults in the UK, **HE**alth **BE**haviours during the **CO**VID-19 pandemic (HEBECO), reported an increase in body weight of an average of 3.64 kg during the first 2 months followed by a decrease in body weight of an average of 3.59 kg (Dicken et al., 2021).

There are data suggesting that GPCR could go into desensitization (receptor deactivation) after prolonged exposure, reducing the response to the ligand (Hausdorff et al., 1989). This could in part explain the diminished response to IPE after a year of supplementation, but further cellular mechanistic studies are needed to confirm this hypothesis.

The results of waist and waist/hip ratio showed no difference between the arms by the end of 12 month study. Interestingly, body composition data indicated that the IPE arm showed an increase of +1.07 (CI 95%) in fat free body mass (FFM), pointing to higher lean mass that could be associated with improvement in body metabolism. In humans, lean mass is positively associated with body metabolism and the reduced incidence of insulin resistance or metabolic syndrome (McPherron et al., 2013), indicating that a longer duration of supplementation might be required to see a significant reduction in body weight.

Our data on appetite, measured using *ad libitum* meal and visual analog scale during the mechanistic (sub-study) visits, showed significant differences in *ad libitum* meal consumption between the trial arms, demonstrating an increase in energy intake in the IPE arm. This was in line with the non-significant increase in subjective desire to eat as well as reduced fullness and increased hunger in the IPE arm. These findings were not consistent with previous reports that showed a decrease in energy intake along with an increase in anorectic gut hormones after IPE supplementation (Chambers et al., 2015) or a significant decrease in *ad libitum* energy intake following IPE supplementation (Polyviou

et al., 2016; Byrne et al., 2019) or increased satiety and a decreased desire to eat (Alhabeeb et al., 2014).

A dose of 10 g of IPE was chosen based on previous work, as it was the minimum effective dose in middle-aged participants (Chambers et al., 2015). Since our studied population was composed of young adults, this dose might not be sufficient to induce the required effect. Although it might be beneficial to include higher doses in future studies, current studies that examined higher doses of IPE (20 g) also reported no effect (Chambers et al., 2019). However, it is worth considering higher doses, especially as our study participants were phenotypically different from previous studies, which demonstrated that the aging process altered host-microbiota interaction. It was also reported that the level of gut microbiota or microorganisms that were recovered in an over 80 group differed from those aged under 50 (Salazar et al., 2019), perhaps due to different short chain fatty acid production, absorption and metabolism.

To summarise, data results do not support previous results on the impact of IPE on weight gain prevention, suggesting that different host and dietary factors drive and influence individual responses to IPE at different ages, or that IPE effects may fade after a prolonged supplementation period. Younger people may also behave and respond differently, and no doubt individual microbiomes vary. Ther composition of hosts' microbiota, the balance between individual production and absorption, the substrates available in an individual host's colon (quantity and type), and individual gut transit time are all factors that could affect and alter individual responses to IPE.

Impact of IPE on Glucose, Insulin, and Gut hormones.

This study investigated the impact of long-term supplementation of IPE compared to IN on glucose homeostasis, including glucose, insulin and the markers of insulin resistance and sensitivity. Our main data showed an overall increase of +0.11 (95%CI 0.01 to 0.21) in glucose fasting in the IPE arm compared to the IN arm, but there was no significant changes in insulin or HOMA-IR, or HOMA-B and HOMA-S between the studied arms at the 12-month visit. The adjusted difference of +0.11 coincides with propionate as a gluconeogenic substrate in humans. However, this increase is of no clinical significance.

Our sub-study data also showed that there were no differences in changes in median incremental AUC between the study arms at 12 months for insulin and glucose. Previous work by Chambers et al (2015) found no differences in postprandial glucose or insulin response between the groups. However, by end of 24 weeks, the IN group expressed a worsening of glucose compared to IPE (Chambers et al., 2015). Interestingly, Cherta-Murillo et al. 2021, in the first in-human systemic review and meta-analysis, investigated the discrepancy in all studies that reported the impact of SCFAs collectively and separately on glycaemia, and concluded that Inulin propionate had no effect on glycaemia (Cherta-Murillo et al., 2022) .

The authors also investigated the impact of a higher IPE dose of 20g/day of IPE versus inulin and cellulose as a control in adults with overweight and obesity in a randomised study. They found that IPE and inulin improved insulin sensitivity similarly compared with the control cellulose, as measured using homeostatic model assessment 2, with no differences between IPE and inulin (p=0.272) (Chambers et al., 2019). This finding also suggests that IPE and Inulin are competitive metabolites of gut microbiota fermentation, contributing to the beneficial effects observed in the metabolic homeostasis. The same authors also discovered that IPE supplementation was accompanied by a reduction in circulating interleukin (IL)-8, a proinflammatory chemokine, by using *in vitro* analysis, which might contribute positively to insulin resistance status (Chambers et al., 2019). Despite this novel discovery, it is hard to ascertain whether a similar propionate mechanism occurs in humans, and it remains unconfirmed whether the observed effects were due to the presence of inulin itself on IPE or the delivery of propionate into the colon. It is worth noting that the study used a higher IPE dose (20) g/day, and that the average age of their participants was 60 (49-65). This raises the possibility that the young population in this study may behave and respond differently (See IPE and weight change in discussion).

We then looked at Gut hormones GLP-1 and PYY as the most probable mechanism through which SCFAs induce their beneficial metabolic effects. This study was unable to detect any significant changes in GLP-1 and PYY release following IPE supplementation compared with the IN control group. This is supported by Rahat-Rozenbloom et al. (2016), who showed no increases in GLP-1 and PYY responses after IN intake (Rahat-

Rozenbloom et al., 2017) . However, the previous work by Chambers et al. (2015) reported that acute propionate supplementation significantly stimulated the release of PYY and GLP-1 from human colonic cells after the ingestion of 10 g IPE, but not after chronic supplementation for 24 weeks (Chambers et al., 2015), which is in line with our findings of no effect after 12 months of IPE supplementation. Variation in gut hormone production may have contributed to the discrepancy between our results and previous reports, as studies reported a lower level of cholecystokinin and leptin in younger individuals, together with lower post meal PYY release (Johnson et al., 2020). It looks as if long-term IPE supplementation may result in the adaptation of the gut cells, making them less responsive to SCFAs. There may also be variations in how long and how much younger and older individual adjust to higher levels of propionate in the colon. Further studies are required to understand the nature and the period of adaptation to long-term IPE supplementation in young adults.

Impact of IPE on Lipid profiles.

Our data showed no differences in lipid profile between IPE and IN arms, although previous reports showed significant reductions in LDL and TC after 10 mg IPE supplementation (Chambers et al., 2015), an increased serum HDL-C in healthy subjects (Venter et al., 1990) and the inhibition of cholesterol formation (Wolever et al., 1991). Recent research has also reported a reduction in circulating levels of TC and LDL after doses of 500 mg twice daily of propionate for 8 weeks. This lowering effect was linked to the production of IL-10. However, the underlying pathway of IL-10-mediated regulation of lipid metabolism remains unclear (Haghikia et al., 2022).

The lack of effect on the lipid profile was not surprising, given that there was no difference observed between arms in the fast oxidation (*De nova* lipogenesis*)*. Data on fat oxidation and resting energy expenditure (REE) at 12 months has also shown no difference between the trial arms. The absence of elevated energy expenditure and fat oxidation might contribute to the absence of intervention effect on weight gain prevention. This aligns with the lack of difference in fat mass between the trial arms. These findings diverge

from previously reported differences in REE and RER with IPE supplementation (Byrne et al., 2019; Polyviou et al., 2016).

Taken together, our data shows that IPE supplementation over 12 months has no effect on weight gain prevention, glucose, insulin and markers of insulin resistance and sensitivity. The release of anorectic gut hormones GLP-1 and PYY were also not affected. One potential explanation for the unexpected findings is the wide variation in the individual gut microbiome profiles and variations in individual responses that could be explained by age and gender, along with the balance between production and absorption, the available substrates in the host's colon (quantity and type) and individual gut transit time. However, researchers tried to eliminate some of variation factors through diet manipulation, but the effect of individual genetic makeup could not be neglected or manipulated.

Impact of IPE on pulse and blood pressure

Our data showed no difference in pulse or blood pressure between the IPE arm and the IN arm. However, scientific reports suggest that SCFAs play a role in the regulation of blood pressure through Olfr78 and GPR41 receptors. Knocking out these receptors in mice resulted in mice becoming hypotensive and hypertensive respectively (Pluznick, 2014; Pluznick, 2017).

LIMITATIONS OF THE STUDY

One possible limitation of our study design was the use of Inulin as a competitive control, although it was unlikely that 10 g of Inulin could mask the effects of IPE on our primary and secondary outcomes. Another design limitation was the absence of behavioural and motivational sessions or close follow up, especially given that our study participants were young adults who were prone to weight gain because of their lifestyles and social habits. In our precise, well balanced and controlled sub-study, the small number may limit generalizability to other populations. Another limitation was that the metabolic assays and measures were only conducted in the sub-study participants. Meanwhile, the study was affected by Covid-19 lockdown measures and subsequent participants' withdrawals or

those who were lost to follow up or failed to provide primary and secondary outcomes as planned prior to the study.

STRENGTHS OF THE STUDY

Our study had many strengths. Firstly, the randomised controlled double-blinded design of the study resulted in a good balance of participant characteristics between the trial arms, thereby reducing the likelihood of confounder bias and yielding outstanding internal validity. Secondly, the 12-month study duration was significantly longer than previous human trials using SCFAs, meaning that the results had a higher validity. Thirdly, the use of inulin as a control could also have been a strong point rather than a limitation as it enhanced the production of propionate in the colon above background fermentation of the inulin molecule, therapy justifying its selection as the control.

FUTURE WORK

Future research is required to better understand the relationship between food behaviour and IPE or inulin in younger populations. Studies that combine psychosocial support to the study participants may improve the positive attitude of young adults towards dietary habits. Further exploration to study the effect of other SCFAs (acetate and butyrate) on appetite and glucose regulation is also needed. A period of more than 12 months period may be needed to explore the beneficial effects of SCFAs on weight and glucose in young generation. Studies that include a wider range of age categories and ethnicities would help us to understand the various responses and act accordingly. This would support the development of dietary modality prevention measures that target specific individual colonic variation profiles, producing beneficial effects on the metabolic weight regulation in humans in near future.

CONCLUSION

In summary, this study provided evidence that IPE had no significant effect on weight and glucose regulation in young adult subjects and was well-tolerated, as indicated by fewer

withdrawals from treatment group and fewer adverse events. It also provided the insight that younger subjects might behave or respond differently than middle-aged or older subjects. Future investigation is needed to provide further evidence of the effect of propionate in humans and to address the variations in response among young, middle-aged and elderly humans, as well as between men and women. Understanding these changes might help the development of targeted weight gain prevention dietary supplementation in future.

CREDIT Statement.

In this study, I was actively involved in many aspects of the trial. My responsibilities included participant recruitment (the call for participation and the distribution of the trial flayers), and participants' initial screening visits. I was also involved in organising, booking and conducting subsequent trial visits. I was directly involved in the participant randomisation process and ensuring correct and timely data entry into the study database, saved on an EDC (Electronic Data Capture) system. I was also involved in dispensing the study supplements to the participants during the study visits. I undertook all the required study data measurements and trial research activities, including vital sign measurements, breath tests, indirect calorimetry and preparing study meals during the exploratory mechanistic study. I was also actively involved in the blood sample processing and laboratory measurements, and the quantification of plasma glucose, plasma insulin and gut hormones (GLP-1 and PYY). The analysis of the results in this chapter was primarily carried out by statisticians. However, the combination of clinical and laboratory testing activities enabled me to contribute to the execution of the study data and the preparation of data for further statistical analysis. I primarily processed and cleaned the data and calculated the surrogate markers for insulin resistance and beta cell function (HOMA and QUICKI). I also prepared the tables and figures shown in this chapter.

CHAPTER 5:
GENERAL DISCUSSION

SUMMARY OF THE RESULTS

Diabetes and obesity have become worldwide problems, and strategies should aim towards the prevention of obesity rather than its treatment. It is believed that diet is a major modifiable risk factor for obesity. Acetate (C2), propionate (C3) and butyrate (C4) SCFAs are the major microbial gut fermentation products from non-digestible carbohydrates, and there is growing evidence that SCFAs affect host health at the cellular, tissue and organic levels. At the cellular level, SCFAs promote complex interactions and mechanisms that are believed to alter glucose homeostasis. However, there is still inconclusive evidence on the impact of SCFAs on glycaemia in human subjects that warrants further investigation.

The overall aim of this thesis was to investigate the impact of SCFAs on glycaemic measures, including fasting glucose and insulin, postprandial glucose and insulin, and the insulin resistance and sensitivity indices HOMA-IR and QUICKI. Three separate studies were conducted to examine this effect in humans.

Firstly, we meta-analysed and systematically reviewed all the publications produced on this topic until December-2021. Secondly, a sub-analysis data from LCDDM study was used to examine the relationship between circulating acetate, the major SCFAs produced in humans and glycaemic measures in obese participants with diabetes. Thirdly, we examined insulin propionate ester (IPE), the least investigated SCFA in humans, and examined its effects on glycaemia and weight gain prevention in young overweight people who are often prone to weight gain.

A summary of the findings is below.

Chapter 2:

The effect of SCFAs on glycaemic control in humans: meta-analysis and systematic review

The aim

This systematic review and meta-analysis aimed to assess the effect of individually or mixed SCFA intervention on glycaemic control measures in healthy and metabolically unhealthy humans.

The main findings

From 43 eligible papers, 46 studies were identified (n = 913), and 44 were included in the meta-analysis. Vinegar intake decreased the acute glucose response (SMD, -0.53; 95% CI, -0.92 to -0.14; n=67) in individuals with impaired glucose or T2DM, as well as in healthy participants (SMD, -0.27; 95% CI, -0.54 to 0.00; n=186). However, the acute and chronic intake of acetate, propionate and butyrate or the administration of mixed SCFAs had no effect on glycaemic measures.

Chapter 3:

The relationship between plasma acetate and glucose in obese diabetes patients on low caloric diet.

The aim:

This chapter examined the relationship between plasma acetate, body weight and glycaemic variables in obese diabetic patients who underwent caloric restriction to induce weight loss.

The main findings:

These included non-significantly higher plasma acetate in the intervention (LCD) arm compared to the control arm, with a mean difference of (-0.0048, p=0.293) between arms. No associations between plasma acetate and glycaemic indices were found, although there was a positive association between plasma acetate and insulin sensitivity index

(QUICKI) in the LCD group (r 0.356, p=0.07). There was an inverse association between plasma acetate and body mass index (BMI) in the control group at visit 9 (24 weeks) (r -0.502, p=0.001) and this association was examined further using multiple regression. This highlighted that BMI is a good predictor of plasma acetate variance, as it was shown that 27.4% of variance in plasma acetate level could be accounted for by changes in BMI.

Chapter 4:

Impact of Inulin Propionate Ester on weight gain prevention and glucose level in overweight young individuals- A randomised double blinded controlled study.

The aim:

To determine the effect of IPE compared to IN on weight gain prevention and glycaemic control in healthy overweight young adult subjects.

The main findings:

At the 12-month visit, there was no significant difference in baseline-adjusted mean weight gain for the IPE group compared to the control group (1.02 kg, 95%CI (-0.37 to 2.41, p= 0.15, n=226)). In the IPE arm, fat-free mass grew by 1.07 kg (95% CI (0.21 to 1.93)), and blood glucose was raised by 0.11 mmol/L (95% CI: 0.01 to 0.21) in the IPE arm. 63% of IPE participants complied on ≥ 50% days. There were no unexpected adverse effects or safety concerns.

DISCUSSION OF THE SUMMARY OF THE RESULTS

The findings of this thesis offer a better understanding to researchers of the effect or impact of short chain fatty acids on glycaemia in humans, indicating future investigations of the role of SCFAs in humans to study the feasibility of introducing SCFAs more in healthy and metabolically compromised humans for longer periods, given that there was no worsening effect of SCFAs on glycaemia. These three studies collectively observed that SCFAs had little effect on glycaemic measures in humans. Firstly, the findings from the systematic review showed that SCFAs – either mixed or individually – had no effect

on glycaemia, although a few studies showed some evidence in terms of improvement of glucose and insulin. In view of the noted lack of effect, the following factors need to be considered: SCFAs when given to humans have no effect on glucose homeostasis, as most of the meta-analysed studies had very low-grade certainty of evidence for all acute outcomes and included the risk of bias from lack of blinding and the measurement or reporting of selective outcomes. Bias also arose from wide confidence intervals and high degrees of heterogeneity that points to inconsistencies between the reported studies. In some of the studies there was a risk related to the incomplete reporting of patients and outcome events, so the results of the systematic review should be interpreted with caution. To overcome these biases and to improve the credibility of the results and decrease variability in the outcomes, using longer-term randomised controlled studies and the same type of SCFA supplementation through the same route of administration could minimise outcome variations. However, this meta-analysis was limited by the total availability of data, as researchers had no control over data quality or study procedures.

A major challenge experienced in this meta-analysis was the shortage of high-quality data, which meant that well controlled randomised double blinded studies should be run over longer periods. Several points should be considered for future meta-analyses to achieve more uniform and precise results, such as conducting separate analysis for cross-over studies and randomised controlled studies to avoid the carryover effect. It is also important to consider sub-analysing the included studies based on the concentration of plasma or faecal SCFAs to gain more of an insight into the relationship between SCFA concentration and their impact on the metabolic and glycaemic measures, as circulating SCFAs are more positively related to metabolic health than faecal SCFAs (Müller et al., 2019). A further consideration would be to sub-group the studies according to whether the intervention was being administered directly or indirectly by including it within food or drink. The mode of SCFA administration and the site of fermentation play significant roles in determining the metabolic response. Studies have shown that infusions of SCFA in the

distal colon, rather than the proximal colon, modulated the whole substrate metabolism, resulting in an increased lipid oxidation, PYY concentration and attenuated lipolysis (van der Beek et al., 2016). Despite its limitations, this systematic review was to our knowledge the first in-human study to summarise and provide strong evidence on the effect of SCFAs on glycaemia, using a meta-analysis of randomised controlled trials (rather than cross-sectional studies) that examined the effect of SCFAs in metabolically normal and metabolically abnormal individuals on glycaemic measures in humans. Our systematic analysis was also broader and more inclusive of different SCFA interventions and different participant profiles, thereby providing a comprehensive assessment of the metabolic benefits of SCFAs. However, several factors should be considered in designing future clinical studies to provide stronger evidence before using SCFAs as an intervention or prevention tool. These studies should include the type of SCFA (acetate, propionate, butyrate or mixed), the form of administration (capsules, powder, drink or incorporated into food/drink), as well as the duration of intervention, the route of intervention (oral or intravenous), and the degree of patient compliance. In the light of these considerations, SCFAs could be considered a safe new treatment for individuals with overweight, obesity or T2DM.

Moving to the second study, we used a pre-existing data set from the LCDDM study, where the primary aim was different. In this sub-analysis, the objective was to examine the effect of a low-calorie diet intervention versus a standardised diabetic diet in patients with long -standing T2DM who were receiving insulin treatment. The current sub-analysis demonstrated the effectiveness of the intervention, as it resulted in a significant weight loss compared to standard dietetic care (-9.4 kg vs -4.98 kg, p=0.01, respectively). Here we attempted to explore the relationship between plasma acetate and glycaemic variables, and to study the relationship between plasma acetate and changes in body weight. The study found a small increase in acetate level in the intervention group, but the mean difference between the arms was not significant (MD = 0.0048 mmol/L,

p=0.293). This showed that weight change may result in increased circulating acetate production in the intervention (0.0388 mmol/L) compared to (0.0321 mmol/L) in the control arm, which could be attributed to the endogenous production of acetate. As caloric restriction continues, the body shifts into a state that resembles a state of fasting, where the body increases hepatic glycogenolysis to produce glucose for vital tissues such as the brain and red blood cells. With extended fasting, the body shift into ketogenesis, resulting in the overproduction of Acetyl- Co A, which is the main substrate for acetate production pathways (Knowles et al., 1974). Since our patients are metabolically compromised and have had T2DM for many years, a such process might be accelerated.

Another possible explanation for the higher acetate levels towards the end of the 12 month study was the re-introduction of structured food and an increase in total caloric intake to 1200-1500 Kcal, which was evidenced by weigh re-gain in both arms towards the end of study. The exogenous food remains the main contributor and modulator for SCFAs production. Meanwhile, the observed increase in plasma acetate was not related to the increase in plasma glucose in those patients, which contradicts reported studies of a positive correlation between plasma acetate and plasma glucose in diabetes (Akanji & Humphreys, 1988; Akanji et al., 1989). Our results showed a negative correlation between plasma acetate and glucose in both arms, although it was weak and not significant. The positive association between plasma acetate and insulin sensitivity index (QUICKI) was seen in the LCD group (R= 0.356, P= 0.07), as was indicated by the favourable metabolic effect of acetate, which is in line with previous studies (van der Beek et al., 2016; Canfora et al., 2017). The discrepancy in our findings on the relationship between acetate and glucose needs to be further investigated in future research, as the original study from which this data was taken was not intended to examine or address these associations. The complex of acetate kinetics in diabetes also needs further exploration in research studies.

Another point to consider while interpreting these results is that other SCFAs, propionate and butyrate, may be important contributors to these correlations. However, these metabolites were not measured. To further understand the endogenous contribution of acetate in diabetes individuals, it would be useful to include measurements of ketone

bodies such as acetoacetate and B-hydroxybutyrate (Poobalan et al., 2014). Studies have shown that medication in general can affect the composition and functionality of gut microbiota (Walsh et al., 2018), and our subjects were all on multiple oral hypoglycaemic drugs such as metformin. Metformin is associated with a favourable effect on gut microbiota, and could contribute to the increase in SCFA-producing bacteria (Rodriguez et al., 2018). However, other studies made different conclusions (Karlsson et al., 2013; Forslund et al., 2017).

The availability of various glycaemic measures at different time points in a large and well-characterized parallel randomized controlled study over 12 months period, together with using circulating plasma acetate rather than faecal acetate, increased the validity of the results and made adjustments for the additional available confounding factors. Future studies that are aimed to address the association between plasma acetate and glucose in diabetes would validate these results. These studies should use matched groups with a stable body weight, as changes in SCFAs are well documented when following weight loss programmes (Sowah et al., 2020; Rad et al., 2023). Studies should include participants who share a similar BMI category as gut dysbiosis is correlated with the degree of obesity and diabetes (Aron-Wisnewsky et al., 2019).

We subsequently examined inulin propionate ester (IPE), in order to examine the effect of inulin propionate ester (IPE) compared to inulin (IN) on glycaemia measures and weight gain prevention in young overweight adults in a 12-month randomised -double-blinded-parallel controlled study. Results showed that supplementation of IPE over 12 months had no effect on body weight prevention as the adjusted mean weight gain for IPE compared with control was 1.02 kg, 95% CI (-0.37 to 2.41, p=0.15, n=226). According to the defined criteria, an overall weight of 79.6 kg in the IPE group could expect a 2.4 kg increase weight. However, the actual mean increase that was observed in the IPE arm was 1.22 kg (Stevens et al., 2006), and the gain of (+1.22 kg) over 12 months is of no clinical significance. Meanwhile, neither the IPE (+1.22 kg) nor the control am (+0.07 kg) reached the expected increase in body weight of 2 kg during the duration of the study. Additionally, the study participants included more female young participants between 20 and 40 years who were at childbearing age, and it could be postulated that hormonal changes might play a role in modulating their body weight. Young adults may be at risk of

weight gain due to dietary behaviour that includes shifts, irregular meal patterns, high levels of social activity and overeating, and these factors have been found to facilitate rapid weight gain in the young adult population (Poobalan et al., 2014). The phenotyping of current study participants also differed from previous studies, where participants reported weight gain prevention using IPE (Chambers et al., 2015). The difference in results could be explained by differences in the composition of the intestinal microbiota and response to IPE between different age categories, as they might have different intestinal microbiome profiles (Xiao et al., 2024). Understanding changers in the gut microbiome between age categories might help us to create specific dietary interventions that target age-specific microbiomes.

In our study, we reported similar dropout rates in the IPE (20) and IN (22) arms, and a higher compliance of >50% in the IPE (63%) compared to IN (53%) arm. This meant that reduced compliance did not account for the study findings. Conversely, the participants with poorest compliance in the IPE group appeared to gain the most weight. Interestingly, the IPE group differed from the inulin control group in terms of body composition. The IPE group in the current study had a greater increase in fat-free mass (+1.07 (0.21 to 1.93)) pointing to an increase in lean mass that usually has a positive effect on human metabolism and insulin resistance (McPherron et al., 2013). There was no change in fat mass in this study, in contrast to a study that reported higher lean mass and lower fat mass (Frampton et al., 2021). It is worth noting that Frampton et al. analysed data from adults aged 40 years and older, which was different from our study age group.

There was a higher fasting glucose level of 0.11 mmol/L (95% CI 0,01 to 0,21) in the IPE arm compared to the IN arm at 12 months, but these levels remained within normal ranges and have no clinical importance or implications. There was also no change in postprandial glucose levels and insulin between the two arms. Insulin resistance and sensitivity indices (HOMA-IR, HOMA-B, HOMA-S and QUICKI) were computed for both groups, and no differences were seen between the groups. This did not align with reports in human and mice studies. For example, a systematic review that examined the effect of dietary and SCFA interventions on insulin and glucose metabolism reported that when treatment baseline and endpoint values were compared, fasting insulin and HOMA-IR were significantly improved at the endpoint, which was not observed for the placebo (Pham,

2022). Additionally, in recent mice studies there was dramatic decrease in insulin and insulin resistance marker (HOMA-IR) after treatment with IPE (Zhu et al., 2020). Several factors should be considered when interpreting these results. Firstly, the age category of current study is different from previous cohorts. Secondly, this study used the lowest effective dose of IPE of 10 g based on previous work (Chambers et al., 2015). Higher doses of 20 g showed no differences in effect, but these studies covered an older age group (49-65years) (Chambers et al., 2019). IPE may also act differently when given to animals. The discrepancies between studies can also be explained by differences in the physiological and behavioural factors that influence energy intake in younger and older adults.

Our study had strengths such as the duration of the intervention, the size of the cohort and the randomised double-blinded placebo-controlled design. This study was also the first in-human study to examine the effect of propionate on weight gain prevention and metabolic marketers in young adults over a relatively long duration. However, the study also had limitations, such as the use of inulin as a control, as inulin is a competitive control (fermentable fibre) that might itself reduce appetite. Another limitation was the absence of behavioural and motivational sessions in the study's design that could make a positive contribution to compliance and eating behaviours. The IPE dose may not have been large enough to regulate appetite and affect other metabolic markers. To overcome these limitations, further research is needed that focuses on young adults and accounts for the psychosocial and behavioural factors that drive eating and weight gain in young adults, as it looks as if findings in the middle-age category cannot be applied to younger adults.

Taken together, this thesis contributes to improving our current understanding of the role of SCFAs in glucose regulation. It shows that SCFAs may not have a direct effect on glucose-insulin regulation in humans but may instead influence glycaemia indirectly through their effects on obesity and energy homeostasis. Although *in vitro* and *in vivo* studies have reported promising effects of SCFAs on glucose and insulin regulation, human studies have not yet produced similar conclusive findings. Despite this, the results presented in this thesis offer valuable insights for future research, particularly as none of the studies demonstrated any worsening of glycaemia. The thesis also addresses an

important gap by examining the effect of SCFAs in young adults – a population that is often underrepresented in obesity and glycaemia research.

CLINICAL SIGNIFICANCE AND APPLICATION

SCFAs play a role in health and disease in humans, and the colonic delivery of SCFAs through selection of dietary fibres that are rich in major SCFAs may represent a novel treatment modality to prevent weight gain and improve glucose homeostasis in obese adults. Increasing SCFA delivery is an attractive method for health regulation. Our thesis confirmed the safety of using IPE on humans for long durations as a preventive dietary supplement. However, future studies are needed to assess its tolerability and safety in metabolically compromised and obese individuals.

CHALLENGES

This thesis faced a major challenge due to the interruption of the clinical studies during the Covid-19 pandemic and associated lockdowns.

THESIS LIMITATIONS AND STRENGTHS

This thesis employed three types of high evidence study designs, including a comprehensive systematic review of randomised controlled trials and randomised double blinded controlled studies. These contributed to the high validity and reliability of the results. In addition, studying different SCFAs and their effects on glycaemia broadens the evidence of the results provided. The use of different age groups in the three studies also adds to the scope and the validity of results, and the inclusion of a wide range of metabolic measurements contributes further to the evidence. However, several limitations need to be mentioned. For example, a major issue in the systematic review is the heterogeneity of the studies included, which hampered the results analysed and affects their

generalizability. In the second study, the major issue was in the data extraction which, was not originally set to determine the relationship between SCFAs and glycaemic markers. It is also worth criticising the lack of behavioural and motivational consultations in the IPE study designs, which would have improved the dietary compliance behaviours of our participants.

FUTURE RESEARCH

High-quality, well-designed studies in specific categories of the population including younger people or people with diabetes are required to further examine the effect of SCFAs on weight and glycaemia regulation. Future work should include studying the mechanism underlying the lack of effect of SCFAs, specifically IPE, on glucose regulation and on young adults. Future analysis is also required to determine whether elevated fasting glucose is linked to the increase of body weight observed in the IPE arm or to other factors not yet identified. To maximise our understanding of the effect of SCFAs on glycaemia, running parallel studies using different types of SCFAs and different routes of administration on the same participants would be helpful to further understand the mechanisms and factors that underlie their effects. In addition, maximising the dose of IPE in future studies would provide an insight into the dose-response effect, as well as tolerability and safety in human subjects.

CONCLUSION

In summary, SCFAs had no effect on the glycaemic measures. This thesis did not replicate the findings of previous reports, but the studies provide pieces of the larger puzzle in terms of fully understanding the effects of SCFAs on glucose regulation in humans, identifying mechanisms that enable SCFAs to regulate host glucose metabolism and providing tools to prevent human obesity and hyperglycaemia. Further investigation is required to understand the reasons behind the divergence of these studies from previous reports.

REFERENCES

REFERENCES

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 (New York, N.Y. 1943)*, *89*(5), 1070–1077. https://doi.org/10.1016/0016-5085(85)90211-2

Akanji, A. O., Ng, L., & Humphreys, S. (1988). Plasma acetate levels in response to intravenous fat or glucose/insulin infusions in diabetic and non-diabetic subjects. *Clinica Chimica Acta*, *178*(1), 85–94. https://doi.org/10.1016/0009-8981(88)90272-0

Akanji, A. O., Peterson, D. B., Humphreys, S., & Hockaday, T. D. R. (1989). Change in plasma acetate levels in diabetic subjects on mixed high fiber diets. *The American Journal of Gastroenterology*, *84*(11), 1365–1370.

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

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), Article E21. https://doi.org/10.1017/S0029665114000354

Ali, Z., Ma, H., Wali, A., Ayim, I., Rashid, M. T., & Younas, S. (2018). 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

Aliasgharzadeh, A., Dehghan, P., Gargari, B. P., & Asghari-Jafarabadi, M. (2015). Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: a randomised controlled clinical trial. *British Journal of Nutrition*, *113*(2), 321–330. https://doi.org/10.1017/S0007114514003675

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. Molecular and Cell Biology of Lipids*, *1801*(11), 1175–1183. https://doi.org/10.1016/j.bbalip.2010.07.007

Al-Lahham, S., Roelofsen, H., Rezaee, F., Weening, D., Hoek, A., Vonk, R., & Venema, K. (2012). Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *European Journal of Clinical Investigation*, *42*(4), 357–364. https://doi.org/10.1111/j.1365-2362.2011.02590.x

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-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*(2010), 171–176. https://doi.org/10.1155/2010/651063

Apovian, C. M. (2016). Obesity: definition, comorbidities, causes, and burden. *The American Journal of Managed Care*, *22*(7 Suppl), s176–s185.

Arathuzik, G. G., & Goebel-Fabbri, A. E. (2011). Nutrition Therapy and the Management of Obesity and Diabetes: An Update. *Current Diabetes Reports*, *11*(2), 106–110. https://doi.org/10.1007/s11892-011-0176-0

Arner, P. (2000). Obesity — a genetic disease of adipose tissue? *British Journal of Nutrition*, *83*(S1), S9–S16. https://doi.org/10.1017/S0007114500000891

Aron-Wisnewsky, J., Prifti, E., Belda, E., Ichou, F., Kayser, B. D., Dao, M. C., Verger, E. O., Hedjazi, L., Bouillot, J.-L., Chevallier, J.-M., Pons, N., Le Chatelier, E., Levenez, F., Ehrlich, S. D., Dore, J., Zucker, J.-D., & Clément, K. (2019). Major microbiota

dysbiosis in severe obesity: fate after bariatric surgery. *Gut*, *68*(1), 70–82. https://doi.org/10.1136/gutjnl-2018-316103

Arora, S., & Anubhuti. (2006). Role of neuropeptides in appetite regulation and obesity – A review. *Neuropeptides (Edinburgh), 40*(6), 375–401. 10.1016/j.npep.2006.07.001

Asp, N.-G. (1987). Dietary fibre-definition, chemistry and analytical determination. *Molecular Aspects of Medicine*, *9*(1), 17–29. https://doi.org/10.1016/0098-2997(87)90014-8

Atkins, D., Eccles, M., Flottorp, S., Guyatt, G. H., Henry, D., Hill, S., Liberati, A., O'Connell, D., Oxman, A. D., Phillips, B., Schünemann, H., Edejer, T. T.-T., Vist, G. E., & Williams, J. W. (2004). Systems for grading the quality of evidence and the strength of recommendations I: Critical appraisal of existing approaches The GRADE Working Group. *BMC Health Services Research*, *4*(1), Article 38. https://doi.org/10.1186/1472-6963-4-38

Ayogu, R. N. B., Oshomegie, H., & Udenta, E. A. (2022). Energy intake, expenditure and balance, and factors associated with energy balance of young adults (20–39 years): a retrospective cross-sectional community-based cohort study. *BMC Nutrition*, *8*(1), Article 142. https://doi.org/10.1186/s40795-022-00628-2

Bauer, P. V., Hamr, S. C., & Duca, F. A. (2016). Regulation of energy balance by a gut–brain axis and involvement of the gut microbiota. *Cellular and Molecular Life Sciences : CMLS*, *73*(4), 737–755. https://doi.org/10.1007/s00018-015-2083-z

Bell, M. L., Fiero, M., Horton, N. J., & Hsu, C.-H. (2014). Handling missing data in RCTs; a review of the top medical journals. *BMC Medical Research Methodology*, *14*(1), Article 118. https://doi.org/10.1186/1471-2288-14-118

Bellahcene, M., O'Dowd, J. F., Wargent, E. T., Zaibi, M. S., Hislop, D. C., Ngala, R. A., Smith, D. M., Cawthorne, M. A., Stocker, C. J., & Arch, J. R. S. (2013). Male mice that lack the G-protein-coupled receptor GPR41 have low energy expenditure and increased body fat content. *British Journal of Nutrition*, *109*(10), 1755–1764. https://doi.org/10.1017/S0007114512003923

Benassi-Evans, B., Clifton, P., Noakes, M., & Fenech, M. (2010). High-protein/high red meat and high-carbohydrate weight-loss diets do not differ in their effect on faecal water genotoxicity tested by use of the WIL2-NS cell line and with other biomarkers of bowel health. *Mutation Research. Genetic Toxicology and Environmental Mutagenesis*, *703*(2), 130–136. https://doi.org/10.1016/j.mrgentox.2010.08.009

Bjursell, M., Admyre, T., Göransson, M., Marley, A. E., Smith, D. M., Oscarsson, J., & Bohlooly-Y, M. (2011). Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *American Journal of Physiology: Endocrinology and Metabolism*, *63*(1).

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., Nauta, A., Scott, K., Stahl, B., van Harsselaar, J., van Tol, R., Vaughan, E. E., & Verbeke, K. (2020). Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes*, *11*(5), 411–455. https://doi.org/10.3920/BM2020.0057

Bornet, F. R. J., Brouns, F., Tashiro, Y., & Duvillier, V. (2002). Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. *Digestive and Liver Disease*, *34*, S111–S120. https://doi.org/10.1016/S1590-8658(02)80177-3

Bouter, K. E., Bakker, G. J., Levin, E., Hartstra, A. V., Kootte, R. S., Udayappan, S. D., Katiraei, S., Bahler, L., Gilijamse, P. W., Tremaroli, V., Stahlman, M., Holleman, F., van Riel, N. A. W., Verberne, H. J., Romijn, J. A., Dallinga-Thie, G. M., Serlie, M. J., Ackermans, M. T., Kemper, E. M., … Nieuwdorp, M. (2018). Differential metabolic effects of oral butyrate treatment in lean versus metabolic syndrome subjects. *Clinical and Translational Gastroenterology*, *9*(5), e155-10. https://doi.org/10.1038/s41424-018-0025-4

Boyle, N. B., Adolphus, K., Caton, S. J., Croden, F. C., Dye, L., Glass, A., Halliwell, K., Hansen, G. L., Holm, L., Jackson, P., Makinwa, F., Stærk, B., & Wilkinson, N. (2024). Increasing fibre intake in the UK: lessons from the Danish Whole Grain Partnership. *British Journal of Nutrition*, *131*(4), 672–685. https://doi.org/10.1017/S0007114523002106

Bray, G. A., & Ryan, D. H. (2021). Evidence-based weight loss interventions: Individualized treatment options to maximize patient outcomes. *Diabetes, Obesity & Metabolism*, *23*(S1), 50–62. https://doi.org/10.1111/dom.14200

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.

Brown, A., Dornhorst, A., McGowan, B., Omar, O., Leeds, A., Taheri, S., & Frost, G. (2020). A low energy total diet replacement intervention in patients with type 2 diabetes mellitus and obesity treated with insulin – a randomized trial. *BMJ Open*

Diabetes Research and Care, *8*, Article e001012. https://doi.org/10.1136/bmjdrc-2019-001012

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

Budak, H. N., & Guzel-Seydim, Z. (2010). Antioxidant activity and phenolic content of wine vinegars produced by two different techniques. *Journal of the Science of Food and Agriculture, 90*(12), 2021–2026. https://doi.org/10.1002/jsfa.4047

Burgos-Morón, E., Abad-Jiménez, Z., Marañón, A. M. d., Iannantuoni, F., Escribano-López, I., López-Domènech, S., Salom, C., Jover, A., Mora, V., Roldan, I., Solá, E., Rocha, M., & Víctor, V.,M. (2019). Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *Journal of Clinical Medicine, 8*(9), 1385. https://doi.org/10.3390/jcm8091385

Burnier, P., Tappy, L., Jequier, E., Schneeberger, D., & Chiolero, R. (1992). Metabolic and respiratory effects of infused sodium acetate in healthy human subjects. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *263*(6), R1271–R1276. https://doi.org/10.1152/ajpregu.1992.263.6.R1271

Bush, R. S., & Milligan, L. P. (1971). Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. *Canadian Journal of Animal Science*, *51*(1), 121–127. https://doi.org/10.4141/cjas71-016

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), 1331–1338. https://doi.org/10.1038/ijo.2015.84

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., Chambers, E., Brignardello, J., Garcia-Perez, I., Holmes, E., Gareth, W., Tom, P., Catriona, T., Douglas, M., & Gary S, F. (2019). Effects of inulin propionate ester incorporated into palatable food products on appetite and resting energy expenditure: a randomised crossover study. *Nutrients*, *11*, Article ARTN 861. https://doi.org/10.3390/nu11040861

Canfora, E. E., & Blaak, E. E. (2017). Acetate: a diet-derived key metabolite in energy metabolism: good or bad in context of obesity and glucose homeostasis? *Current Opinion in Clinical Nutrition and Metabolic Care, 20*(6), 477–483. https://doi.org/10.1097/MCO.0000000000000408

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), Article 2360. https://doi.org/10.1038/s41598-017-02546-x

Cardon, L. R., Carmelli, D., Fabsitz, R. R., & Reed, T. (1994). Genetic and Environmental Correlations between Obesity and Body Fat Distribution in Adult Male Twins. *Human Biology, 66*(3), 465–479.

Cassidy, M.M., Vahouny, G.V. (1987). Dietary Fiber and Atherosclerosis, *Cardiovascular Disease.* (pp. 533–555). Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-5296-9_57

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

Chambers, E., Byrne, C., Morrison, D., Murphy, K., Preston, T., Tedford, M. C., Garcia Perez, I., Fountana, S., Serrano Contreras, J., Holmes, E., Roberts, J., Reynolds, C., Boyton, R., Altmann, D., McDonald, J., Marchesi, J., Akbar, A., Riddell, N., Wallis, G., & Frost, G. (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), 1430-1428. https://10.1136/gutjnl-2019-318424

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

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

Chiu, C., & Taylor, A. (2011). Dietary hyperglycaemia, glycaemic index and metabolic retinal diseases. *Progress in Retinal and Eye Research, 30*(1), 18–53. https://doi.org/10.1016/j.preteyeres.2010.09.001

Chiurazzi, M., Cozzolino, M., Orsini, R. C., Di Maro, M., Di Minno, M. N. D., & Colantuoni, A. (2020). Impact of Genetic Variations and Epigenetic Mechanisms on the Risk of Obesity. *International Journal of Molecular Sciences*, *21*(23), 9035. https://doi.org/10.3390/ijms21239035

Clément, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J.-M., Basdevant, A., Bougnères, P., Lebouc, Y., Froguel, P., & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature (London)*, *392*(6674), 398–401. https://doi.org/10.1038/32911

Cox, C. E. (2017). Role of Physical Activity for Weight Loss and Weight Maintenance. *Diabetes Spectrum, 30*(3), 157–160. https://doi.org/10.2337/ds17-0013

Crudele, L., Gadaleta, R. M., Cariello, M., & Moschetta, A. (2023). Gut microbiota in the pathogenesis and therapeutic approaches of diabetes. *EBioMedicine, 97*, Article 104821. https://doi.org/10.1016/j.ebiom.2023.104821

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

Dao, M. C., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., Verger, E. O., Kayser, B. D., Levenez, F., Chilloux, J., Hoyles, L., Dumas, M., Rizkalla, S. W., Doré, J., Cani, P. D., & Clément, K. (2016). Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut, 65*(3), 426–436 https://doi.org/10.1136/gutjnl-2014-308778

Darwiche, G., Östman, E., Elmståhl, H., Kallinen, N., Björgell, O., Björck, I., & Almér, L. (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

Davis, S. R., Castelo-Branco, C., Chedraui, P., Lumsden, M. A., Nappi, R. E., Shah, D., & Villaseca, P. (2012). Understanding weight gain at menopause. *Climacteric : The Journal of the International Menopause Society, 15*(5), 419–429. https://doi.org/10.3109/13697137.2012.707385

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*(1-2), 84–96. https://doi.org/10.1016/j.cell.2013.12.016

De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., Duchampt, A., Bäckhed, F., & Mithieux, G. (2016). Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. *Cell Metabolism, 24*(1), 151–157. https://doi.org/10.1016/j.cmet.2016.06.013

DeCaria, J. E., Sharp, C., & Petrella, R. J. (2012). Scoping review report: obesity in older adults. *International Journal of Obesity*, *36*(9), 1141–1150. https://doi.org/10.1038/ijo.2012.29

Delzenne, N. M., & Cani, P. D. (2005). A place for dietary fibre in the management of the metabolic syndrome. *Current Opinion in Clinical Nutrition and Metabolic Care, 8*(6), 636–640. https://doi.org/10.1097/01.mco.0000171124.06408.71

den Besten, G., Bleeker, A., Gerding, A., Eunen, K. v., Havinga, R., van Dijk, T.,H., Oosterveer, M. H., Jonker, J. W., Groen, A. K., Reijngoud, D., & Bakker, B. M. (2015). Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPAR?-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes (New York, N.Y.), 64*(7), 2398. https://doi.org/10.2337/db14-1213

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D., & 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., & Entezari, M. (2014). Effect of honey vinegar syrup on blood sugar and lipid profile in healthy subjects. *International Journal of Preventive Medicine, 5*(12), 1608–1615.

Dicken, S. J., Mitchell, J. J., Newberry Le Vay, J., 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

Dona, A. C., Jiménez, B., Schäfer, H., Humpfer, E., Spraul, M., Lewis, M. R., Pearce, J. T. M., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2014). Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. *Analytical Chemistry (Washington), 86*(19), 9887–9894. https://doi.org/10.1021/ac5025039

Duncan, S. H., Lobley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., & Flint, H. J. (2008). Human colonic microbiota associated with diet, obesity and weight loss. *International Journal of Obesity, 32*(11), 1720–1724. https://doi.org/10.1038/ijo.2008.155

ElSayed, N. A., Aleppo, G., Aroda, V. R., Bannuru, R. R., Brown, F. M., Bruemmer, D., Collins, B. S., Hilliard, M. E., Isaacs, D., Johnson, E. L., Kahan, S., Khunti, K., Leon,

J., Lyons, S. K., Perry, M. L., Prahalad, P., Pratley, R. E., Seley, J. J., Stanton, R. C., & Gabbay, R. A. (2023). 6. Glycemic Targets: Standards of Care in Diabetes—2023. *Diabetes Care*, *46*(Supplement_1), S97–S110. https://doi.org/10.2337/dc23-S006

Engin, A. (2017). The Definition and Prevalence of Obesity and Metabolic Syndrome*Adv Exp Med Biol* (pp. 1–17). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-48382-5_1

Farb, M. G., & Gokce, N. (2015). Visceral adiposopathy: a vascular perspective. *Hormone Molecular Biology and Clinical Investigation, 21*(2), 125–136. https://doi.org/10.1515/hmbci-2014-0047

Farooqi, I. S., & O'Rahilly, S. (2006). Genetics of Obesity in Humans. *Endocrine Reviews*, *27*(7), 710–718. https://doi.org/10.1210/er.2006-0040

Feinle-Bisset, C., & Horowitz, M. (2021). Appetite and Satiety Control—Contribution of Gut Mechanisms. *Nutrients*, *13*(10), 3635. https://doi.org/10.3390/nu13103635

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(2020), 1–5. https://doi.org/10.1155/2020/9098739*

Fernandes, J., Vogt, J., & Wolever, T. M. S. (2014). Kinetic model of acetate metabolism in healthy and hyperinsulinaemic humans. *European Journal of Clinical Nutrition*, *68*(9), 1067–1071. https://doi.org/10.1038/ejcn.2014.136

Fernandes, J., Vogt, J., & Wolever, T. M. S. (2012). Intravenous acetate elicits a greater free fatty acid rebound in normal than hyperinsulinaemic humans. *European Journal of Clinical Nutrition*, *66*(9), 1029–1034. https://doi.org/10.1038/ejcn.2012.98

Ferro-Luzzi, A., & Martino, L. (1996). Obesity and Physical Activity. *Ciba Foundation Symposium 201 - The Origins and Consequences of Obesity*, *201*, 207–227. https://doi.org/10.1002/9780470514962.ch13

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 (1971), 105*(9), e54–e59. https://doi.org/10.2105/AJPH.2015.302773

Fitch, A. K., & Bays, H. E. (2022). Obesity definition, diagnosis, bias, standard operating procedures (SOPs), and telehealth: An Obesity Medicine Association (OMA) Clinical Practice Statement (CPS) 2022. *Obesity Pillars (Online), 1*, Article 100004. https://doi.org/10.1016/j.obpill.2021.100004

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity, 24*(1), 38–48. https://doi.org/10.1038/sj.ijo.0801083

Ford, E. S., Williamson, D. F., & Liu, S. (1997). Weight Change and Diabetes Incidence: Findings from a National Cohort of US Adults. *American Journal of Epidemiology, 146*(3), 214–222. https://doi.org/10.1093/oxfordjournals.aje.a009256

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H. K., Arumugam, M., Kristiansen, K., Voigt, A. Y., Vestergaard, H., Hercog, R., Costea, P. I., Kultima, J. R., Li, J., Jørgensen, T., . . . Pedersen, O. (2017). Corrigendum: Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature (London), 545*(7652), 116. https://doi.org/10.1038/nature22318

Frampton, J., Murphy, K. G., Frost, G., & Chambers, E. S. (2021). Higher dietary fibre intake is associated with increased skeletal muscle mass and strength in adults aged 40 years and older. *Journal of Cachexia, Sarcopenia and Muscle*, *12*(6), 2134–2144. https://doi.org/10.1002/jcsm.12820

Freeland, K. R., Anderson, G. H., & Wolever, T. M. S. (2009). Acute effects of dietary fibre and glycaemic carbohydrate on appetite and food intake in healthy males. *Appetite, 52*(1), 58–64. https://doi.org/10.1016/j.appet.2008.08.001

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

Frobish, R. A., & Davis, C. L. (1977). Theory Involving Propionate and Vitamin B12 in the Low-Milk Fat Syndrome. *Journal of Dairy Science, 60*(2), 268–273. https://doi.org/10.3168/jds.S0022-0302(77)83863-0

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), Article 3611. https://doi.org/10.1038/ncomms4611

Frühbeck, G., Jebb, S. A., & Prentice, A. M. (1998). Leptin: physiology and pathophysiology. *Clinical Physiology (Oxford), 18*(5), 399–419. 10.1046/j.1365-2281. https://doi.org/10.1046/j.1365-2281.1998.00129.x

Fuller, S., Beck, E., Salman, H., & Tapsell, L. (2016). New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods for Human Nutrition (Dordrecht), 71*(1), 1–12. https://doi.org/10.1007/s11130-016-0529-6

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

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), 205–9. Article 205. https://doi.org/10.1186/s12944-019-1127-z

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 (New York, N.Y.), 58*(7), 1509–1517. https://doi.org/10.2337/db08-1637

Ge, H., Li, X., Weiszmann, J., Wang, P., Baribault, H., Chen, J., Tian, H., & Li, Y. (2008). Activation of G Protein-Coupled Receptor 43 in Adipocytes Leads to Inhibition of Lipolysis and Suppression of Plasma Free Fatty Acids. *Endocrinology (Philadelphia), 149*(9), 4519–4526. https://doi.org/10.1210/en.2008-0059

Gheflati, A., Bashiri, R., Ghadiri-Anari, A., Reza, J. Z., Kord, M. T., & Nadjarzadeh, A. (2019). The effect of apple vinegar consumption on glycaemic indices, blood pressure, oxidative stress, and homocysteine in patients with type 2 diabetes and dyslipidaemia: A randomized controlled clinical trial. *Clinical Nutrition ESPEN, 33*, 132–138. https://doi.org/10.1016/j.clnesp.2019.06.006

Glenny, A.-M., O'Meara, S., Melville, A., Sheldon, T. A., & Wilson, C. (1997). The treatment and prevention of obesity: a systematic review of the literature.

International Journal of Obesity, *21*(9), 715–737. https://doi.org/10.1038/sj.ijo.0800495

González Hernández, M.,A., Canfora, E. E., Pasmans, K., Astrup, A., Saris, W. H. M., & Blaak, E. E. (2020). The Relationship between Circulating Acetate and Human Insulin Resistance before and after Weight Loss in the DiOGenes Study. *Nutrients, 12*(2), 339. https://doi.org/10.3390/nu12020339

González, N., Moreno-Villegas, Z., González-Bris, A., Egido, J., & Lorenzo, Ó. (2017). Regulation of visceral and epicardial adipose tissue for preventing cardiovascular injuries associated to obesity and diabetes. *Cardiovascular Diabetology, 16*(1), 44. https://doi.org/10.1186/s12933-017-0528-4

Gordon, J. I., Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., & Mardis, E. R. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*(7122), 1027– 1031. https://doi.org/10.1038/nature05414

Gratz, S. W., Hazim, S., Richardson, A. J., Scobbie, L., Johnstone, A. M., Fyfe, C., Holtrop, G., Lobley, G. E., & Russell, W. R. (2019). Dietary carbohydrate rather than protein intake drives colonic microbial fermentation during weight loss. *European Journal of Nutrition, 58*(3), 1147–1158. https://doi.org/10.1007/s00394-018-1629-x

Grundler, F., Mesnage, R., Ruppert, P. M. M., Kouretas, D., & Françoise Wilhelmi, d. T. (2024). Long-Term Fasting-Induced Ketosis in 1610 Subjects: Metabolic Regulation and Safety. *Nutrients, 16*(12), 1849. https://doi.org/10.3390/nu16121849

Haghikia, A., Zimmermann, F., Schumann, P., Jasina, A., Roessler, J., Schmidt, D., Heinze, P., Kaisler, J., Nageswaran, V., Aigner, A., Ceglarek, U., Cineus, R., Hegazy, A. N., van der Vorst, E.,P.C., Döring, Y., Strauch, C. M., Nemet, I., Tremaroli, V., Dwibedi, C., . . . Landmesser, U. (2022). Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. *European Heart Journal, 43*(6), 518–533. https://doi.org/10.1093/eurheartj/ehab644.

Haimoto, H., Sasakabe, T., Wakai, K., & Umegaki, H. (2009). Effects of a low-carbohydrate diet on glycemic control in outpatients with severe type 2 diabetes. *Nutrition & Metabolism, 6*(1), 21–21. https://doi.org/10.1186/1743-7075-6-21

Han, H., Kang, G., Kim, J. S., Choi, B. H., & Koo, S. (2016). Regulation of glucose metabolism from a liver-centric perspective. *Experimental & Molecular Medicine, 48*(3), e218–e218. https://doi.org/10.1038/emm.2015.122

Hardy, D. S., Garvin, J. T., & Xu, H. (2020). Carbohydrate quality, glycaemic index, glycaemic load and cardiometabolic risks in the US, Europe and Asia: A dose–response meta-analysis. *Nutrition, Metabolism, and Cardiovascular Diseases, 30*(6), 853–871. https://doi.org/10.1016/j.numecd.2019.12.050

Haslam, D. (2010). Obesity and diabetes: the links and common approaches. *Primary Care Diabetes, 4*(2), 105–112. https://doi.org/10.1016/j.pcd.2010.04.002

Hausdorff, W. P., Bouvier, M., O'Dowd, B. F., Irons, G. P., Caron, M. G., & Lefkowitz, R. J. (1989). Phosphorylation Sites on Two Domains of the β2-Adrenergic Receptor Are Involved in Distinct Pathways of Receptor Desensitization. *The Journal of Biological Chemistry, 264*(21), 12657–12665. https://doi.org/10.1016/S0021-9258(18)63907-9

He, J., Zhang, P., Shen, L., Niu, L., Tan, Y., Chen, L., Zhao, Y., Bai, L., Hao, X., Li, X., Zhang, S., & Zhu, L. (2020). Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. *International Journal of Molecular Sciences, 21*(17), 6356. https://doi.org/10.3390/ijms21176356

He, L., Zhou, X., Huang, N., Li, H., Tian, J., Li, T., Yao, K., Nyachoti, C. M., Kim, S. W., & Yin, Y. (2017). AMPK Regulation of Glucose, Lipid and Protein Metabolism: Mechanisms and Nutritional Significance. *Current Protein & Peptide Science, 18*(6), 562–570. https://doi.org/10.2174/1389203717666160627071125

Heitmann, B., & Garby, L. (1999). Patterns of long-term weight changes in overweight developing Danish men and women aged between 30 and 60 years. *International Journal of Obesity*, *23*(10), 1074–1078. https://doi.org/10.1038/sj.ijo.0801035

Henry, R. R., & Gumbiner, B. (1991). Benefits and limitations of very-low-calorie diet therapy in obese NIDDM. *Diabetes Care*, *14*(9), 802–823. https://doi.org/10.2337/diacare.14.9.802

Hernández, M. A. G., 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), 1943. https://doi.org/10.3390/nu11081943

Higgins, J. P. T., Green, S., & Ben Van Den, A. (2020). [Rev. of *Cochrane Handbook for Systematic Reviews of Interventions*]. *International Coaching Psychology Review*, *15*(2), 123–125. https://doi.org/10.53841/bpsicpr.2020.15.2.123

Higgins, J. P. T., Altman, D. G., Gøtzsche, P.,C., Jüni, P., Moher, D., Oxman, A. D., Savović, J., Schulz, K. F., Weeks, L., & Sterne, J. A. C. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ, 343*(7829), 889–893. https://doi.org/10.1136/bmj.d5928

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

Hildebrand, S., & Pfeifer, A. (2025). The obesity pandemic and its impact on non-communicable disease burden. *Pflügers Archiv*, *477*(5), 657–668. https://doi.org/10.1007/s00424-025-03066-8

Hong, Y. H. (Shinshu U., Hishikawa, D., Miyahara, H., Nishimura, Y., Tsuzuki, H., Gotoh, C., Iga, T., Suzuki, Y., Song, S. H., Choi, K. C., Lee, H. G., Sasaki, S., & Roh, S. G. (2005). Up-regulation of the claudin-6 gene in adipogenesis. *Bioscience, Biotechnology, and Biochemistry*, *69*(11), 2117–2121. https://doi.org/10.1271/bbb.69.2117

Hong, Y., Nishimura, Y., Hishikawa, D., Tsuzuki, H., Miyahara, H., Gotoh, C., Choi, K., Feng, D. D., Chen, C., Lee, H., Katoh, K., Roh, S., & Sasaki, S. (2005). Acetate and Propionate Short Chain Fatty Acids Stimulate Adipogenesis via GPCR43. *Endocrinology (Philadelphia), 146*(12), 5092–5099. https://doi.org/10.1210/en.2005-0545

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–S226. https://doi.org/10.1016/j.clinbiochem.2011.08.543

Huang, T., Xu, M., Lee, A., Cho, S., & Qi, L. (2015). *Erratum: Consumption of whole grains and cereal fiber and total and cause-specific mortality: prospective analysis of 367,442 individuals*. https://doi.org/10.1186/s12916-015-0338-z

Hussain, S. S., & Bloom, S. R. (2013). The regulation of food intake by the gut-brain axis: implications for obesity. *International Journal of Obesity, 37*(5), 625–633. https://doi.org/10.1038/ijo.2012.93

Ibrahim, O., & Menkovska, M. (2022). Dietary Fibers-Classification, Properties, Analysis and Function: A Review. *Advances in Bioscience and Biotechnology, 13*(12), 527–544. https://doi.org/10.4236/abb.2022.1312036

Iván, J., Major, E., Sipos, A., Kovács, K., Horváth, D., Tamás, I., Bay, P., Dombrádi, V., & Lontay, B. (2017). The Short-Chain Fatty Acid Propionate Inhibits Adipogenic Differentiation of Human Chorion-Derived Mesenchymal Stem Cells Through the Free Fatty Acid Receptor 2. *Stem Cells and Development, 26*(23), 1724–1733. https://doi.org/10.1089/scd.2017.0035

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, 1*(11), 7343–7355. https://doi.org/10.1039/c9fo01082c

Jayedi, A., Soltani, S., Motlagh, S. Z., Emadi, A., Shahinfar, H., Moosavi, H., & Shab-Bidar, S. (2022). Anthropometric and adiposity indicators and risk of type 2 diabetes: systematic review and dose-response meta-analysis of cohort studies. *BMJ (Online), 376*, e067516. https://doi.org/10.1136/bmj-2021-067516

Jiang, L., Gulanski, B. I., De Feyter, H.,M., Weinzimer, S. A., Pittman, B., Guidone, E., Koretski, J., Harman, S., Petrakis, I. L., Krystal, J. H., & Mason, G. F. (2013). Increased brain uptake and oxidation of acetate in heavy drinkers. *The Journal of Clinical Investigation, 123*(4), 1605–1614. https://doi.org/10.1172/JCI65153

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., 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 Antiglycaemic 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

Joly-Amado, A., Cansell, C., PhD., Denis, R. G. P., PhD., Delbes, A., M.D., Castel, J., B.D., Martinez, S., M.D., & Luquet, S., PhD. (2014). The hypothalamic arcuate nucleus and the control of peripheral substrates. *Baillière's Best Practice & Research. Clinical Endocrinology & Metabolism, 28*(5), 725–737. https://doi.org/10.1016/j.beem.2014.03.003

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., Nielsen, J., & Bäckhed, F. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature (London), 498*(7452), 99–103. https://doi.org/10.1038/nature12198

Kelly, T., Yang, W., Chen, C. S., Reynolds, K., & He, J. (2008). Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity, 32*(9), 1431–1437. https://doi.org/10.1038/ijo.2008.102

Kenzig, M. (2013). *Health Status During College Students' Transition to Adulthood: Health Behaviors, Negative Experiences, and the Mediating Effects of Personal Development*. ProQuest Dissertations & Theses.

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 - PNAS, 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*(1), Article 1829. https://doi.org/10.1038/ncomms2852

Klein, S., Gastaldelli, A., Yki-Järvinen, H., & Scherer, P. E. (2022). Why does obesity cause diabetes? *Cell Metabolism, 34*(1), 11–20. https://doi.org/10.1016/j.cmet.2021.12.012

Knowles, S. E., Jarrett, I. G., Filsell, O. H., & Ballard, F. J. (1974). Production and utilization of acetate in mammals. *Biochemical Journal, 142*(2), 401–411. https://doi.org/10.1042/bj1420401

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell; Cell, 165*(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Koh-Banerjee, P., Franz, M., Sampson, L., Liu, S., Jacobs, D. R. J., Spiegelman, D., Willett, W., & Rimm, E. (2004). Changes in whole-grain, bran, and cereal fibre consumption in relation to 8-y weight gain among men. *The American Journal of Clinical Nutrition, 80*(5), 1237–1245. https://doi.org/10.1093/ajcn/80.5.1237

Koh-Banerjee, P., Wang, Y., Hu, F. B., Spiegelman, D., Willett, W. C., & Rimm, E. B. (2004). Changes in Body Weight and Body Fat Distribution as Risk Factors for Clinical Diabetes in US Men. *American Journal of Epidemiology, 159*(12), 1150–1159. https://doi.org/10.1093/aje/kwh167

Kondo, T., Kishi, M., Fushimi, T., & Kaga, T. (2009). Acetic Acid Upregulates the Expression of Genes for Fatty Acid Oxidation Enzymes in Liver To Suppress Body Fat Accumulation. *Journal of Agricultural and Food Chemistry, 57*(13), 5982–5986. https://doi.org/10.1021/jf900470c

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature (London), 404*(6778), 635–643. https://doi.org/10.1038/35007508

Korner, J., Woods, S. C., & Woodworth, K. A. (2009). Regulation of Energy Homeostasis and Health Consequences in Obesity. *The American Journal of Medicine, 122*(4, Supplement), I–CO4. https://doi.org/10.1016/j.amjmed.2009.01.003

Kreymann, B., Ghatei, M. A., Williams, G., & Bloom, S. R. (1987). GLUCAGON-LIKE PEPTIDE-1 7-36: A PHYSIOLOGICAL INCRETIN IN MAN. *The Lancet (British Edition), 330*(8571), 1300–1304. https://doi.org/10.1016/S0140-6736(87)91194-9

Król, E., & Krejpcio, Z. (2010). Chromium (III) propionate complex supplementation improves carbohydrate metabolism in insulin-resistance rat model. *Food and Chemical Toxicology, 48*(10), 2791–2796. https://doi.org/10.1016/j.fct.2010.07.008

Lanza, I. R., Zhang, S., Ward, L. E., Karakelides, H., Raftery, D., & Nair, K. S. (2010). Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. *PloS One, 5*(5), e10538. https://doi.org/10.1371/journal.pone.0010538

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fibre and its components on metabolic health. *Nutrients, 2*(12), 1266–1289. https://doi.org/10.3390/nu2121266

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.

Layden, B. T., Yalamanchi, S. K., Wolever, T. M., Dunaif, A., & Lowe, J.,William L. (2012). Negative association of acetate with visceral adipose tissue and insulin levels. *Diabetes, Metabolic Syndrome and Obesity, 5*, 49–55. https://doi.org/10.2147/DMSO.S29244

Li, Q., Liu, Y., Yang, X., Zhang, J., Lu, B., & Chen, R. (2020). Kinetic and thermodynamic effects of temperature on methanogenic degradation of acetate, propionate, butyrate and valerate. *Chemical Engineering Journal, 396*, Article 125366. https://doi.org/10.1016/j.cej.2020.125366

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

Lin, X., Xu, Y., Xu, J., Pan, X., Song, X., Shan, L., Zhao, Y., & Shan, P.-F. (2020). Global burden of noncommunicable disease attributable to high body mass index in 195 countries and territories, 1990–2017. *Endocrine*, *69*(2), 310–320. https://doi.org/10.1007/s12020-020-02352-y

Lin, H. V., Frassetto, A., Kowalik, J.,Edward 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

Ling, C., & Rönn, T. (2019). Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metabolism*, *29*(5), 1028–1044. https://doi.org/10.1016/j.cmet.2019.03.009

Liu, L., Zhang, J., Cheng, Y., Zhu, M., Xiao, Z., Ruan, G., & Wei, Y. (2022). Gut microbiota: A new target for T2DM prevention and treatment. *Frontiers in Endocrinology (Lausanne), 13*, 958218. https://doi.org/10.3389/fendo.2022.958218

Liu, X., Shao, J., Liao, Y., Wang, L., Jia, Y., Dong, P., Liu, Z., He, D., Li, C., & Zhang, X. (2023). Regulation of short-chain fatty acids in the immune system. *Frontiers in Immunology, 14*, 1186892. https://doi.org/10.3389/fimmu.2023.1186892

Liu, X., Cooper, D. E., Cluntun, A. A., Warmoes, M. O., Zhao, S., Reid, M. A., Liu, J., Lund, P. J., Lopes, M., Garcia, B. A., Wellen, K. E., Kirsch, D. G., & Locasale, J. W. (2018). Acetate Production from Glucose and Coupling to Mitochondrial Metabolism in Mammals. *Cell, 175*(2), 502–513.e13. https://doi.org/10.1016/j.cell.2018.08.040

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology, 19*(1), 29–41. https://doi.org/10.1111/1462-2920.13589

Louis, S., Tappu, R., Damms-Machado, A., Huson, D. H., & Bischoff, S. C. (2016). Characterization of the Gut Microbial Community of Obese Patients Following a Weight-Loss Intervention Using Whole Metagenome Shotgun Sequencing: e0149564. *PloS One, 11*(2). https://doi.org/10.1371/journal.pone.0149564

Lu, B., Christian, M., Hanyaloglu, A., Frampton, A., & Frost, G. (2023). Impact of the short-chain fatty acid propionate on mesenteric adipose tissue and insulin sensitivity. *Proceedings of the Nutrition Society, 82(OCE1), Article E34. https://doi.org/10.1017/S0029665123000423*

Luo, J., Van Yperselle, M., Rizkalla, S. W., Rossi, F., Slama, G., & Bornet, F. R. J. (2000). Chronic Consumption of Short-Chain Fructooligosaccharides Does Not Affect Basal Hepatic Glucose Production or Insulin Resistance in Type 2 Diabetics. *The Journal of Nutrition, 130*(6), 1572–1577. https://doi.org/10.1093/jn/130.6.1572

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

Mahmoud, A. M. (2022). An Overview of Epigenetics in Obesity: The Role of Lifestyle and Therapeutic Interventions. *International Journal of Molecular Sciences*, *23*(3), 1341. https://doi.org/10.3390/ijms23031341

Mallik, R., Carpenter, J., & Zalin, A. (2023). Assessment of obesity. *Clinical Medicine (London, England), 23*(4), 299–303. https://doi.org/10.7861/clinmed.2023-0148

Martinez, T. M., Meyer, R. K., & Duca, F. A. (2021). Therapeutic Potential of Various Plant-Based Fibers to Improve Energy Homeostasis via the Gut Microbiota. *Nutrients, 13*(10), 3470. https://doi.org/10.3390/nu13103470

Mayeda, E. R., Torgal, A. H., & Westhoff, C. L. (2014). Weight and Body Composition Changes During Oral Contraceptive Use in Obese and Normal Weight Women. *Journal of Women's Health (Larchmont, N.Y. 2002)*, *23*(1), 38–43. https://doi.org/10.1089/jwh.2012.4241

Mayorga-Ramos, A., Barba-Ostria, C., Simancas-Racines, D., & Guamán, L.,P. (2022). Protective role of butyrate in obesity and diabetes: New insights. *Frontiers in Nutrition (Lausanne), 9*, 1067647. https://doi.org/10.3389/fnut.2022.1067647

McPherron, A. C., Guo, T., Bond, N. D., & Gavrilova, O. (2013). Increasing muscle mass to improve metabolism. *Adipocyte, 2*(2), 92–98. https://doi.org/10.4161/adip.22500

McPherson, R. (2007). Genetic contributors to obesity. *Canadian Journal of Cardiology*, *23*(Suppl A), 23A-27A. https://doi.org/10.1016/S0828-282X(07)71002-4

Meiners, F., Ortega-Matienzo, A., Fuellen, G., & Barrantes, I. (2025). Gut microbiome-mediated health effects of fiber and polyphenol-rich dietary interventions. *Frontiers in Nutrition (Lausanne)*, *12*, 1647740. https://doi.org/10.3389/fnut.2025.1647740

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

Meyer, K. A., Kushi, L. H., Jacobs, D. R., Slavin, J., Sellers, T. A., & Folsom, A. R. (2000). Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *The American Journal of Clinical Nutrition*, *71*(4), 921–930. https://doi.org/10.1093/ajcn/71.4.921

Minari, T. P., Tácito, L.,Helena Bonalume, Yugar, L. B. T., Ferreira-Melo, S., Manzano, C. F., Pires, A. C., Moreno, H., Vilela-Martin, J., Cosenso-Martin, L., & Yugar-Toledo, J. (2023). Nutritional Strategies for the Management of Type 2 Diabetes Mellitus: A Narrative Review. *Nutrients, 15*(24), 5096. https://doi.org/10.3390/nu15245096

Moffett, J. R., Puthillathu, N., Vengilote, R., Jaworski, D. M., & Namboodiri, A. M. (2020a). Acetate Revisited: A Key Biomolecule at the Nexus of Metabolism,

Epigenetics and Oncogenesis—Part 1: Acetyl-CoA, Acetogenesis and Acyl-CoA Short-Chain Synthetases. *Frontiers in Physiology, 11*, 580167. https://doi.org/10.3389/fphys.2020.580171

Moffett, J. R., Puthillathu, N., Vengilote, R., Jaworski, D. M., & Namboodiri, A. M. (2020b). Acetate Revisited: A Key Biomolecule at the Nexus of Metabolism, Epigenetics, and Oncogenesis - Part 2: Acetate and ACSS2 in Health and Disease. *Frontiers in Physiology, 11*, 580171. https://doi.org/10.3389/fphys.2020.580171

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

Müller, M. J., Mast, M., Asbeck, I., Langnäse, K., & Grund, A. (2001). Prevention of obesity - is it possible? *Obesity Reviews, 2*(1), 15–28. 10.1046/j.1467- https://doi.org/10.1080/19490976.2015.1134082

Müller, M., Hernández, M. A. G., Goossens, G. H., Reijnders, D., Holst, J. J., Jocken, J. W. E., van Eijk, H., Canfora, E. E., & Blaak, E. E. (2019). Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. *Scientific Reports, 9*(1), 12515–9. Article 12515. https://doi.org/10.1038/s41598-019-48775-0

Mundt, D. (2007). [Rev. of *The glycaemia index - a physiological classification of dietary carbohydrate*]. *Journal of Human Nutrition and Dietetics*, *20*(4), 360–360. https://doi.org/10.1111/j.1365-277X.2007.00802.x

Murphy, K. G., Dhillo, W. S., & Bloom, S. R. (2006). Gut Peptides in the Regulation of Food Intake and Energy Homeostasis. *Endocrine Reviews, 27*(7), 719–727. https://doi.org/10.1210/er.2006-0028

Nogal, A., Louca, P., Zhang, X., Wells, P. M., Steves, C. J., Spector, T. D., Falchi, M., Valdes, A. M., & Menni, C. (2021). Circulating Levels of the Short-Chain Fatty Acid Acetate Mediate the Effect of the Gut Microbiome on Visceral Fat. *Frontiers in Microbiology, 12*, 711359. https://doi.org/10.3389/fmicb.2021.711359

Nogal, A., Valdes, A. M., & Menni, C. (2021). The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes, 13*(1), 1–24. https://doi.org/10.1080/19490976.2021.1897212

Nicolaidis, S. (2019). Environment and obesity. *Metabolism, Clinical and Experimental*, *100*, Article 153942. https://doi.org/10.1016/j.metabol.2019.07.006

O'Keefe, K. L., Kemmeter, P. R., & Kemmeter, K. D. (2010). Bariatric Surgery Outcomes in Patients Aged 65 Years and Older at an American Society for Metabolic and Bariatric Surgery Center of Excellence. *Obesity Surgery, 20*(9), 1199–1205. https://doi.org/10.1007/s11695-010-0201-4

O'Dea, R. E., Lagisz, M., Jennions, M. D., Koricheva, J., Noble, D. W. A., Parker, T. H., Gurevitch, J., Page, M. J., Stewart, G., Moher, D., & Nakagawa, S. (2021). Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. *Biological Reviews of the Cambridge Philosophical Society, 96*(5), 1695–1722. https://doi.org/10.1111/brv.12721

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

Oliphant, K., & Allen-Vercoe, E. (2019). Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome, 7*(1), 91–15. Article 91. https://doi.org/10.1186/s40168-019-0704-8

Opperman, C., Majzoobi, M., Farahnaky, A., Shah, R., Van, T. T. H., Ratanpaul, V., Blanch, E. W., Brennan, C., & Eri, R. (2025). Beyond soluble and insoluble: A comprehensive framework for classifying dietary fibre's health effects. *Food Research International*, *206*, Article 115843. https://doi.org/10.1016/j.foodres.2025.115843

Parretti, H., Johns, D., Lewis, A., Christian, A., Jebb, S., & Aveyard, P. (2015). Clinical effectiveness of very low calorie diets. Systematic review and meta-analysis of randomised controlled trials. *Appetite, 87*, 379. https://doi.org/10.1016/j.appet.2014.12.126

Patterson, M., Bloom, S. R., & Gardiner, J. V. (2011). Ghrelin and appetite control in humans—Potential application in the treatment of obesity. *Peptides (New York, N.Y.: 1980), 32*(11), 2290–2294. https://doi.org/10.1016/j.peptides.2011.07.021

Pearson-Stuttard, J., Banerji, T., Capucci, S., de Laguiche, E., Faurby, M. D., Haase, C. L., Sommer Matthiessen, K., Near, A. M., Tse, J., Zhao, X., & Evans, M. (2023). Real-world costs of obesity-related complications over eight years: a US retrospective cohort study in 28,500 individuals. *International Journal of Obesity, 47*(12), 1239–1246. https://doi.org/10.1038/s41366-023-01376-4

Pearson-Stuttard, J., Holloway, S., Sommer Matthiessen, K., Thompson, A., & Capucci, S. (2024). Ten-year progression of obesity-related complications in a population with overweight and obesity in the UK: A retrospective open cohort study. *Diabetes, Obesity & Metabolism*, *26*(11), 5056–5064. https://doi.org/10.1111/dom.15836

Perry, B., & Wang, Y. (2012). Appetite regulation and weight control: the role of gut hormones. *Nutrition & Diabetes, 2*(1), e26–e26. https://doi.org/10.1038/nutd.2011.21

Perry, R. J., Peng, L., Barry, N. A., Cline, G. W., Zhang, D., Cardone, R. L., Petersen, K. F., Kibbey, R. G., Goodman, A. L., & Shulman, G. I. (2016). Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. *Nature (London), 534*(7606), 213–217. https://doi.org/10.1038/nature18309

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. *Nature Reviews. Endocrinology, 13*(10), 572–587. https://doi.org/10.1038/nrendo.2017.80

Petrelli, A., Cugnata, F., Carnovale, D., Bosi, E., Libman, I. M., Piemonti, L., Cuthbertson, D., & Sosenko, J. M. (2024). HOMA-IR and the Matsuda Index as predictors of progression to type 1 diabetes in autoantibody-positive relatives. *Diabetologia, 67*(2), 290–300. https://doi.org/10.1007/s00125-023-06034-y

Pham, H. T. N. (2022). *Role of Short-Chain Fatty Acids in Obesity and Type 2 Diabetes*. ProQuest Dissertations & Theses.

Pham, N. H. T., Joglekar, M. V., Wong, W. K. M., Nassif, N. T., Simpson, A. M., & Hardikar, A. A. (2024). Short-chain fatty acids and insulin sensitivity: a systematic review and meta-analysis. *Nutrition Reviews, 82*(2), 193–209. https://doi.org/10.1093/nutrit/nuad042

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 & Metabolism, 19*(2), 257–265. https://doi.org/10.1111/dom.12811

Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., Chilloux, J., Ottman, N., Duparc, T., Lichtenstein, L., Myridakis, A., Delzenne, N. M., Klievink, J., Bhattacharjee, A., van der Ark, K. C. H., Aalvink, S., Martinez, L. O., Dumas, M.-E.,

Maiter, D., … Cani, P. D. (2017). A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine*, *23*(1), 107–113. https://doi.org/10.1038/nm.4236

Pluznick, J. (2014). A novel SCFA receptor, the microbiota, and blood pressure regulation. *Gut Microbes, 5*(2), 202–207. https://doi.org/10.4161/gmic.27492

Pluznick, J. L. (2017). Microbial Short-Chain Fatty Acids and Blood Pressure Regulation. *Current Hypertension Reports, 19*(4), 25. https://doi.org/10.1007/s11906-017-0722-5

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

Poobalan, A. S., Aucott, L. S., Clarke, A., & Smith, W. C. S. (2014). Diet behaviour among young people in transition to adulthood (18-25 year old): a mixed method study. *Health Psychology & Behavioural Medicine, 2*(1), 909–928. https://doi.org/10.1080/21642850.2014.931232

Price, R. A., & Gottesman, I. I. (1991). Body fat in identical twins reared apart: Roles for genes and environment. *Behavior Genetics*, *21*(1), 1–7. https://doi.org/10.1007/BF01067662

Priyadarshini, M., Navarro, G., & Layden, B. T. (2018). Gut Microbiota: FFAR Reaching Effects on Islets. *Endocrinology (Philadelphia), 159*(6), 2495–2505. https://doi.org/10.1210/en.2018-00296

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, 39*(3), 424–429. https://doi.org/10.1038/ijo.2014.153

Pugh, J., 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): A

multi-centre, double-blind, randomised, parallel-group study to investigate the efficacy of inulin-propionate ester versus inulin (control) in the prevention of weight gain over 12 months. *F1000Research*, *11*(1157), 1–14. https://doi.org/10.12688/f1000research.125950.1

Rad, Z. A., Mousavi, S. N., & Chiti, H. (2023). A low-carb diet increases fecal short-chain fatty acids in feces of obese women following a weight-loss program: randomized feeding trial. *Scientific Reports, 13*(1), Article 18146. https://doi.org/10.1038/s41598-023-45054-x

Rahat-Rozenbloom, S., Fernandes, J., Cheng, J., & Wolever, T. M. S. (2017). Acute increases in serum colonic short-chain fatty acids elicited by inulin do not increase GLP-1 or PYY responses but may reduce ghrelin in lean and overweight humans. *European Journal of Clinical Nutrition, 71*(8), 953–958. https://doi.org/10.1038/ejcn.2016.249

Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B., & Wolever, T. M. S. (2014). Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *International Journal of Obesity*, *38*(12), 1525–1531. https://doi.org/10.1038/ijo.2014.46

Rauf, A., Khalil, A. A., Rahman, U., Khalid, A., Naz, S., Shariati, M. A., Rebezov, M., Urtecho, E. Z., de Albuquerque, R. D.,Duarte Galhardo, Anwar, S., Alamri, A., Saini, R. K., & Rengasamy, K. R. R. (2022). Recent advances in the therapeutic application of short-chain fatty acids (SCFAs): An updated review. *Critical Reviews in Food Science and Nutrition, 62*(22), 6034–6054. https://doi.org/10.1080/10408398.2021.1895064

Ravussin, E. (., Valencia, M. E., Esparza, J., Bennett, P. H., & Schulz, L. O. (1994). Effects of a traditional lifestyle on obesity in Pima Indians. *Diabetes Care, 17*(9), 1067–1074. https://doi.org/10.2337/diacare.17.9.1067

Régnier, M., Hul, M. V., Knauf, C., & Cani, P. D. (2021). Gut microbiome, endocrine control of gut barrier function and metabolic diseases. *Journal of Endocrinology, 250*(1), X1–X1. https://doi.org/10.1530/JOE-20-0473e

Rissanen AM, Heliövaara M, Knekt P, Reunanen A, Aromaa A. (1991). Determinants of weight gain and overweight in adult Finns. *Eur J Clin Nutr., 45*(9), 419–30.

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

Rodriguez, J., Hiel, S., & Delzenne, N. M. (2018). Metformin: old friend, new ways of action–implication of the gut microbiome? *Current Opinion in Clinical Nutrition and Metabolic Care, 21*(4), 294–301. https://doi.org/10.1097/MCO.0000000000000468

Roelofsen, H., Priebe, M., & Vonk, R. (2010). The interaction of short-chain fatty acids with adipose tissue: relevance for prevention of type 2 diabetes. *Beneficial Microbes, 1*(4), 433–438. 10.3920/BM2010.0028

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

Rosin, O. (2008). THE ECONOMIC CAUSES OF OBESITY: A SURVEY. *Journal of Economic Surveys, 22*(4), 617–647. https://doi.org/10.1111/j.1467-6419.2007.00544.x

Rui, L. (2014). Energy Metabolism in the Liver. *Comprehensive Physiology, 4*(1), 177–197. https://doi.org/10.1002/j.2040-4603.2014.tb00548.x

Rumberger, J. M., Arch, J. R. S., & Green, A. (2014). Butyrate and other short-chain fatty acids increase the rate of lipolysis in 3T3-L1 adipocytes. *PeerJ (San Francisco, CA), 2*, e611. https://doi.org/10.7717/peerj.611

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. (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

Sakakibara, S., Yamauchi, T., Oshima, Y., Tsukamoto, Y., & Kadowaki, T. (2006). Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. *Biochemical and Biophysical Research Communications, 344*(2), 597–604. https://doi.org/10.1016/j.bbrc.2006.03.176

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

Salonen, A., Lahti, L., Salojärvi, J., Holtrop, G., Korpela, K., Duncan, S. H., Date, P., Farquharson, F., Johnstone, A. M., Lobley, G. E., Louis, P., Flint, H. J., & de Vos, W.,M. (2014). Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *The ISME Journal, 8*(11), 2218–2230. https://doi.org/10.1038/ismej.2014.63

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 - PNAS, 105*(43), 16767–16772. https://doi.org/10.1073/pnas.0808567105

Scheppach, W., Cummings, J. H., Branch, W. J., & Schrezenmeir, J. (1988). Effect of gut-derived acetate on oral glucose tolerance in man. *Clinical Science (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. (1988a). Effect of gut-derived acetate on glucose turnover in man. *Clinical Science (1979), 75*(4), 363–370. https://doi.org/10.1042/cs0750363

Schulze, M. B., Manson, J. E., Ludwig, D. S., Colditz, G. A., Stampfer, M. J., Willett, W. C., & Hu, F. B. (2004). Sugar-Sweetened Beverages, Weight Gain, and Incidence of Type 2 Diabetes in Young and Middle-Aged Women. *JAMA: The Journal of the American Medical Association, 292*(8), 927–934. https://doi.org/10.1001/jama.292.8.927

Schulze, M., Liu, S., Rimm, E., Manson, J., Willett, W., & Hu, F. (2004). Glycaemic index, glycaemic load, and dietary fibre intake and incidence of type 2 diabetes in younger and middle-age women. https://doi.org/10.1093/ajcn/80.2.348

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., & Hardt, P. D. (2010). Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity (Silver Spring, Md.), 18*(1), 190–195. https://doi.org/10.1038/oby.2009.167

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

Shi, H., Hu, F. B., Huang, T., Schernhammer, E. S., Willett, W. C., Sun, Q., & Wang, M. (2024). Sedentary Behaviors, Light-Intensity Physical Activity, and Healthy Aging. *JAMA Network Open*, *7*(6), e2416300. https://doi.org/10.1001/jamanetworkopen.2024.16300

Sivasubramanian, R., & Malhotra, S. (2023). Genetic Contributors to Obesity. *Gastroenterology Clinics of North America*, *52*(2), 323–332. https://doi.org/10.1016/j.gtc.2023.03.005

Sivaprasad, S., Prof, Prevost, A. T., Vasconcelos, J. C., M.Sc, Riddell, A., PhD., Murphy, C., M.S., Kelly, J., M.Sc, Bainbridge, J., Prof, Tudor-Edwards, R., Hopkins, D., F.R.C.P., & Hykin, P., F.R.C.Ophth. (2017). Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): a multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial. *The Lancet (British Edition), 389*(10085), 2193–2203. https://doi.org/10.1016/S0140-6736(17)31193-5

Skutches, C. L., Holroyde, C. P., Myers, R. N., Paul, P., & Reichard, G. A. (1979). Plasma acetate turnover and oxidation. *The Journal of Clinical Investigation, 64*(3), https://doi.org/10.1172/JCI109513

Smethers, A. D., & Rolls, B. J. (2018). Dietary Management of Obesity: Cornerstones of Healthy Eating Patterns. *The Medical Clinics of North America, 102*(1), 107–124. https://doi.org/10.1016/j.mcna.2017.08.009

Solianik, R., Židonienė, K., Eimantas, N., & Brazaitis, M. (2023). Prolonged fasting outperforms short-term fasting in terms of glucose tolerance and insulin release: a

randomised controlled trial. *British Journal of Nutrition, 130*(9), 1500–1509. https://doi.org/10.1017/S0007114523000557

Sowah, S. A., Hirche, F., Milanese, A., Johnson, T. S., Grafetstätter, M., Schübel, R., Kirsten, R., Ulrich, C. M., Kaaks, R., Zeller, G., Kühn, T., & Stangl, G. I. (2020). Changes in Plasma Short-Chain Fatty Acid Levels after Dietary Weight Loss Among Overweight and Obese Adults over 50 Weeks. *Nutrients, 12*(2), 452. https://doi.org/10.3390/nu12020452

Stevens, J., Truesdale, K. P., McClain, J. E., & Cai, J. (2006). The definition of weight maintenance. *International Journal of Obesity*, *30*(3), 391–399. https://doi.org/10.1038/sj.ijo.0803175

Stribling, P., & Ibrahim, F. (2023). Dietary fibre definition revisited - The case of low molecular weight carbohydrates. *Clinical Nutrition ESPEN*, *55*, 340–356. https://doi.org/10.1016/j.clnesp.2023.04.014

Sugiyama, S., Fushimi, T., Kishi, M., Irie, S., Tsuji, S., Hosokawa, N., & Kaga, T. (2010). Bioavailability of Acetate from Two Vinegar Supplements: Capsule and Drink. *Journal of Nutritional Science and Vitaminology, 56*(4), 266–269. https://doi.org/10.3177/jnsv.56.266

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, M., Wu, W., Liu, Z., & Cong, Y. (2017). Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *Journal of Gastroenterology, 52*(1), 1–8. https://doi.org/10.1007/s00535-016-1242-9

Susan, M. G., Daniel Castracane, V., & Christos, S. (2004). Energy Homeostasis, Obesity and Eating Disorders: Recent Advances in Endocrinology. *Ozhirenie i Metabolism, 1*(1), 39. https://doi.org/10.14341/2071-8713-5181

Suzuki, K., Jayasena, C., & Bloom, S. (2012). Obesity and Appetite Control. *Experimental Diabetes Research*, *2012*, Article 824305. https://doi.org/10.1155/2012/824305

Suzuki, K., Jayasena, C. N., & Bloom, S. R. (2011). The Gut Hormones in Appetite Regulation. *Journal of Obesity*, *2011*(2011), 585–594. https://doi.org/10.1155/2011/528401

Tang, R., & Li, L. (2021). Modulation of Short-Chain Fatty Acids as Potential Therapy Method for Type 2 Diabetes Mellitus. *The Canadian Journal of Infectious Diseases & Medical Microbiology, 2021*, 6632266– 1–3.https://doi.org/10.1155/2021/9756586

Tay, J.,B.NutrDiet (Hons), Brinkworth, G. D., PhD., Noakes, M., PhD., Keogh, J., M.Sc, & Clifton, P. M., PhD. (2008). Metabolic Effects of Weight Loss on a Very-Low-Carbohydrate Diet Compared with an Isocaloric High-Carbohydrate Diet in Abdominally Obese Subjects. *Journal of the American College of Cardiology, 51*(1), 59–67. https://doi.org/10.1016/j.jacc.2007.08.050

Telleria-Aramburu, N., & Arroyo-Izaga, M. (2022). Risk factors of overweight/obesity-related lifestyles in university students: Results from the EHU12/24 study. *British Journal of Nutrition, 127*(6), 914–926. https://doi.org/10.1017/S0007114521001483

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) https://doi.org/10.1126/scitranslmed.aav0120

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

Todesco, T., Zamboni, M., Armellini, F., Bissoli, L., Turcato, E., Piemonte, G., Venketshwer Rao, A., Jenkins, D. J. A., & Bosello, O. (1993). Plasma acetate levels in a group of obese diabetic, obese normoglycemic, and control subjects and their relationships with other blood parameters. *The American Journal of Gastroenterology, 88*(5), 751–755. https://doi.org/10.1111/j.1572-0241.1993.tb03026.x

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 (New York, N.Y.), 61*(2), 364–371. https://doi.org/10.2337/db11-1019

Tristan Asensi, M., Napoletano, A., Sofi, F., & Dinu, M. (2023). Low-Grade Inflammation and Ultra-Processed Foods Consumption: A Review. *Nutrients, 15*(6), 1546. https://doi.org/10.3390/nu15061546

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), 1397–1407. https://doi.org/10.1038/sj.ijo.0803307

Ulijaszek, S. J. (2003). [Rev. of *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. Pp. 252. (World Health Organization, Geneva, 2000.) SFr 56.00, ISBN 92-4-120894-5, paperback*]. *Journal of Biosocial Science*, *35*(4), Article S0021932003245508. https://doi.org/10.1017/S0021932003245508

Umirah, F., Neoh, C. F., Ramasamy, K., & Lim, S. M. (2021). Differential gut microbiota composition between type 2 diabetes mellitus patients and healthy controls: A systematic review. *Diabetes Research and Clinical Practice, 173*, Article 108689. https://doi.org/10.1016/j.diabres.2021.108689

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). OP038: Colonic Acetate Infusions Promote Fat Oxidation and Improve Metabolic Parameters in Overweight Males. *Clinical Nutrition (Edinburgh, Scotland), 33*, S16–S17. https://doi.org/10.1016/S0261-5614(14)50039-8

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 (1979), 130*(22), 2073–2082. https://doi.org/10.1042/CS20160263

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. https://doi.org/10.1111/j.1572-0241.1990.tb06643.x

Venter, C. S., Vorster, H. H., & van der Nest, D. G. (1990). Comparison between Physiological Effects of Konjac-Glucomannan and Propionate in Baboons Fed “Western” Diets. *The Journal of Nutrition, 120*(9), 1046–1053. https://doi.org/10.1093/jn/120.9.1046

Vogt, J. A., & Wolever, T. M. S. (2003). Fecal Acetate Is Inversely Related to Acetate Absorption from the Human Rectum and Distal Colon. *The Journal of Nutrition, 133*(10), 3145–3148. https://doi.org/10.1093/jn/133.10.3145

Walsh, J., Griffin, B. T., Clarke, G., & Hyland, N. P. (2018). Drug–gut microbiota interactions: implications for neuropharmacology. *British Journal of Pharmacology, 175*(24), 4415–4429. https://doi.org/10.1111/bph.14366

Wang, Li Y.,M.B.A., M.A., Denniston, M., M.S., Lee, S., Ph.D., Galuska, Deborah,Ph.D., M.P.H., & Lowry, Richard,M.D., M.S. (2010). Long-term Health and Economic Impact of Preventing and Reducing Overweight and Obesity in Adolescence. *Journal of Adolescent Health, 46*(5), 467–473. https://doi.org/10.1016/j.jadohealth.2009.11.204

Weickert, M. O., & Pfeiffer, A. F. H. (2018). Impact of Dietary Fiber Consumption on Insulin Resistance and the Prevention of Type 2 Diabetes. *The Journal of Nutrition, 148*(1), 7–12. https://doi.org/10.1093/jn/nxx008

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

Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., Qizilbash, N., Collins, R., & Peto, R. (2009). Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *LANCET*, *373*(9669), 1083–1096. https://doi.org/10.1016/S0140-6736(09)60318-4

Williams, D. L., Spray, G. H., & Williamson, D. H. (1971). Metabolic effects of propionate administration to normal and vitamin B12-deficient rats. *Biochemical Journal, 121*(1), 16P–17P. https://doi.org/10.1042/bj1210016Pb

Williams, B. A., Mikkelsen, D., Flanagan, B. M., & Gidley, M. J. (2019). “Dietary fibre”: moving beyond the “soluble/insoluble” classification for monogastric nutrition, with an emphasis on humans and pigs. *Journal of Animal Science and Biotechnology*, *10*(1), Article 45. https://doi.org/10.1186/s40104-019-0350-9

Williamson, D. F. (1990). The 10-year incidence of overweight and major weight gain in US adults. *Archives of Internal Medicine (1960), 150*(3), 665–672. https://doi.org/10.1001/archinte.150.3.665

Wing, R. R. (1995). Use of Very-Low-Calorie Diets in the Treatment of Obese Persons with Non-Insulin-Dependent Diabetes Mellitus. *Journal of the American Dietetic Association, 95*(5), 569–572. https://doi.org/10.1016/S0002-8223(95)00155-7

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

Wolever, T. M., 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., Jenkins, D. J., Ocana, A. M., Rao, V. A., & Collier, G. R. (1988). Second-meal effect: low-glycaemic-index foods eaten at dinner improve subsequent breakfast glycaemic response. *The American Journal of Clinical Nutrition, 48*(4), 1041–1047. https://doi.org/10.1093/ajcn/48.4.1041

Wolever, T. M. S. (University of T., Josse, R. G., Leiter, L. A., & Chiasson, J. L. (1997). Time of day and glucose tolerance status affect serum short-chain fatty acid concentrations in humans. *Metabolism, Clinical and Experimental, 46*(7), 805–811. https://doi.org/10.1016/S0026-0495(97)90127-X

Wolfe, A. J. (2005). The Acetate Switch. *Microbiology and Molecular Biology Reviews, 69*(1), 12–50. https://doi.org/10.1128/MMBR.69.1.12-50.2005

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Mannerås-Holm, L., Ståhlman, M., Olsson, L. M., Serino, M., Planas-Fèlix, M., Xifra, G., Mercader, J. M., Torrents, D., Burcelin, R., Ricart, W., Perkins, R., Fernàndez-Real, J. M., & Bäckhed, F. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, *23*(7), 850–858. https://doi.org/10.1038/nm.4345

Xiao, Y., Feng, Y., Zhao, J., Chen, W., & Lu, W. (2024). Achieving healthy aging through gut microbiota-directed dietary intervention: Focusing on microbial biomarkers and host mechanisms. *Journal of Advanced Research, 68*, 179–200. https://doi.org/10.1016/j.jare.2024.03.005

Yadav, H., Lee, J., Lloyd, J., Walter, P., & Rane, S. G. (2013). Beneficial Metabolic Effects of a Probiotic via Butyrate-induced GLP-1 Hormone Secretion. *The Journal of Biological Chemistry, 288*(35), 25088–25097. https://doi.org/10.1074/jbc.M113.452516

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 (OKETF) rats. *Bioscience, Biotechnology, and Biochemistry, 73*(3), 570–576. https://doi.org/10.1271/bbb.80634

York, A. (2021). A. muciniphila boosts metabolic health. *Nature Reviews. Microbiology, 19*(6), 343 –343. https://doi.org/10.1038/s41579-021-00556-1

Yoshida, H., Ishii, M., & Akagawa, M. (2019). Propionate suppresses hepatic gluconeogenesis via GPR43/AMPK signalling pathway. *Archives of Biochemistry and Biophysics, 672*, Article 108057. https://doi.org/10.1016/j.abb.2019.07.022

Zeigler, Z. (2021). COVID-19 Self-quarantine and Weight Gain Risk Factors in Adults. *Current Obesity Reports, 10*(3), 423–433. https://doi.org/10.1007/s13679-021-00449-7

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., Fu, H., Xue, X., Lu, C., Ma, J., Yu, L., Xu, C., Ren, Z., Xu, Y., Xu, S., Shen, H., Zhu, X., Shi, Y., Shen, Q., . . . Zhang, C. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science (American Association for the Advancement of Science), 359*(6380), 1151–1156. https://doi.org/10.1126/science.aao5774

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 (New York, N.Y.), 83*, 108–118. https://doi.org/10.1016/j.nutres.2020.09.003

Zhu, X., Jia, C., Meng, X., Xing, M., Yi, Y., & Gao, X. (2018). Synthesis, Characterization of Inulin Propionate Ester, and Evaluation of its in Vitro Effect on SCFA Production. *Starch, 70*(9-10), https://doi.org/10.1002/star.201800037

Zhu, X., Zhang, X., Gao, X., Yi, Y., Hou, Y., Meng, X., Jia, C., Chao, B., Fan, W., Li, X., & Zhang, H. (2020). Effects of Inulin Propionate Ester on Obesity-Related Metabolic Syndrome and Intestinal Microbial Homeostasis in Diet-Induced Obese Mice. *ACS Omega, 5*(22), 12865–12876. https://doi.org/10.1021/acsomega.0c00649

APPENDICES

APPENDIX CHAPTER # 2

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Supplemental Figure 14.2 Galbraith plot sensitivity analysis with chronic propionate intervention investigating fasting blood insulin

Supplemental Table 1. Search algorithms for each database.	
Data base	**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)

Supplemental Table 2. Excluded papers upon full-text screening		
Year of Publication	**Main Author**	**Reason for exclusion**
1990	Akanji (1)	Population: Chronic renal failure end stage
1994	Alamowitch (2)	Conference paper
1992	Amaral (3)	Not available
2001	Ball (4)	Poster
2019	Blessy Pusparatha (5)	Control: single-arm
1994	Bornet (6)	Review
2018	Bouter (7)	Control: healthy population not intervention
2015	Canfora (8)	Abstract paper
2015	Canfora (9)	Conference paper
2019	Cani (10)	Review
2019	Chambers (11)	Population: fatty liver disease
2012	Diaz-Buxo (12)	Outcomes: not glycemic with sufficient detail
2009	Ebrahimi-Mamaghani*	Not available
2012	Fernandes (13)	Control: against type population not intervention
2007	Geng*	Not available
2018	Johnston (14)	Abstract meeting
2019	Khatib (15)	Abstract paper
2015	Kohn (16)	Commentary
1998	Liljeberg (17)	Results: no error bars
2016	Lim (18)	Review
2020	Malkova (19)	Outcomes: not glycemic
2018	Marzocco (20)	Study design: no control or randomized
2010	Mitrou(21)	Population: type 1 diabetes

2013	Nosrati*	Not available in English
2020	Pagliai (22)	Outcomes: not glycemic
2019	Petersen (23)	Control: healthy population not intervention
2014	Petsiou (24)	Review
1967	Piccardo (25)	Not available in English
2016	Polyviou (26)	Outcomes: not glycemic
2009	Salbe (27)	Intervention: administered with octreotide
2016	Sasaki (28)	Conference paper
2007	Smith(29)	Outcomes: not glycemic
2003	Sugiyama (30)	Design: comparators not matched
2013	Suzuki*	Not available
1993	Todesco (31)	Control: against type population not intervention
2019	Vahid (32)	Design: control also contains vinegar
2012	Van Dijk*	Comment to editor
2014	Van der Beek (33)	Conference paper
1988	Yki-Jarvinen (34)	Results: no error bars
*citation not available		

Supplemental Table 3. Control for study design confounders for glycemic outcomes							
	Weight Change	**Standard Evening Meal**	**Strenuous Exercise Avoided**	**Alcohol Avoided**	**Body Fat Change**	**Low fiber diet Prior Study Visit**	**Overnight Fast (>6)**
Acetate (acute)							
Scheppach (1988)	NA	Yes	NI	Yes	NA	Yes (3 days)	Yes (10-12 h)
Laurent (1995)	NA	No	NI	NI	NA	Yes (3 days)	Yes (12 h)
Freeland (2010)	NA	NI	NI	NI	NA	NI	Yes (12 h)
Johnston (2010)	NA	Yes	Yes	No	NA	No	Yes (>10 h)
Van der Beek (2016)	NA	No	NI	NI	NA	Yes (3 days)	Yes (16 h)
Vinegar (Acute)							
Brighenti (1995)	NA	No	No	No	No	NA	NI
Johnston (2004)	NA	No	No	No	NA	No	Yes (NI)
Johnston (2005)	NA	No	No	No	NA	No	Yes (NI)
Ostman (2005)	NA	No	No	No	NA	No	Yes (NI)
Leeman (2005)	NA	No	No	No	NA	No	Yes (NI)
Hlebowicz (2007)	NA	No	No	No	NA	No	Yes (8 h)
Mettler (2009)	NA	NI	NI	NI	NA	NI	Yes (10 h)
Liatis (2010)	NA	No	No	No	NA	No	Yes (12 h)
Johnston (2010)	NA	Yes	Yes	No	NA	No	Yes (>10 h)

Darzi (2014)	NA	Yes	Yes	Yes	NA	Yes (evening meal)	Yes (>12 h)
Mitrou (2015)	NA	No	No	No	NA	No	Yes (NI)
Mitrou (2015)	NA	No	No	No	NA	No	Yes (NI)
Zhao (2020)	NA	Yes	Yes	Yes	NA	No	Yes (>12 h)
Feise (2020)	NA	No	Yes	Yes	NA	No	Yes (10 h)
Vinegar (Chronic)							
Ali (2019)	No	No	NI	Yes	NI	No	Yes (NI)
Gheflati (2019)	No	No	No	No	NI	No	Yes (NI)
Hosseini (2011)	NI	No	NI	NI	NI	No	Yes (NI)
Jasbi (2019)	No	No	No	No	No	No	Yes (10 h)
White (2007)	NA	No	No	No	NA	No	Yes (NI)
Derakhshandeh-Risheheri (2014)	No	No	No	No	No	No	Yes (12 h)
Propionate (Acute)							
Todesco (1991)	Yes	No	NI	NI	NI	NI	Yes (12 h)
Laurent (1995)	NA	No	NI	NI	No	Yes (3 days)	Yes (12 h)
Darwiche (2001)	NA	No	No	NI	NI	NI	Yes (NI)
Darzi (2012)	NA	No	Yes (24 h)	Yes	No	Yes (night before)	Yes (12 h)
Byrne (2016)	NA	No	Yes (24 h)	Yes	NI	NI	Yes (NI)
Chambers (2018)	No	No	No	Yes	Yes	No	Yes (NI)

Tirosh (2019)	NA	NA	NA	NA	NA	NA	Yes (8 h)
Adler (2021)	NA	No	NI	NI	NI	No (propionate-free diet for 7 days prior)	Yes (8 h)
Propionate (chronic)							
Venter (1990)	No	No	NI	NI	NI	NI	Yes (12 h)
Todesco (1991)	Yes	No	NI	NI	NI	NI	Yes (12 h)
Chambers (2015)	Yes	Yes	Yes (24 h)	Yes	Yes	No	Yes (10 h)
Pingitore (2017)	Yes	Yes	Yes (24 h)	Yes	Yes	No	Yes (10 h)
Byrne (2019) (69)	Yes	No	Yes (24 h)	Yes	Yes	No	Yes (12 h)
Chambers (2019)	Yes	Yes	Yes (24 h)	Yes	Yes	No	Yes (10 h)
Butyrate (Chronic)							
Roshanravan (2017)	Yes	No	No	NI	Yes	NI	Yes (12 h)
Roshanravan (2018)	Yes	No	No	NI	Yes	NI	Yes (12 h)
Mixed SCFAs (Acute)							
Wolever (1988)	NA	No	No	No	NA	No	Yes (12 h)
Wolever (1991)	NA	No	No	No	NA	No	Yes (NI)
Laurent (1995)	NA	No	NI	NI	NA	Yes (3 days)	Yes (12 h)
Alamowitch (1996)	NA	No	No	No	NA	Yes (meal prior)	Yes (NI)

Canfora (2017)	NA	No	No	No	NA	No	Yes (NI)

Supplemental Table 4. Summary of adverse events by study											
		Gastrointestinal (%)									
Reference	**Treatment groups**	**Nausea**	**Constipation**	**Flatulence**	**Vomiting**	**StD**	**GERD**	**Bloating**	**Belching**	**Diarrhea**	**Others**
Acetate (Acute)											
Scheppach 1988 (Study 1)	Na Acetate + K Acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Chloride	NI	NI	NI	IN	NI	NI	NI	NI	NI	NI
Scheppach 1988 (Study 3)	Na Acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Chloride	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Laurent (1995)	Na Acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Freeland (2010)	Acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Johnston (2010) (study 4)	Na acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Van der Beek (2016)	Na Chloride (proximal)	0	0	0	0	0	0	0	0	0	0
	Na Chloride (distal)	0	0	0	0	0	0	0	0	0	0
	Na Acetate (proximal)	0	0	0	0	0	0	0	0	0	0
	Na Acetate (distal)	0	0	0	0	0	0	0	0	0	0

Propionate (Acute)											
Todesco (1991) Study 1	Propionate Bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Plain bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Darwiche (2001)	Propionate Bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Plain bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Darzi (2012)	Propionate Bread	0	0	0	0	0	0	0	0	0	0
	Plain bread	0	0	0	0	0	0	0	0	0	0
Byrne (2016)	IPE	0	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Inulin	5 (1)	NI	NI	NI	NI	NI	NI	NI	NI	NI
Chambers (2018)	IPE	0	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Na Chloride	0	NI	NI	NI	NI	NI	NI	NI	NI	NI
Tirosh (2019)	Propionate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Adler (2021)	Ca Propionate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Ca Carbonate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Propionate (Chronic)											
Venter (1990)	Na Propionate	30 (6)	10 (2)	5 (1)	20 (4)	0	0	0	0	0	0
	Control	15 (3)	10 (2)	5 (1)	0	0	0	0	0	0	0
Todesco (1991) Study 2	Propionate bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Plain bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

Laurent (1995)	Na Acetate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Chambers (2015)	IPE	0	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Inulin	0	NI	NI	NI	NI	NI	NI	NI	NI	NI
Pingitore (2017)	IPE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Inulin	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Byrne (2019)	IPE bread	0	0	14.3 (3)	NI	0	0	0	0	0	0
	Plain bread	0	0	0 (0)	NI	0	0	0	0	0	0
Chambers (2019)	IPE	NS	NI	NS	NI	NI	NI	NS	NS	NI	NS
	Cellulose	NS	NI	NS	NI	NI	NI	NS	NS	NI	NS
Butyrate (Chronic)											
Roshanravan (2017)	Butyrate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Starch	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Roshanravan (2018)	Butyrate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Starch	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Vinegar (Acute)											
Brighenti (1995)	Acetic acid from vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Acetate from vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Johnston (2004)	Sodium chloride	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

	Vinegar (healthy)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo (healthy)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Vinegar (insulin resistant)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo (insulin resistant)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Vinegar (T2D)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo (T2D)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Johnston (2005)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Leeman (2005)	Vinegar with potatoes	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Potatoes	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Ostman (2005)	Acetic acid	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Hlebowicz (2007)*	White wine+ White bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	White bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	White wine+ Wholemeal bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Wholemeal bread	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Mettler (2009)	Vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

	Milk rice	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Johnston (2010) (study 1)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
(study 2)	Red raspberry vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
(study 3)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
(study 4)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Liatis (2010)	Vinegar (high glycemic index meal)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo (high glycemic index meal)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Vinegar (low glycemic index meal)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo (low glycemic index meal)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Darzi (2014)	White wine	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Water	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Mitrou (2015)	White wine vinegar	0	0	0	0	0	0	0	0	0	0
	Water	0	0	0	0	0	0	0	0	0	0

Mitrou (2015)	White wine vinegar	0	NI	NI	0	NI	NI	NI	NI	NI	NI
	Water	0	NI	NI	0	NI	NI	NI	NI	NI	NI
Feise (2020)	Control	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Acetic acid pill	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Acetic acid crushed pill	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Zhao (2020)	White rice vinegar	0	0	0	0	0	0	0	0	0	0
	White rice	0	0	0	0	0	0	0	0	0	0
Vinegar (chronic)											
White (2007)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Water	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Hosseini (2011)	Vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Water	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Derakhshandeh-Rishehri (2014)**	Honey vinegar syrup	1.63 (1)	NI	NI	NI	NI	NI	NI	NI	NI	1.63 (1), Headache 1.63 (1), Stomach ache
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Ali (2019)	Date vinegar	0	0	0	0	0	0	0	0	0	0
	Placebo	0	0	0	0	0	0	0	0	0	0

Gheflati (2019)	Apple cider vinegar	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Placebo	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Jasbi (2019)	Red wine vinegar	0	0	0	0	0	0	0	0	0	0
	Placebo tablets	0	0	0	0	0	0	0	0	0	0
Mixed SCFA											
Wolever (1988)	Acetate (90 mmol) + Propionate (30mmol)	NI	NI	NI	NI	NI	NI	NI	66.6 (4)	NI	NI
	Acetate (180 mmol) + Propionate (60mmol)	NI	NI	NI	NI	NI	NI	NI	100 (6)	NI	NI
	Isotonic Saline	NI	NI	NI	NI	NI	NI	NI	83.3 (5)	NI	NI
Wolever (1991)	Acetate (180 mmol) + propionate (60 mmol)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline solution	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Laurent (1995)	Acetate + Propionate	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Alamowitch (1996)	SCFA mixture [Acetate (60%) + propionate (25%) + butyrate (15%)] for 18 h	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	SCFA mixture for 12 h + saline solution 6h	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Saline solution	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

Canfora (2017)	SCFA mixture high in acetate (200 mmol/L)	0	0	0	0	0	0	0	0	0	0
	SCFA mixture high in propionate (200 mmol/L)	0	0	0	0	0	0	0	0	0	0
	SCFA mixture high in butyrate (200 mmol/L)	0	0	0	0	0	0	0	0	0	0
	Placebo	0	0	0	0	0	0	0	0	0	0

*Only mention: “If the subject reported gastrointestinal symptoms (diarrhea or constipation) on the study day, the examination was postponed”, but no mention of participants suffering these symptoms.
**Side effects appeared after 15 days.

0, none; Ca, calcium, Na, sodium, NI, no information, NS, not significantly different between groups, (), participant number; IPE, inulin propionate ester; GERD, gastro-oesophageal reflux disease; StD, stomach discomfort.

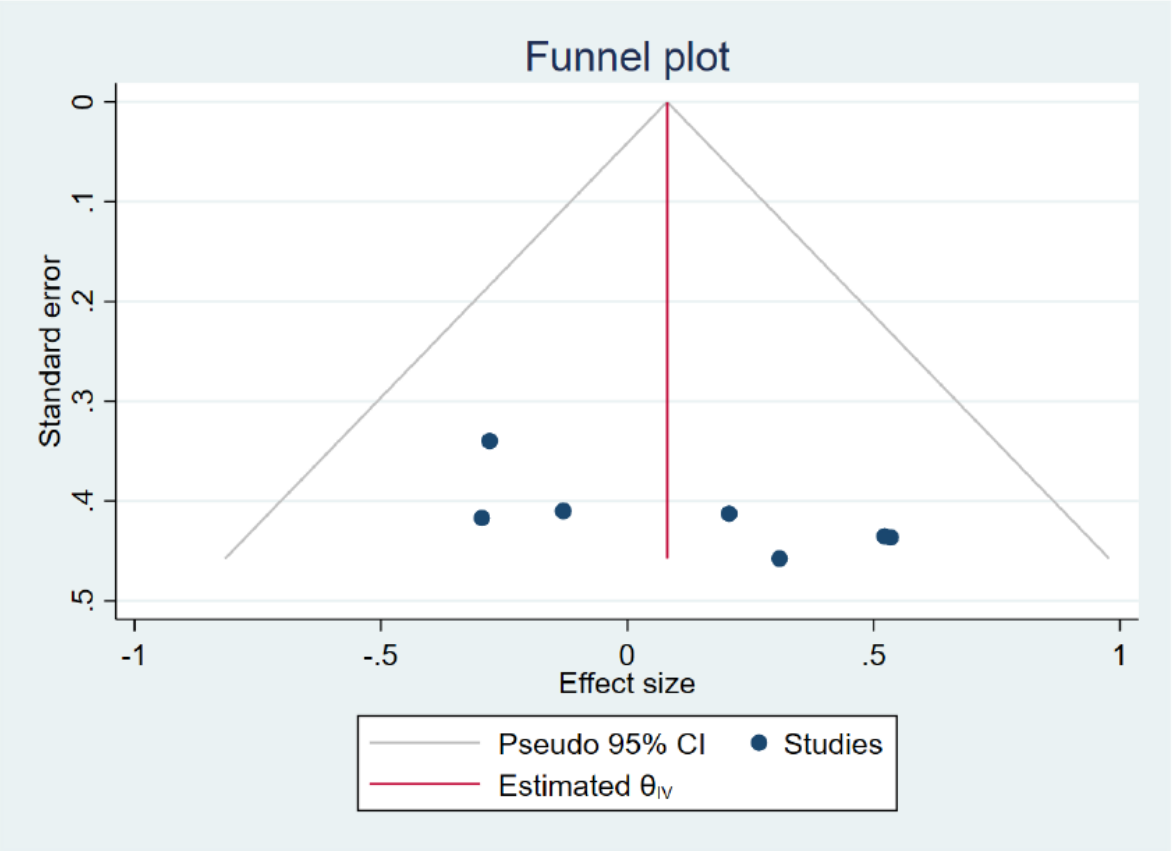
Supplemental Table 5. Summary of adherence by study		
	Percentage withdrawal (n)	**Reasons for withdrawal**
Acetate (Acute)		
Scheppach (1988)	0	NA
Laurent (1995)	0	NA
Freeland (2010)	0	NA
Johnston (2010)		
Van der Beek (2016)	40 (4)	Two failed attempts to clip the catheter to the colonic mucosa on two separate days
Propionate (Acute)		
Todesco (1991)	0	NA
Laurent (1995)	0	NA
Darwiche (2001)	0	NA
Darzi (2012)	0	NA
Byrne (2016)	0	NA
Chambers (2018)	0	NA
Tirosh (2019)	0	NA
Adler (2021)	3.44 (1)	Unable to follow protocol (n=1)
Propionate (Chronic)		
Venter (1990)	5 (1)	Nausea
Todesco (1991)	0	NA

Chambers (2015)	18.3 (11)	Did not complete the 24-week supplementation
Pingitore (2017)	0	NA
Byrne (2019)	0	NA
Chambers (2019)	0	NA
Butyrate (Chronic)		
Roshanravan (2017)	1.7 (1)	Lost to follow up
Roshanravan (2018)	1.7 (1)	Lost to follow up
Vinegar (Acute)		
Brighenti (1995)	0	NA
Johnston (2004)	0	NA
Johnston (2005)	0	NA
Ostman (2005)	0	NA
Leeman (2005)	0	NA
Hlebowicz (2007)	0	NA
Mettler (2009)	0	NA
Johnston (2010) (study 1)	0	NA
(study 2)	0	NA
(study 3)	0	NA
(study 4) (4)	0	NA
Liatis (2010)	0	NA

Darzi (2014)	0	NA
Mitrou (2015)	0	NA
Mitrou (2015)	0	NA
Zhao (2020)	17 (3)	Time conflicts (n=2) and dislike of vinegar (n=1)
Feise (2020)	0	NA
Vinegar (Chronic)		
White (2007)	0	NA
Hosseini (2011)	0	NA
Derakhshandeh-Rishehri (2014)	84.7 (11)	Personal reasons (n=5), viral infection and drug therapy (n=4), seasonal allerglycemic indexes (n=1), adverse effects (nausea, stomachache, headache) (n=1)
Ali (2019)	8.33 (5)	Protocol variance (n=3), consent withdrawal (n=2)
Gheflati (2019)	11 (8)	Vinegar intolerance, not intention to continue the trial, medication changes (no number of participants provided for each withdrawal reason)
Jasbi (2019)	29 (19)	Dislike vinegar taste (n=2), no mention of withdrawal reasons for the rest of the participants.
Mixed SCFA		
Wolever (1988)	0	NA
Wolever (1991)	0	NA
Laurent (1995)	0	NA
Alamowitch (1996)	0	NA
Canfora (2017)	7.6 (1)	The participant decided to withdraw before starting.

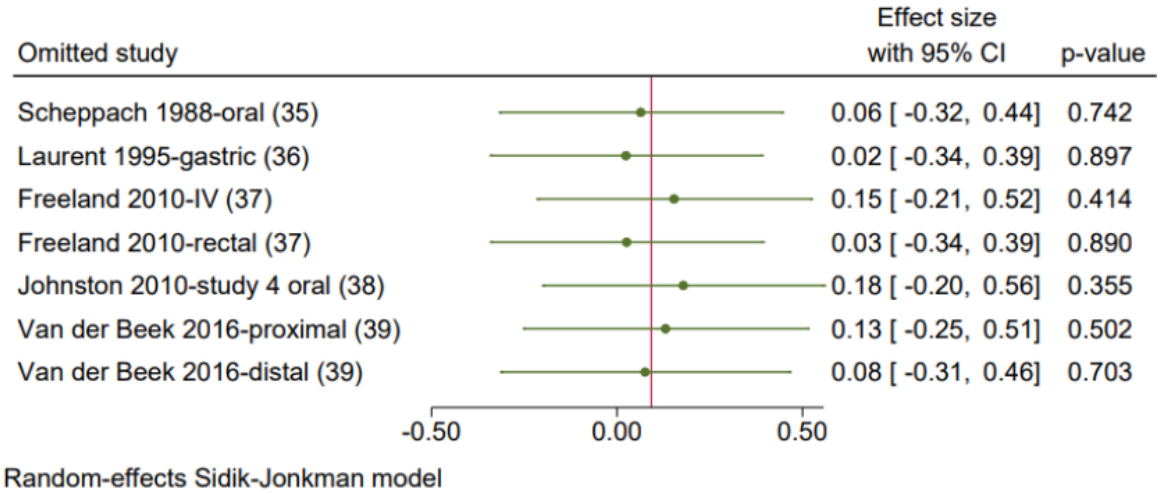
NA, not applicable.

Supplemental Figure 1. Funnel plot for acute interventions with acetate investigating postprandial blood glucose



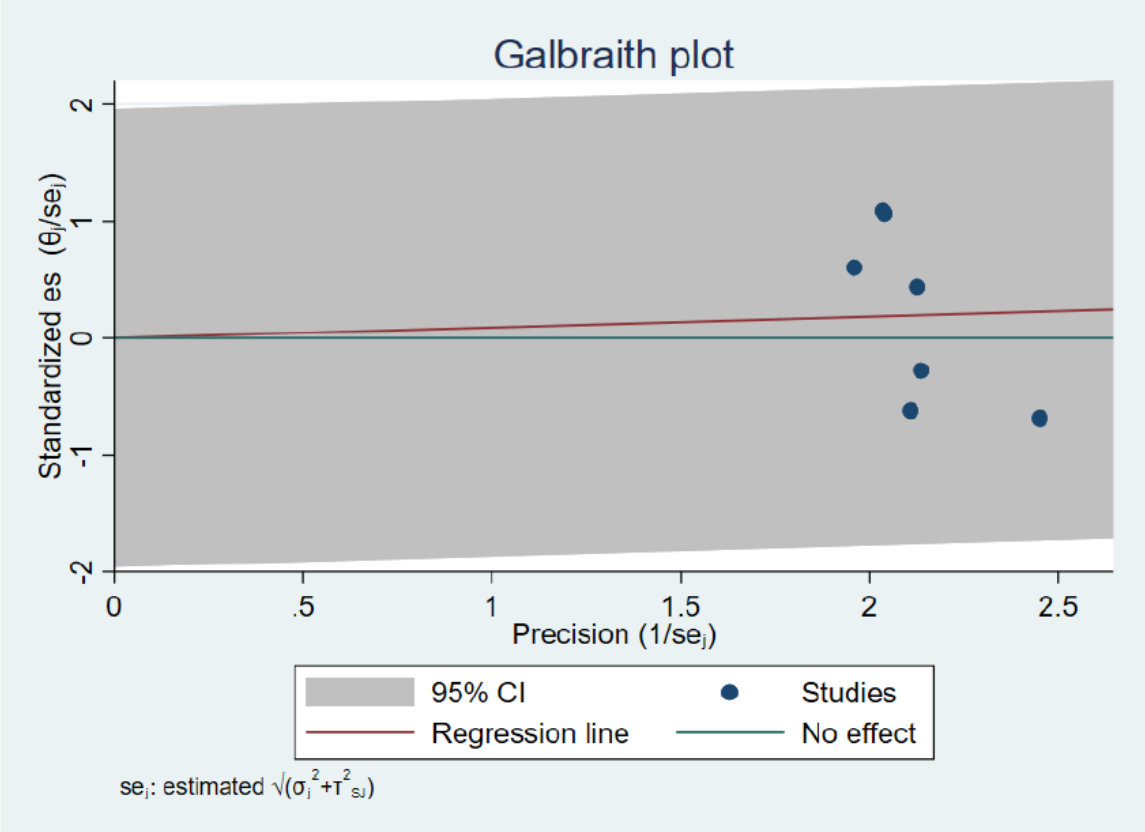
Supplemental Figure 1. Funnel plots to assess publication bias in randomized controlled trials of acute acetate on postprandial blood glucose. n= 44. CI, confidence interval.

Supplemental Figure 1.1 Leave one out analysis for acute interventions with acetate investigating postprandial blood glucose



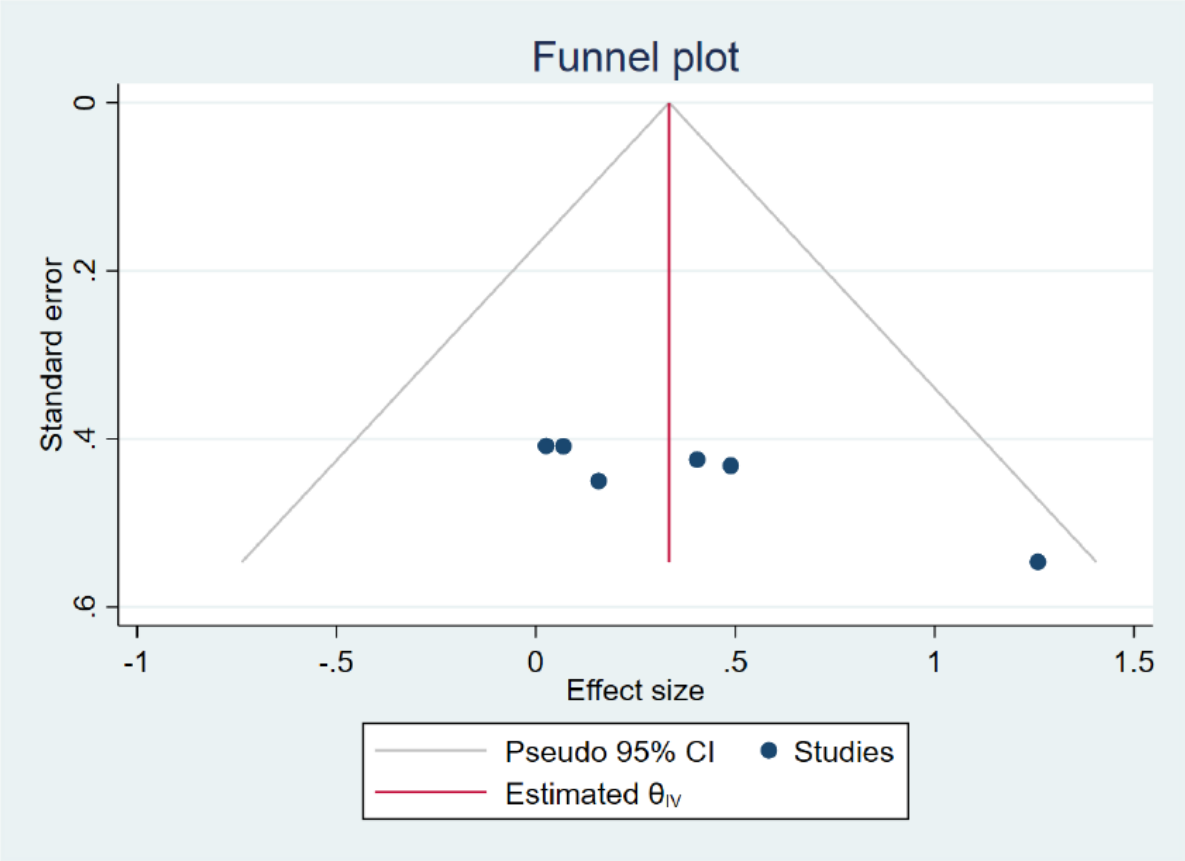
Supplemental Figure 1.1. Leave one out sensitivity analysis for randomized controlled trials of acute acetate on postprandial blood glucose. Random-effects Sidik-Johnkman model. n= 44. P-value ≤0.05 was considered statistically significant. CI, confidence interval, IV, intravenous.

Supplemental Figure 1.2. Galbraith plot for acute interventions with acetate investigating postprandial blood glucose



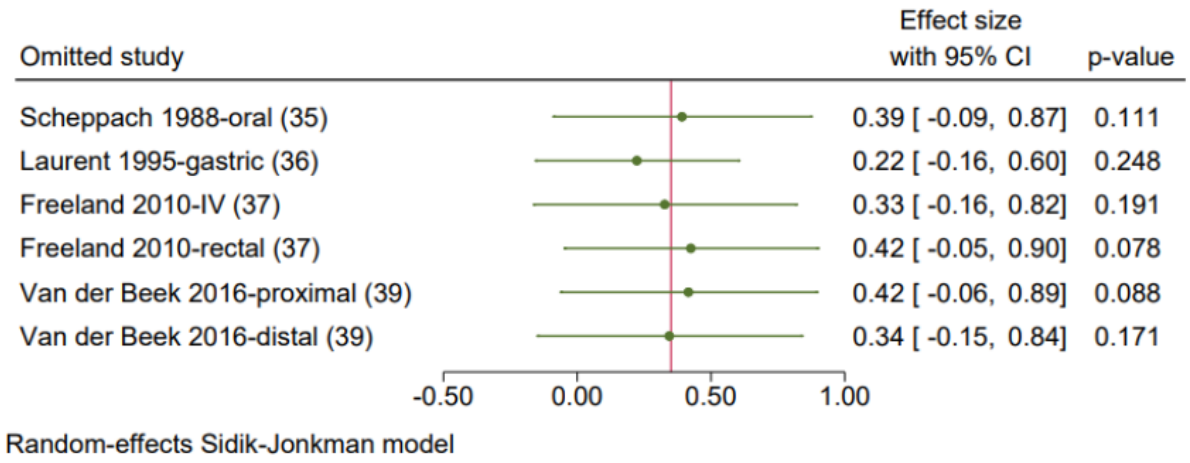
Supplemental Figure 1.2. Galbriath plot to assess for heterogeneity for randomized controlled trials of acute acetate on postprandial blood glucose. n= 44. CI, confidence interval.

Supplemental Figure 2. Funnel plot for acute interventions with acetate investigating postprandial insulin



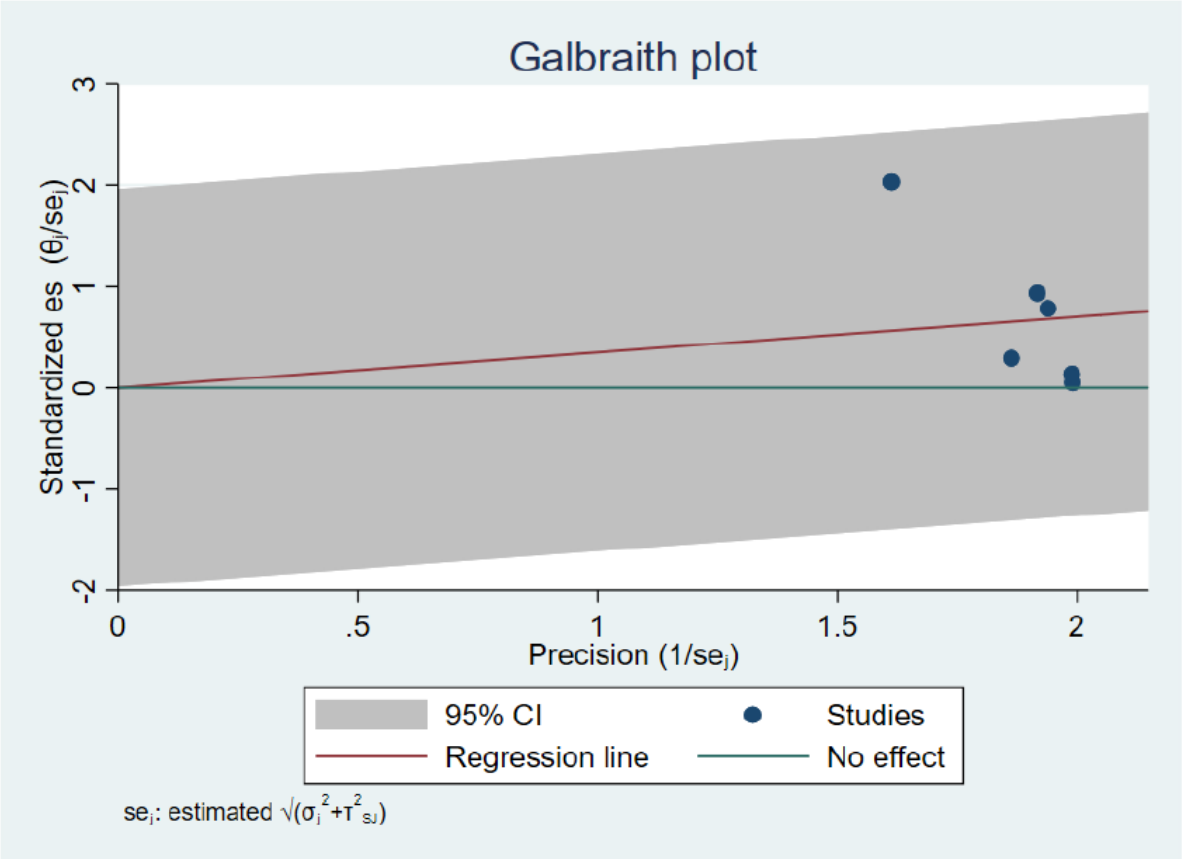
Supplemental Figure 2. Funnel plots to assess publication bias in randomized controlled trials of acute acetate on postprandial blood insulin. n=35. CI, confidence interval.

Supplemental Figure 2.1 Leave one out analysis for acute interventions with acetate investigating postprandial insulin



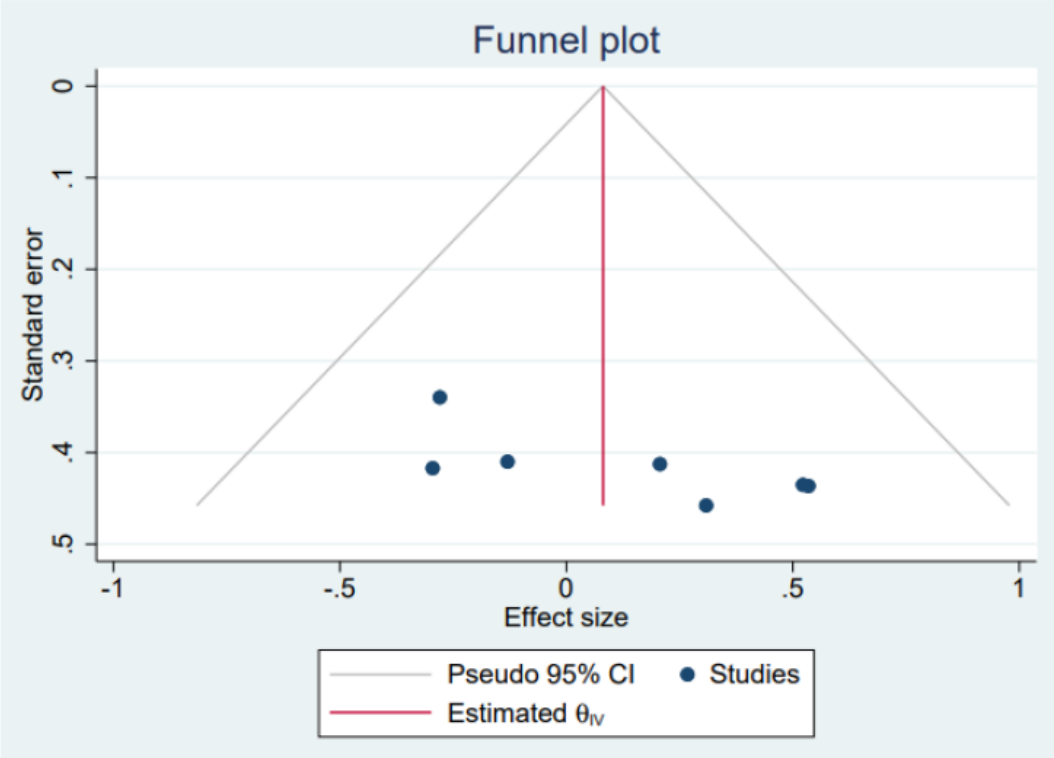
Supplemental Figure 2.1. Leave one out sensitivity analysis for randomized controlled trials of acute acetate on postprandial blood insulin. n=35. Random-effects Sidik-Johnkman model. n=35. P-value ≤0.05 was considered statistically significant. CI, confidence interval, IV, intravenous.

Supplemental Figure 2.2. Galbraith plot for acute interventions with acetate investigating postprandial insulin



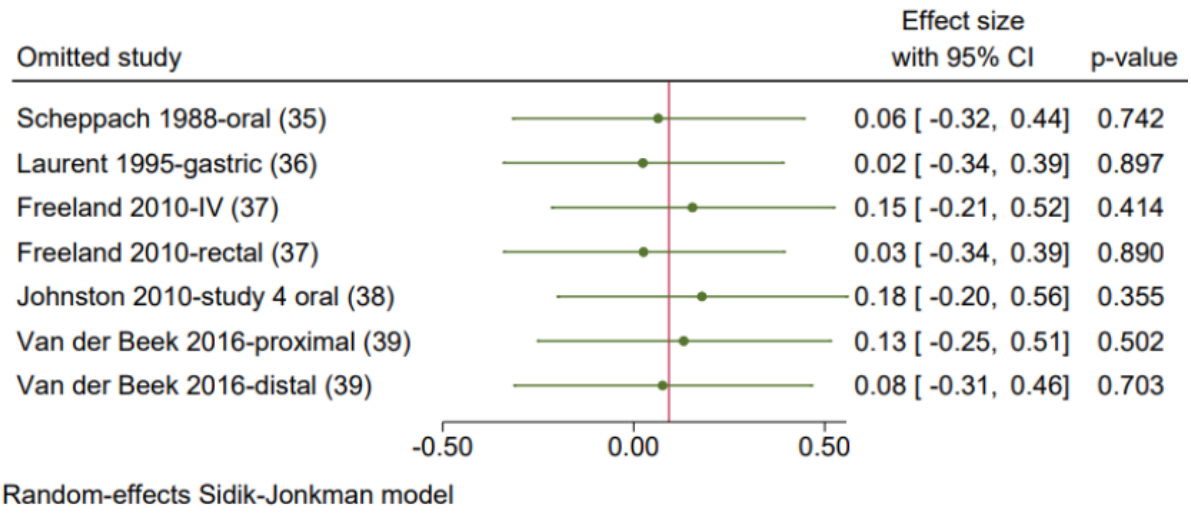
Supplemental Figure 2.2. Galbriath plot to assess for heterogeneity for randomized controlled trials of acute acetate on postprandial blood insulin. n=35. CI, confidence interval.

Supplemental Figure 3. Funnel plot for acute interventions with vinegar investigating postprandial blood glucose in healthy individuals



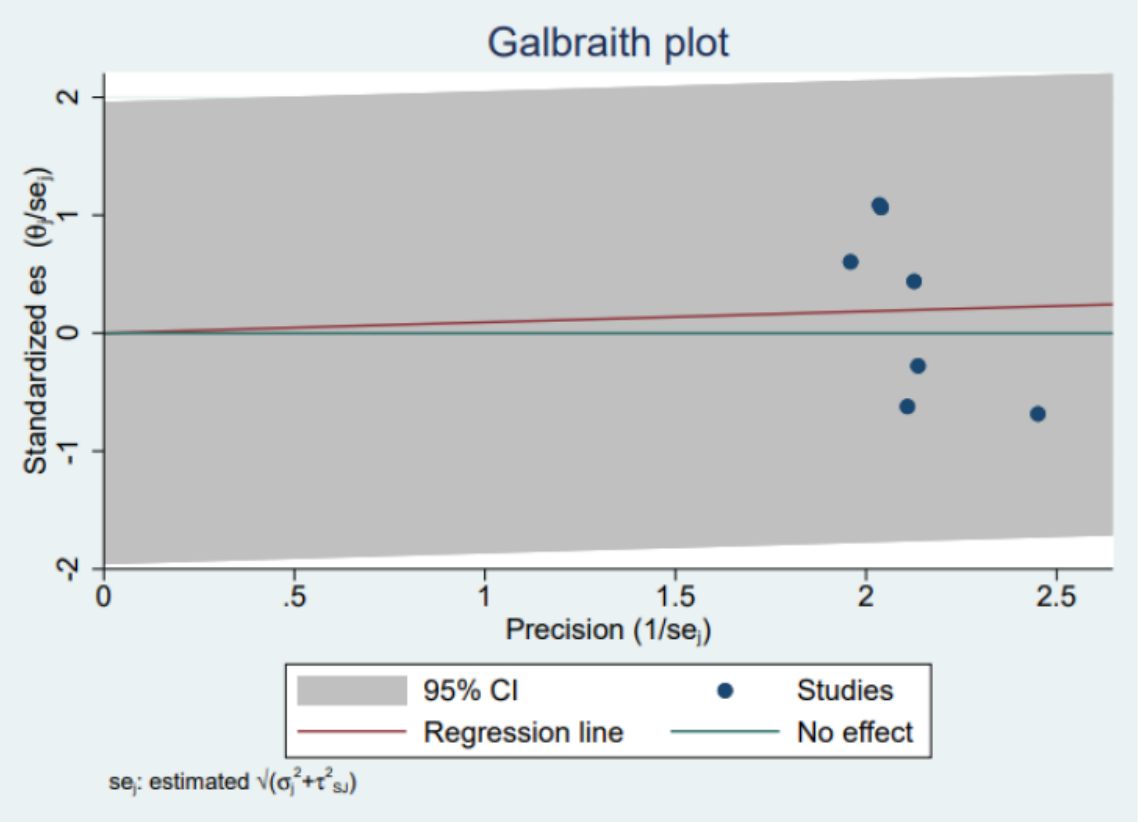
Supplemental Figure 3. Funnel plots to assess publication bias in acute vinegar intake on postprandial blood glucose in healthy volunteers. n=186. CI, confidence interval.

Supplemental Figure 3.1 Leave one out analysis for scute interventions with vinegar investigating postprandial blood glucose in healthy individuals



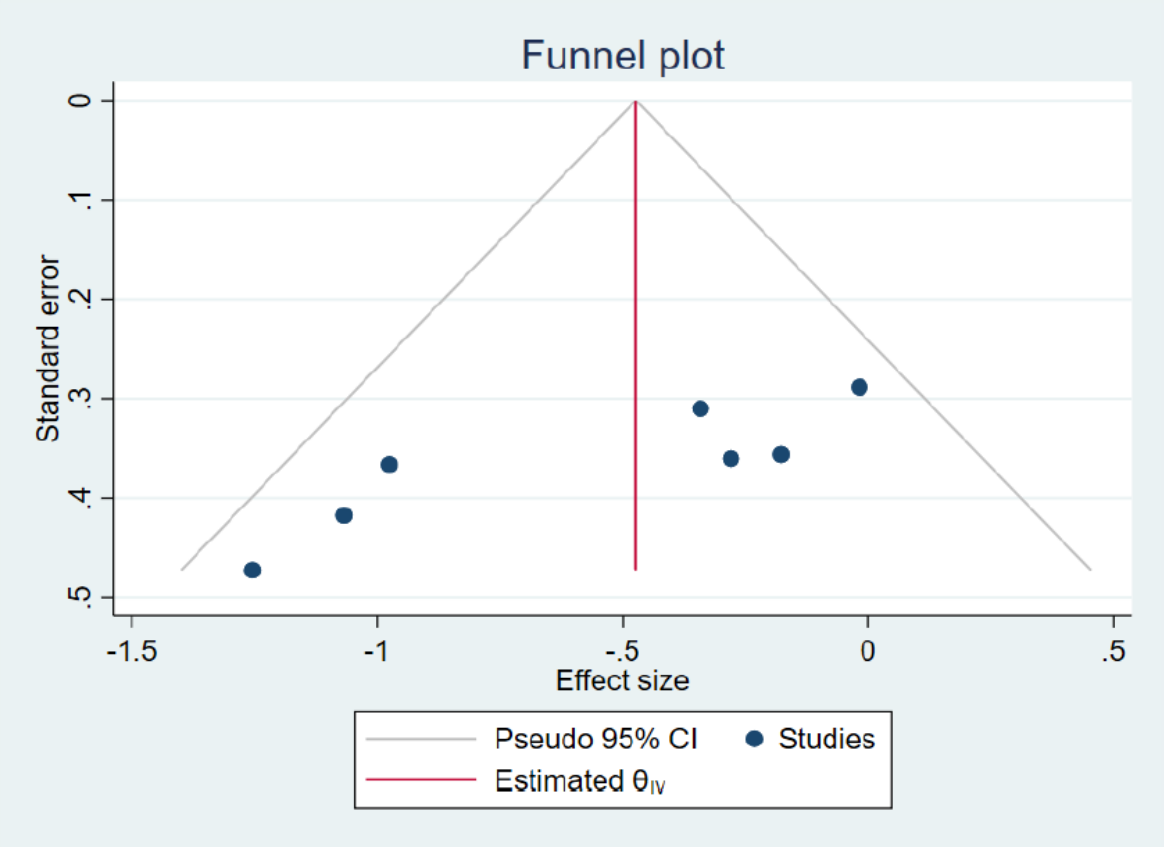
Supplemental Figure 3.1. Leave one out sensitivity analysis for acute vinegar intake on postprandial blood glucose in healthy volunteers. n=186.Random-effects Sidik-Johnkman model. n=35. P-value ≤0.05 was considered statistically significant. CI, confidence interval, IV, intravenous.

Supplemental Figure 3.2. Galbraith plot for acute interventions with vinegar investigating postprandial blood glucose in healthy individual



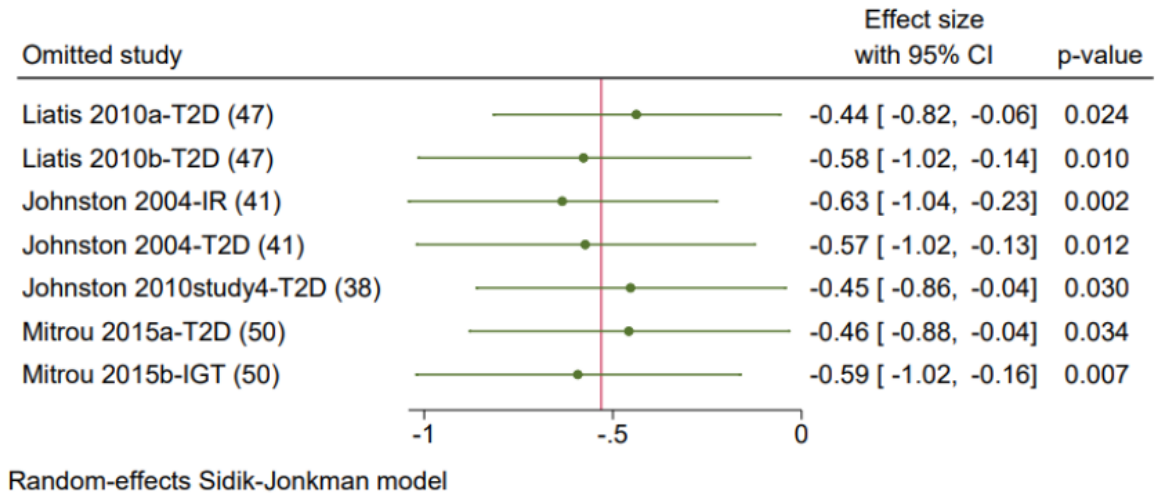
Supplemental Figure 3.2. Galbriath plot to assess for heterogeneity for acute vinegar intake on postprandial blood glucose in healthy volunteers. n=186. CI, confidence interval.

Supplemental Figure 4. Funnel plot for acute interventions with vinegar investigating postprandial blood glucose in non-healthy individuals



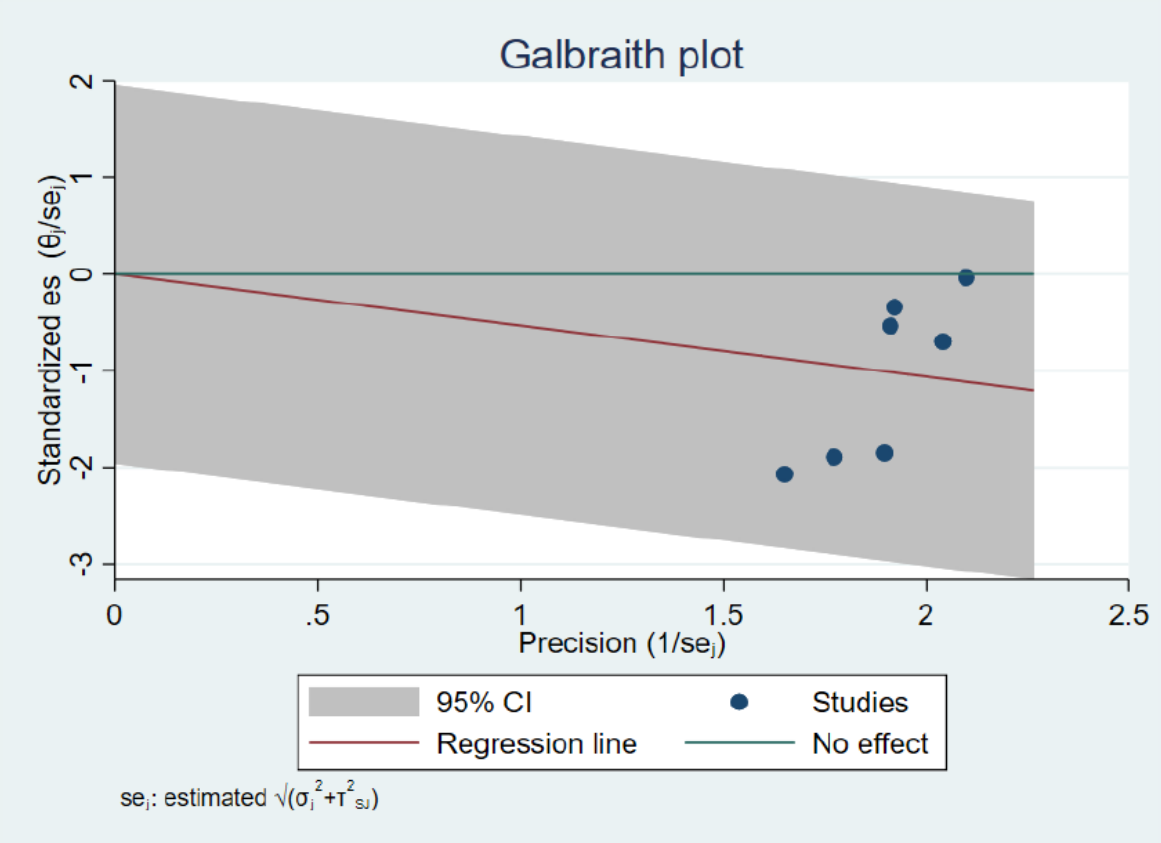
Supplemental Figure 4. Funnel plots to assess publication bias in acute vinegar intake on postprandial blood glucose in non-healthy adults. n=67. CI, confidence interval.

Supplemental Figure 4.1 Leave one out analysis for scute interventions with vinegar investigating postprandial blood glucose in non-healthy individuals



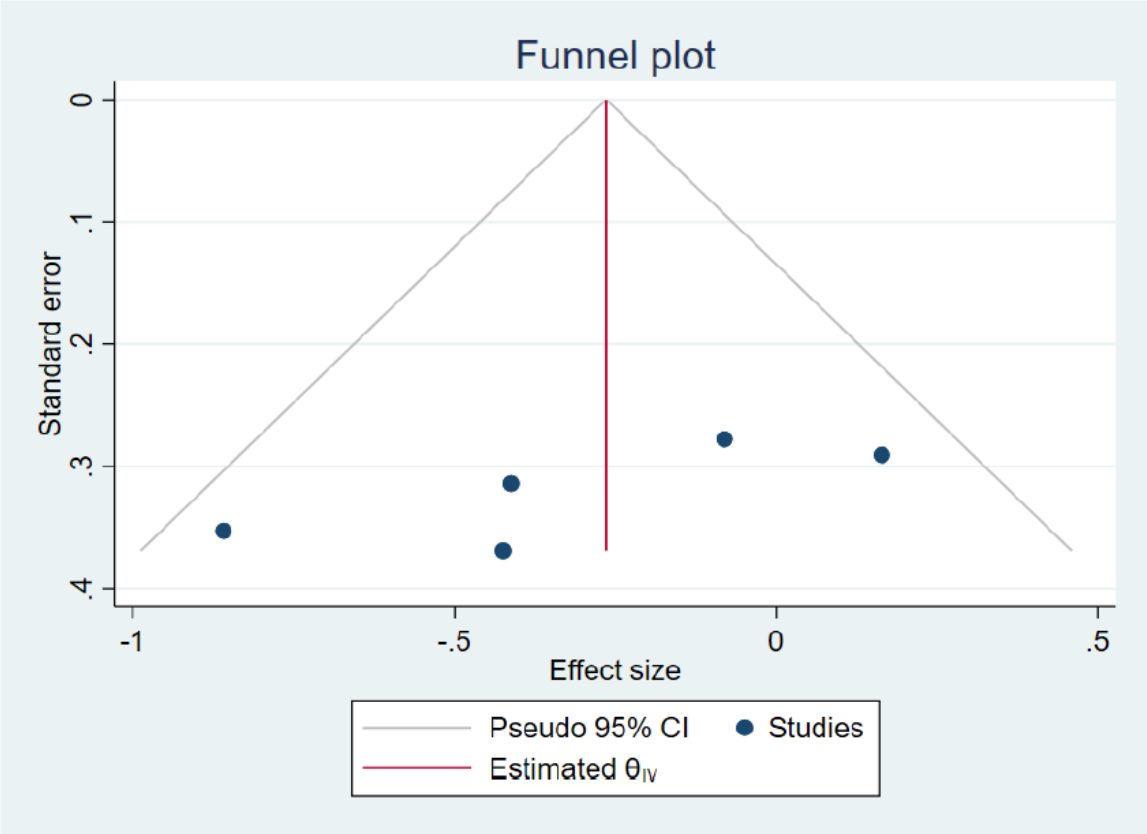
Supplemental Figure 4.1. Leave one out sensitivity analysis for acute vinegar intake on postprandial blood glucose in non-healthy adults. n=67. Random-effects Sidik-Johnkman model. P-value ≤0.05 was considered statistically significant. CI, confidence interval, IGT, impaired glucose tolerance, T2D, type 2 diabetes

Supplemental Figure 4.2. Galbraith plot for acute interventions with vinegar investigating postprandial blood glucose in non-healthy individuals



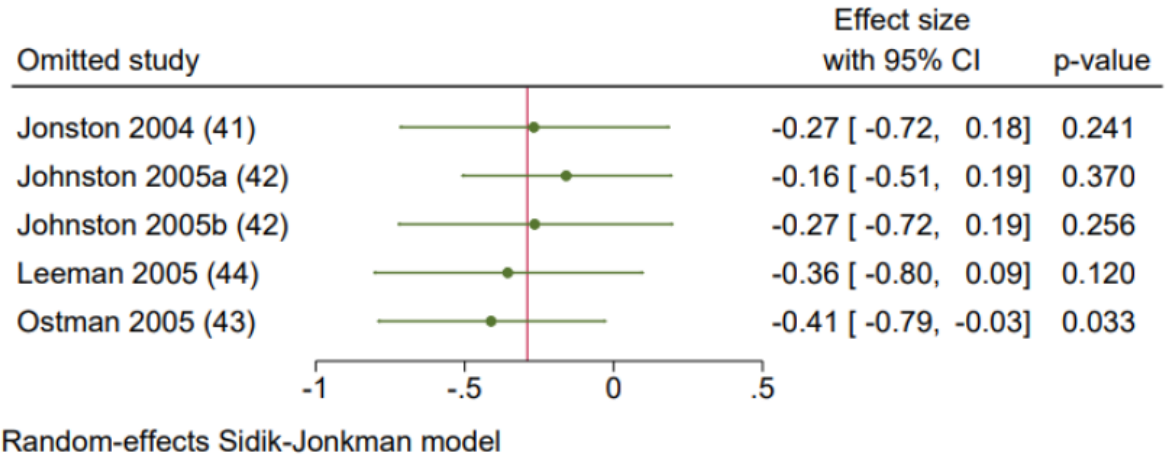
Supplemental Figure 4.2. Galbriath plot to assess for acute vinegar intake on postprandial blood glucose in non-healthy adults. n=67. CI, confidence interval.

Supplemental Figure 5. Funnel plot for acute interventions with acetate investigating postprandial insulin



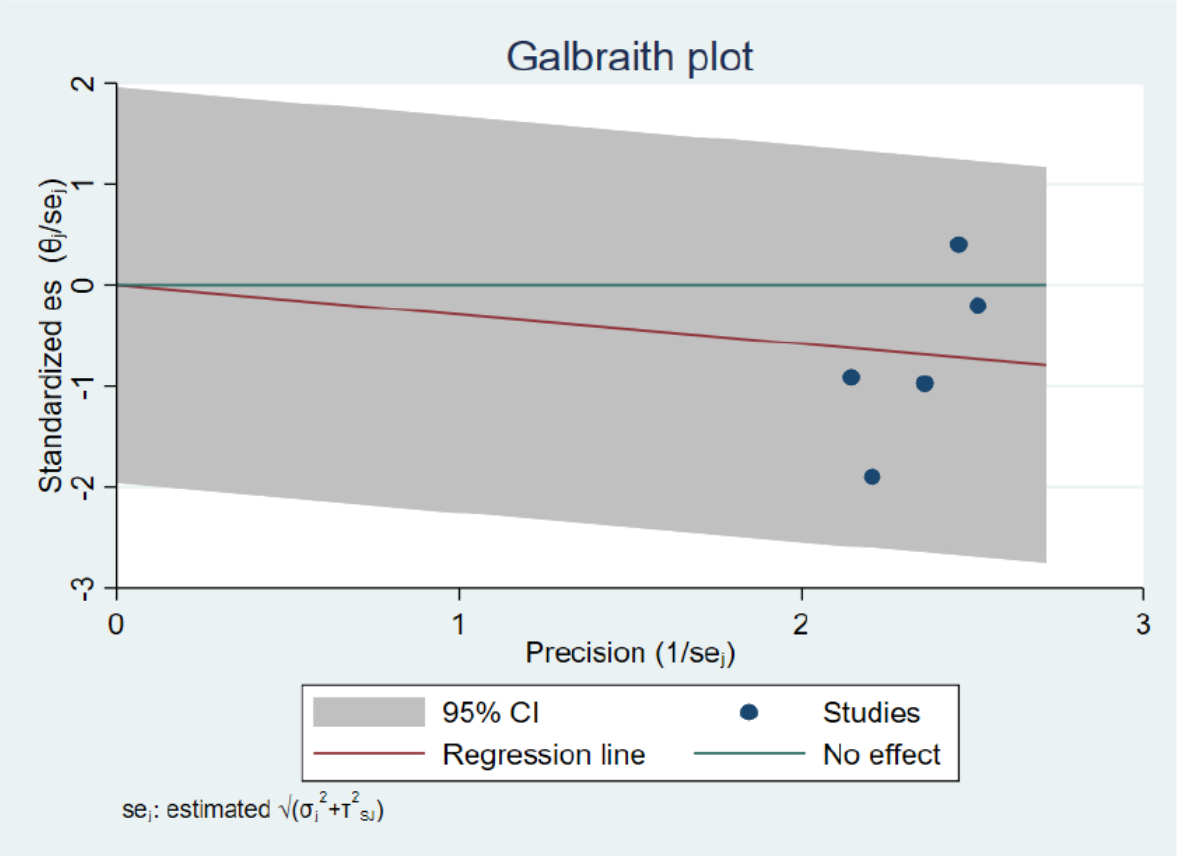
Supplemental Figure 5. Funnel plots to assess publication bias in acute vinegar intake on postprandial blood insulin in healthy volunteers. n=55. CI, confidence interval.

Supplemental Figure 5.1 Leave one out analysis for acute interventions with vinegar investigating postprandial insulin in healthy individuals



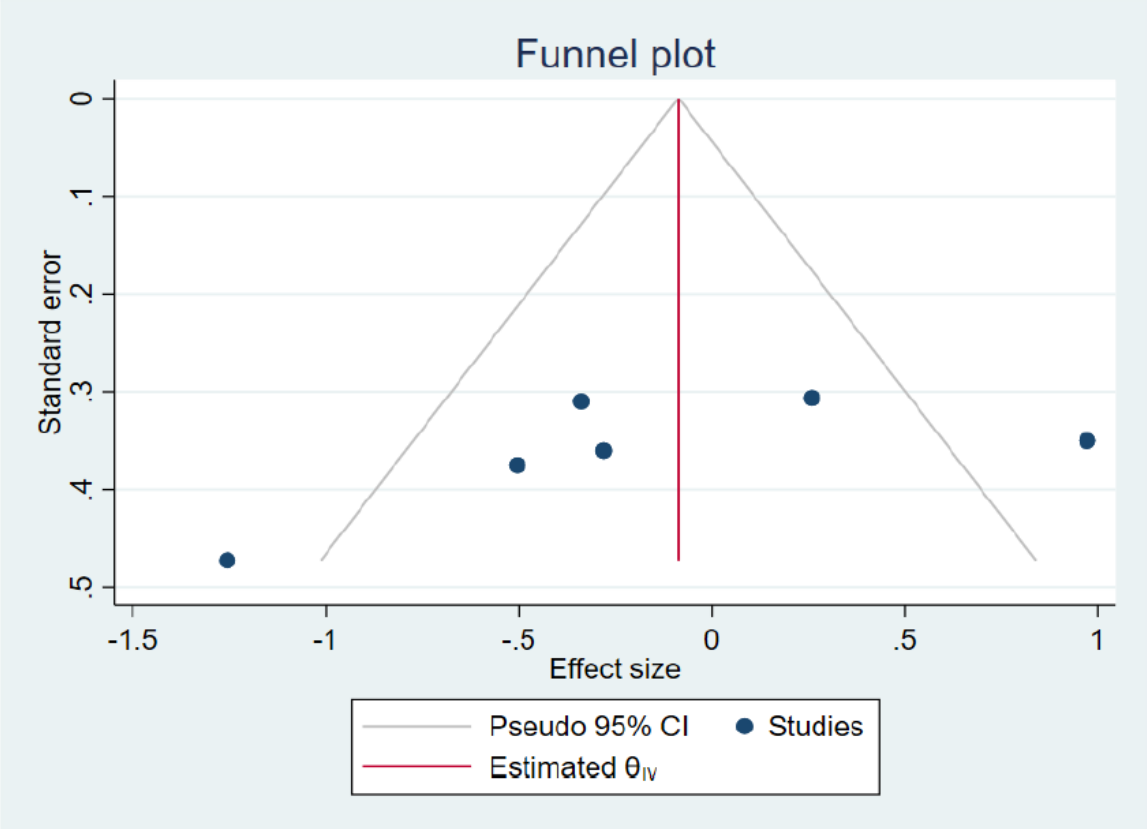
Supplemental Figure 5.1. Leave one out sensitivity analysis for acute vinegar intake on postprandial blood insulin in healthy volunteers. n=55. Random-effects Sidik-Johnkman model. P-value ≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 5.2. Galbraith plot for acute interventions with vinegar investigating postprandial insulin



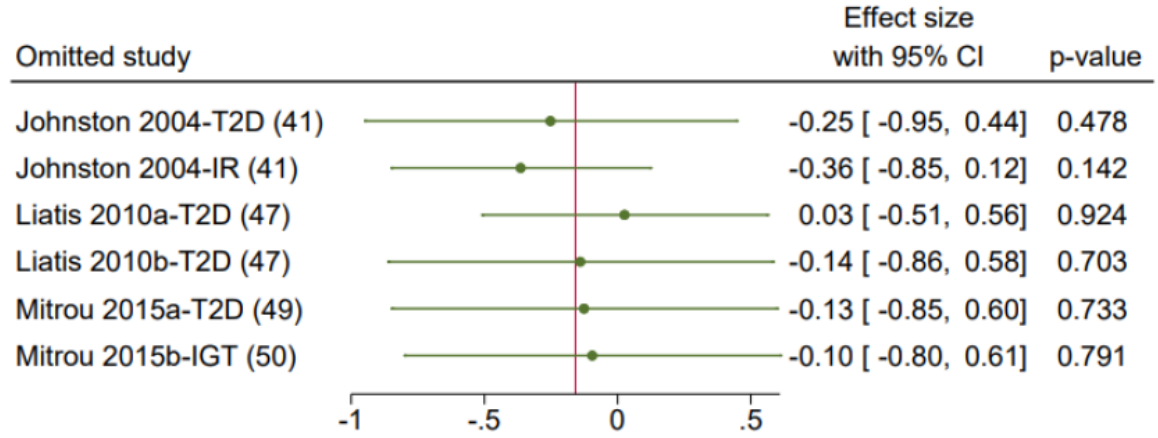
Supplemental Figure 5.2. Galbriath plot to assess for acute vinegar intake on postprandial blood insulin in healthy volunteers. n=55. CI, confidence interval.

Supplemental Figure 6. Funnel plot for acute interventions with vinegar investigating postprandial insulin in non-healthy



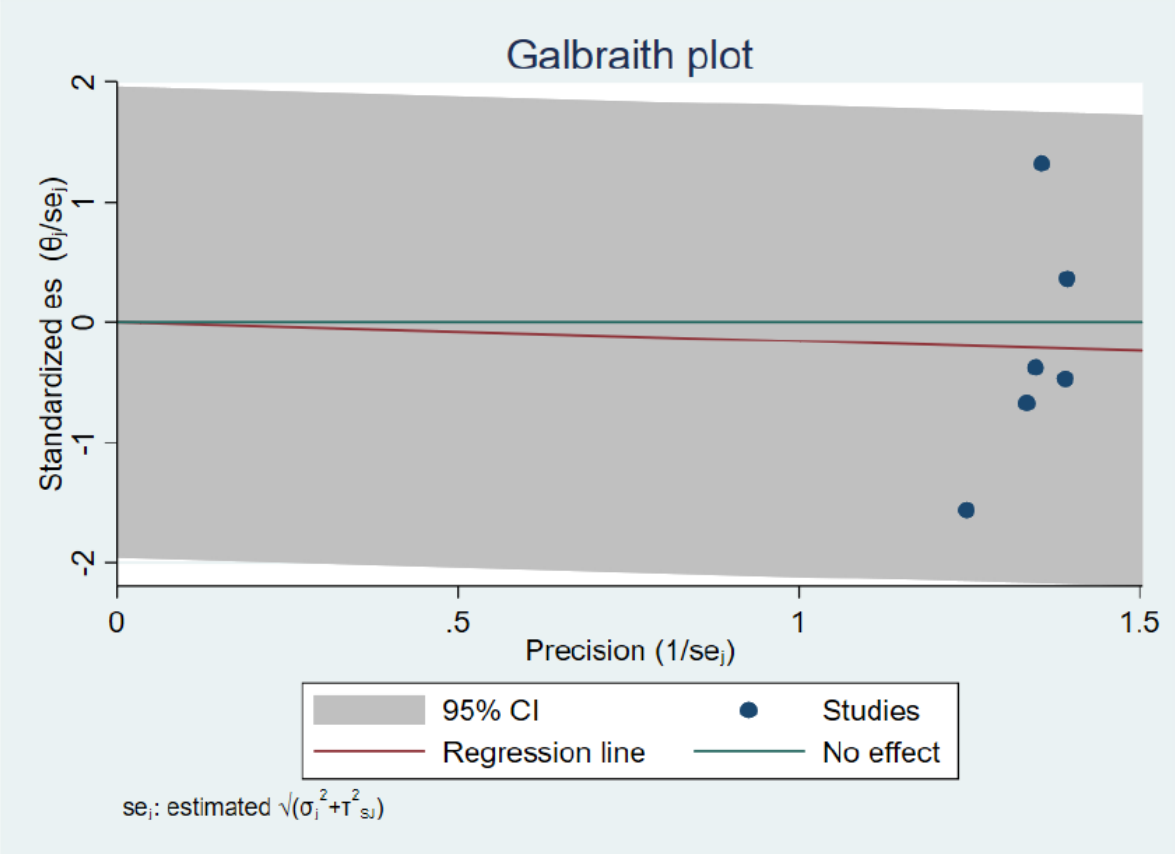
Supplemental Figure 6. Funnel plots to assess publication bias in acute vinegar intake on postprandial blood insulin in non-healthy adults. n=58. CI, confidence interval.

Supplemental Figure 6.1 Leave one out analysis for acute interventions with vinegar investigating postprandial insulin in non-healthy individuals



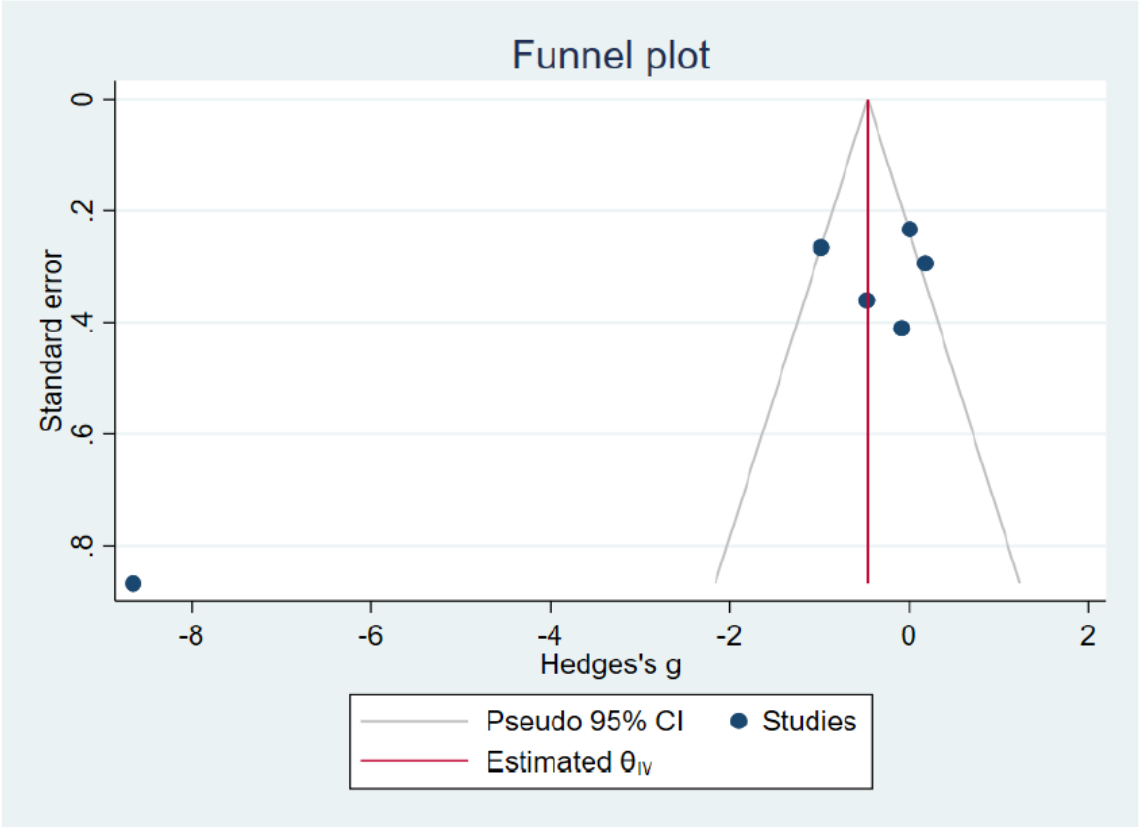
Supplemental Figure 6.1. Leave one out sensitivity analysis for acute vinegar intake on postprandial blood insulin in non-healthy adults. n=58. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 6.2. Galbraith plot for acute interventions with vinegar investigating postprandial insulin in non-healthy individuals



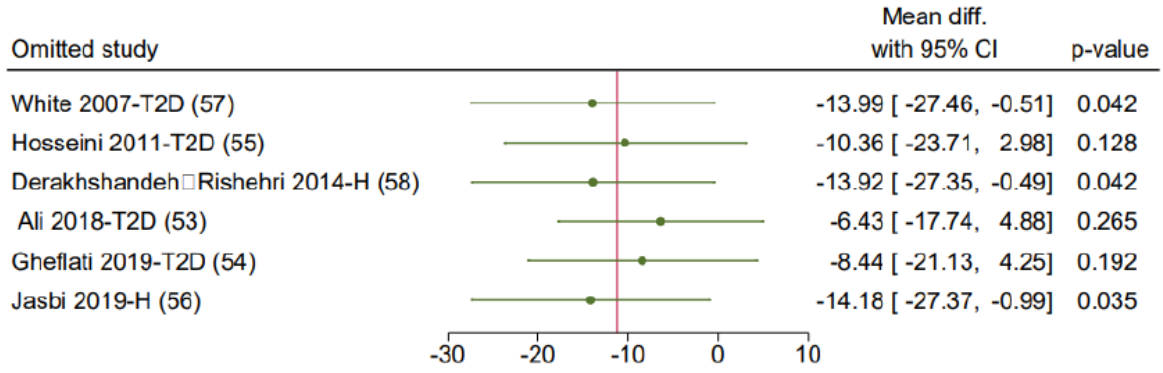
Supplemental Figure 6.2. Galbriath plot to assess for acute vinegar intake on postprandial blood insulin in non-healthy adults. n=58. CI, confidence interval.

Supplemental Figure 7. Funnel plot for chronic interventions with vinegar investigating postprandial blood glucose



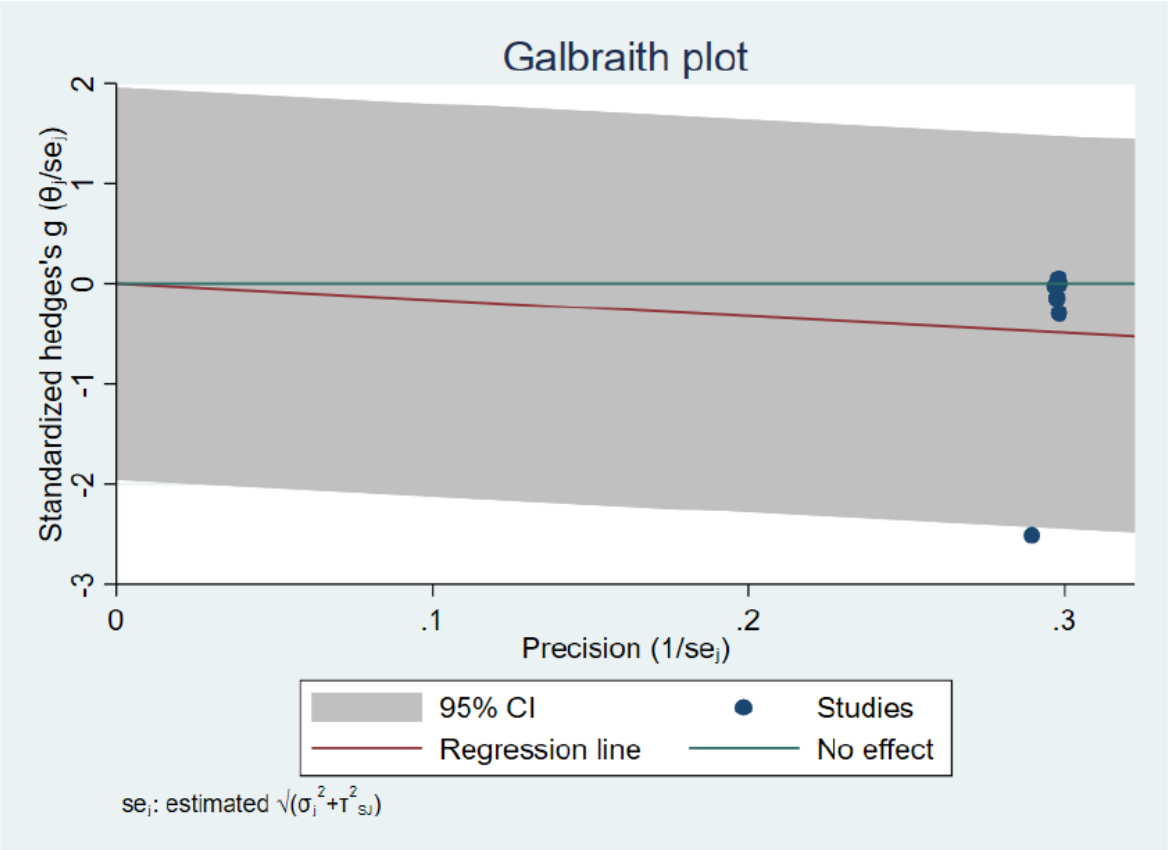
Supplemental Figure 7. Funnel plots to assess publication bias in chronic vinegar intake on postprandial blood glucose. n=143. CI, confidence interval.

Supplemental Figure 7.1 Leave one out analysis for chronic interventions with vinegar investigating postprandial blood glucose



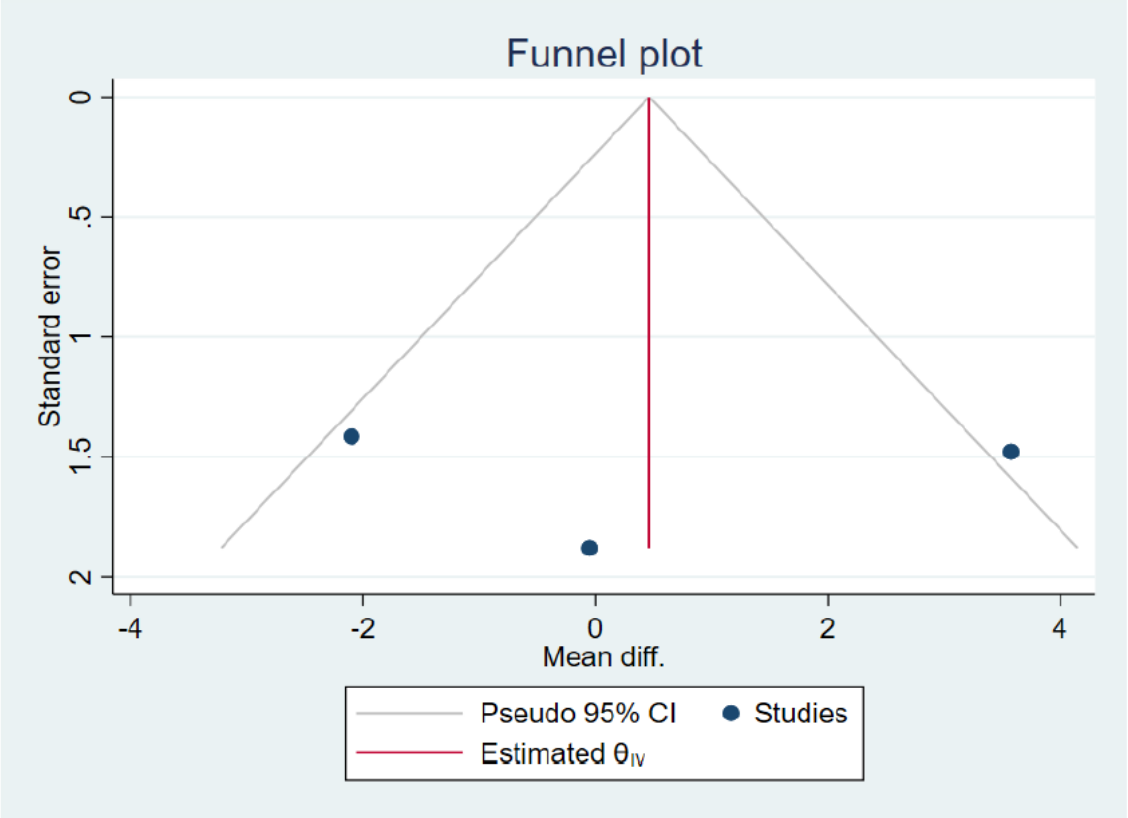
Supplemental Figure 7.1. Leave one out sensitivity analysis for chronic vinegar intake on postprandial blood glucose. n=143. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval, H, healthy, T2D, type 2 diabetes.

Supplemental Figure 7.2. Galbraith plot for chronic interventions with vinegar investigating postprandial blood glucose



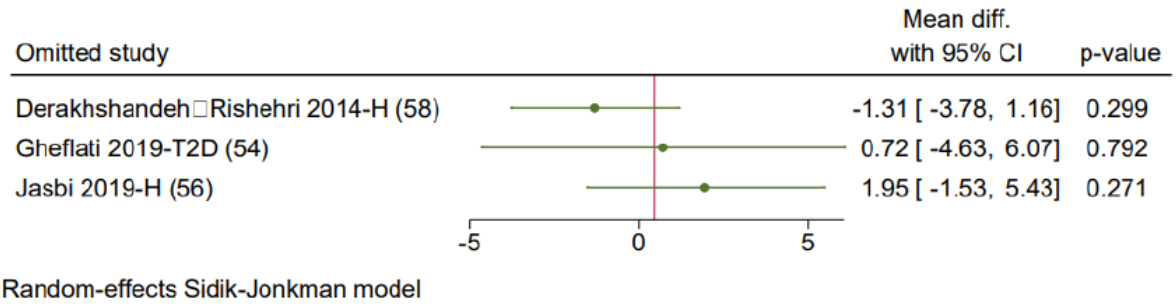
Supplemental Figure 7.2. Galbriath plot to assess for chronic vinegar intake on postprandial blood glucose. n=143. CI, confidence interval.

Supplemental Figure 8. Funnel plot for chronic interventions with vinegar investigating postprandial insulin



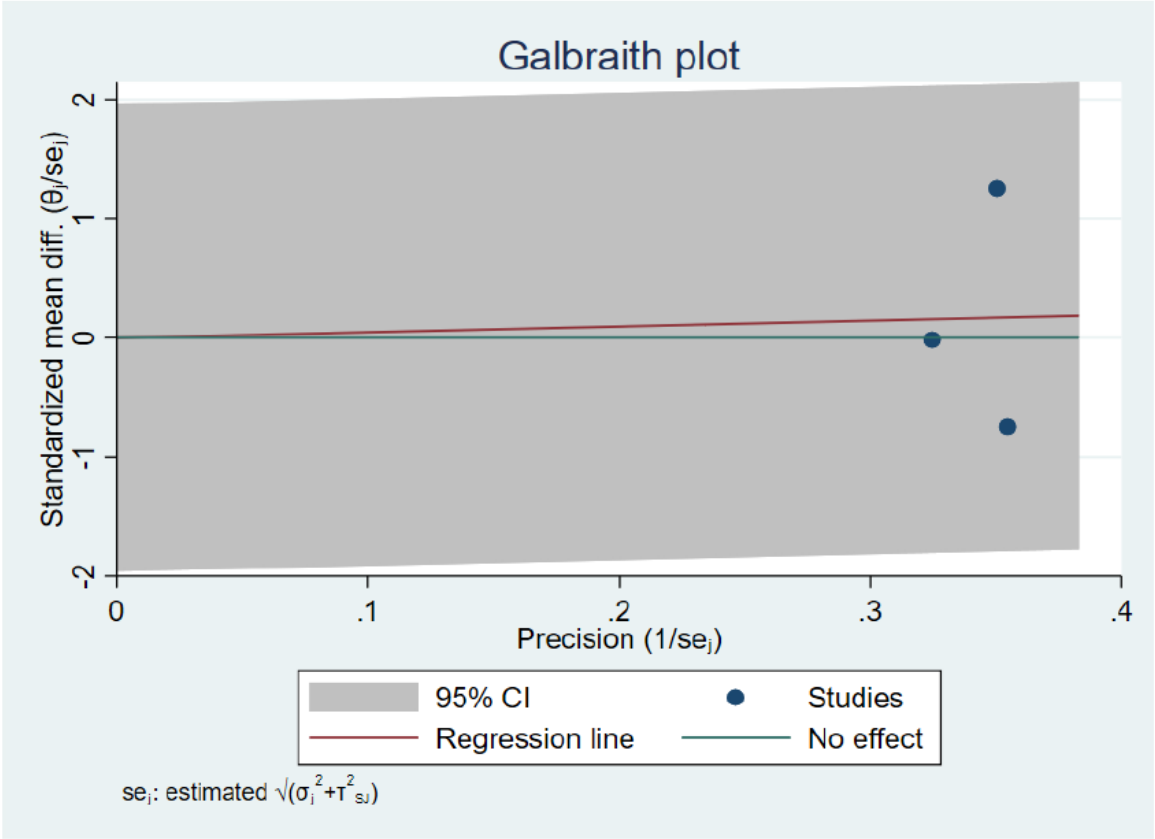
Supplemental Figure 8. Funnel plots to assess publication bias in chronic vinegar intake on postprandial blood insulin. n=89. CI, confidence interval.

Supplemental Figure 8.1 Leave one out analysis for chronic interventions with vinegar investigating postprandial insulin



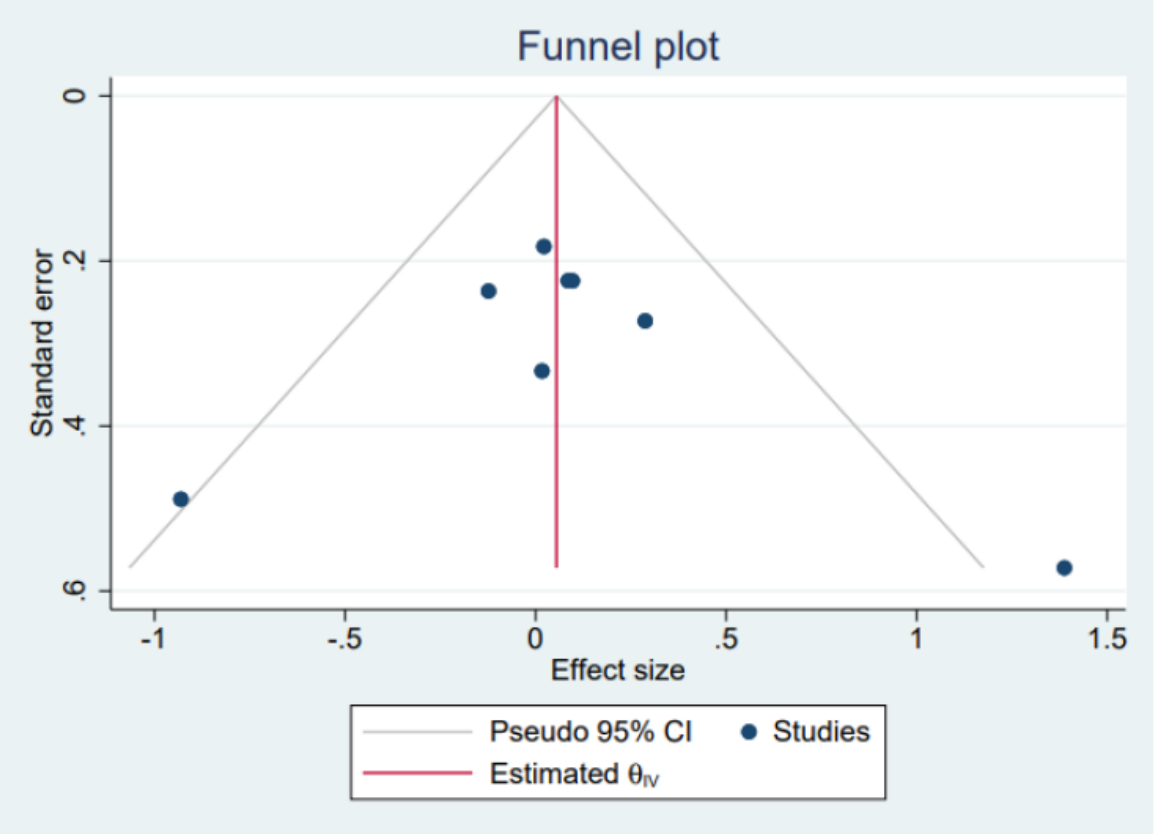
Supplemental Figure 8.1. Leave one out sensitivity analysis for chronic vinegar intake on postprandial blood insulin. n=89. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval, H, healthy, T2D, type 2 diabetes.

Supplemental Figure 8.2. Galbraith plot for chronic interventions with vinegar investigating postprandial insulin



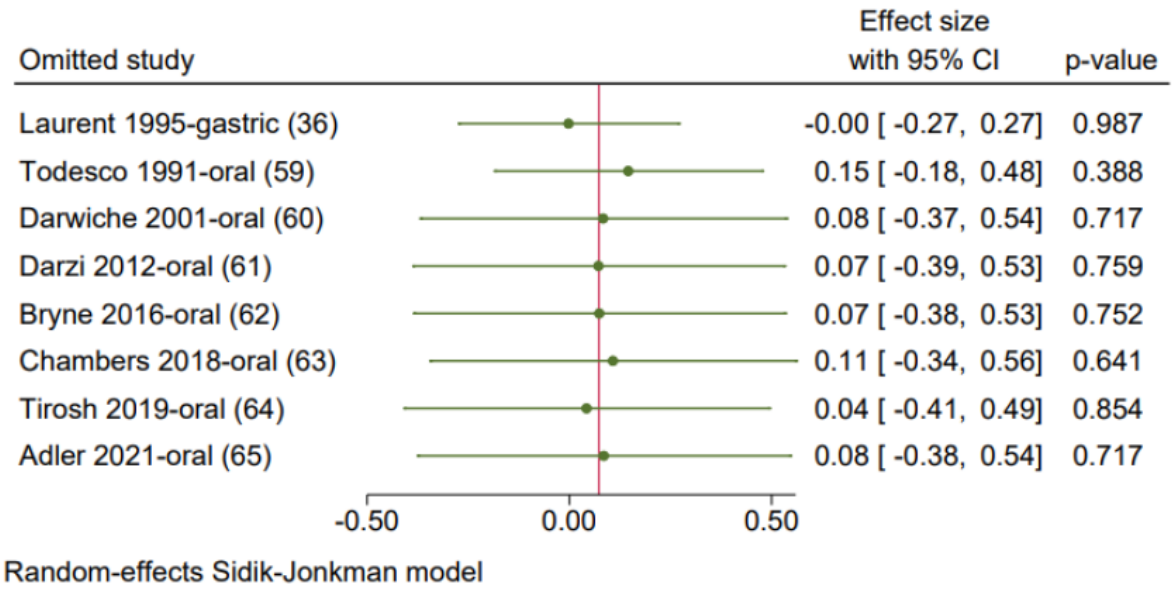
Supplemental Figure 8.2. Galbriath plot to assess for chronic vinegar intake on postprandial blood insulin. n=89. CI, confidence interval.

Supplemental Figure 9 Funnel plot for acute interventions with propionate investigating postprandial blood glucose



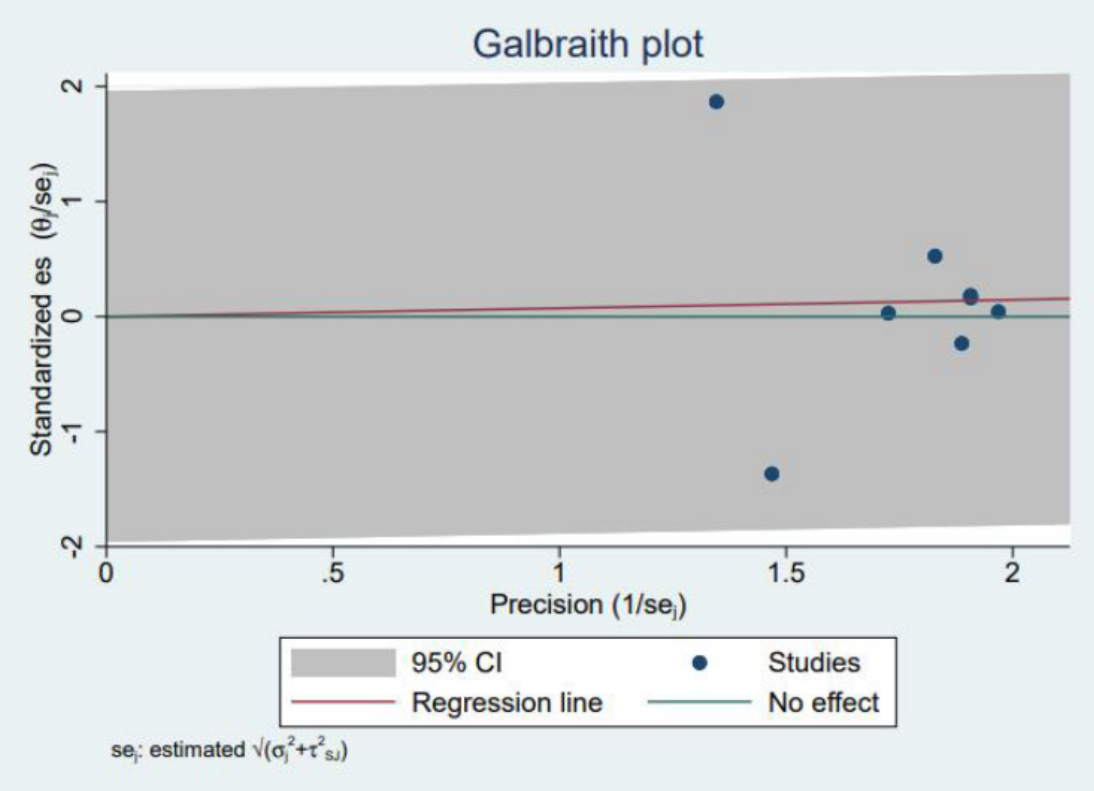
Supplemental Figure 9. Funnel plots to assess publication bias in acute propionate on postprandial blood glucose. n=123. CI, confidence interval.

Supplemental Figure 9.1 Leave one out analysis for acute interventions with propionate investigating postprandial blood glucose



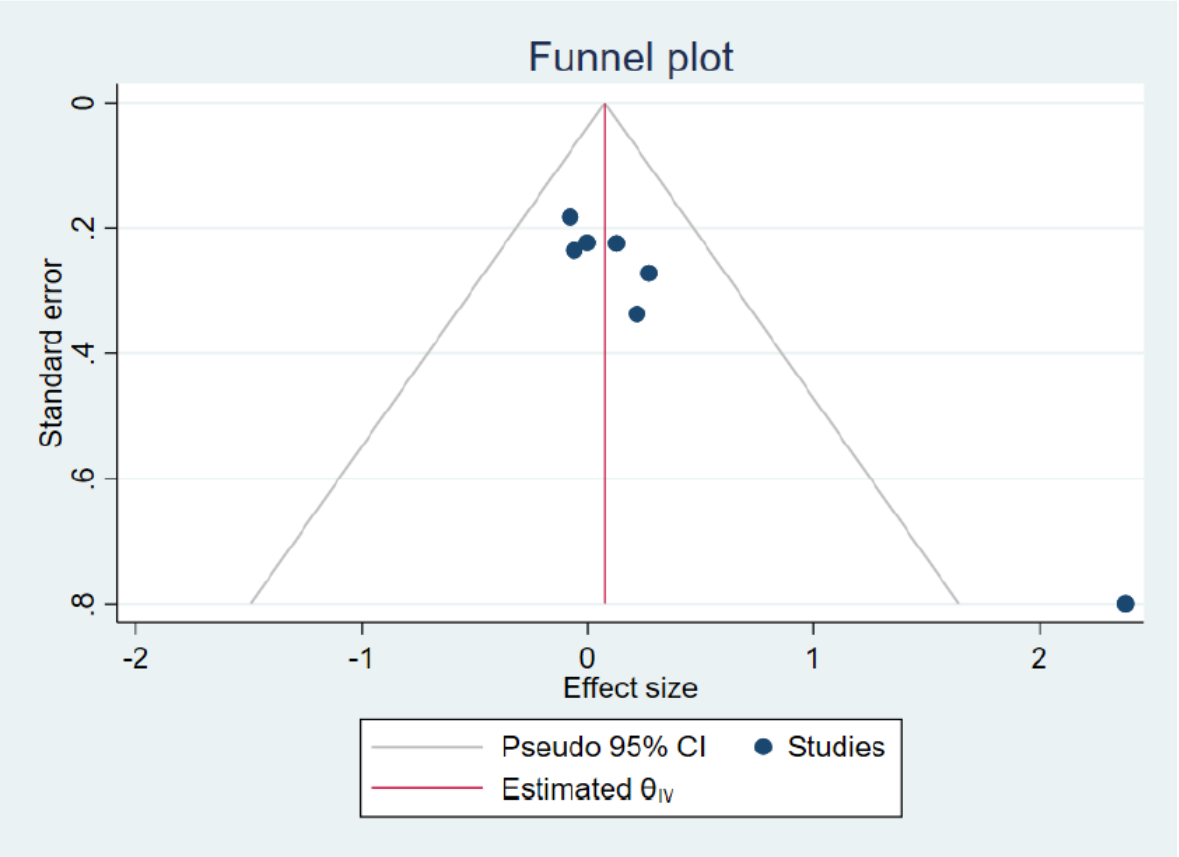
Supplemental Figure 9.1. Leave one out sensitivity analysis for acute propionate on postprandial blood glucose. n=123. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 9.2. Galbraith plot for acute interventions with propionate investigating postprandial blood glucose



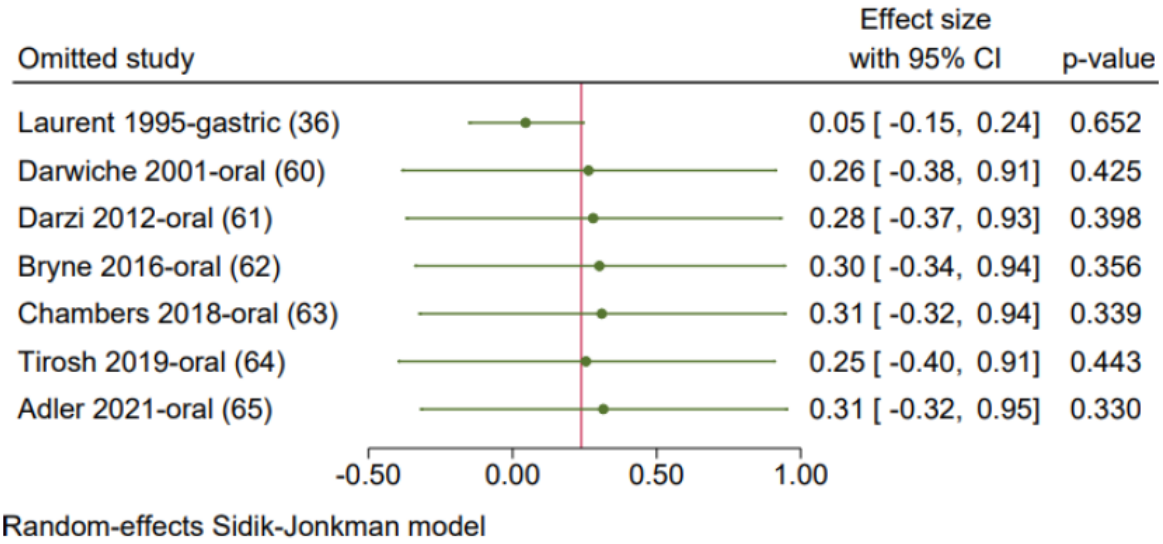
Supplemental Figure 9.2. Galbriath plot to assess for acute propionate on postprandial blood glucose. n=123. CI, confidence interval.

Supplemental Figure 10. Funnel plot for acute interventions with propionate investigating postprandial insulin



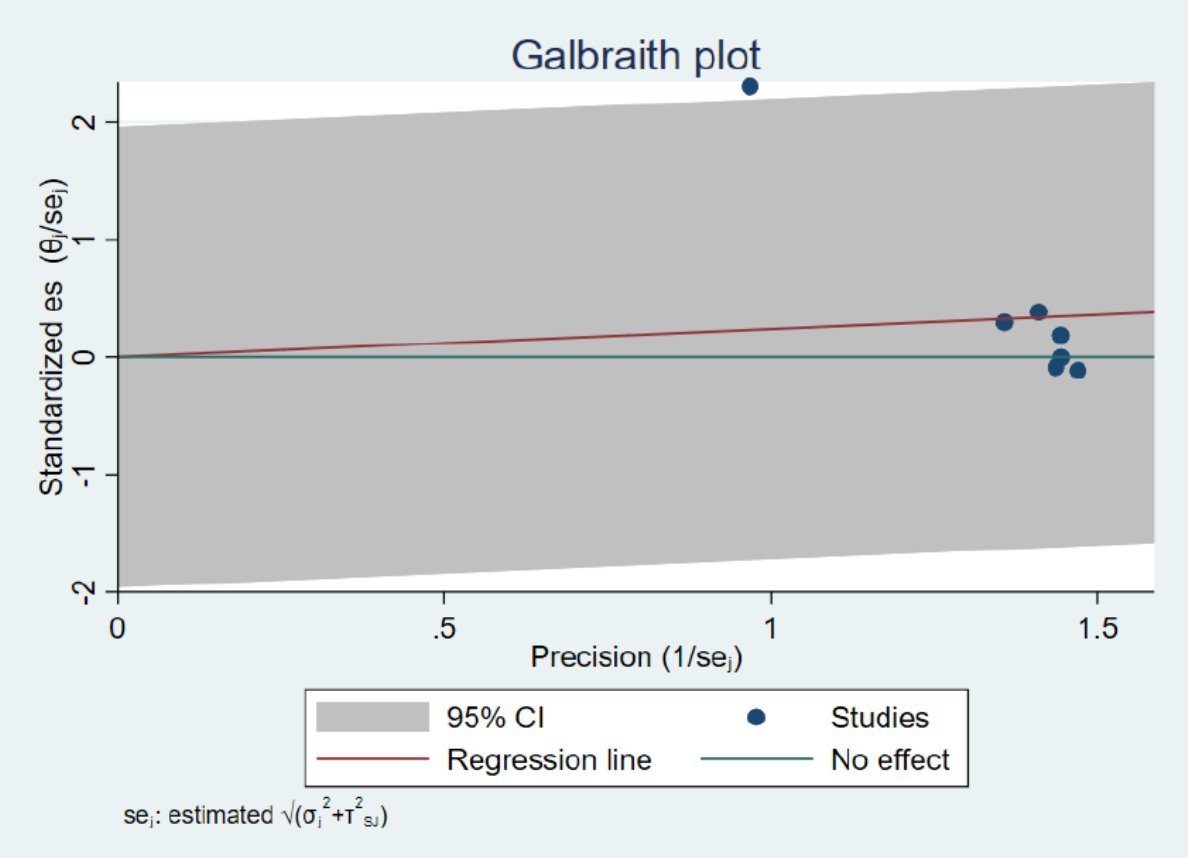
Supplemental Figure 10. Funnel plots to assess publication bias in acute propionate on postprandial blood insulin. n=117. CI, confidence interval.

Supplemental Figure 10.1 Leave one out analysis for acute interventions with propionate investigating postprandial insulin



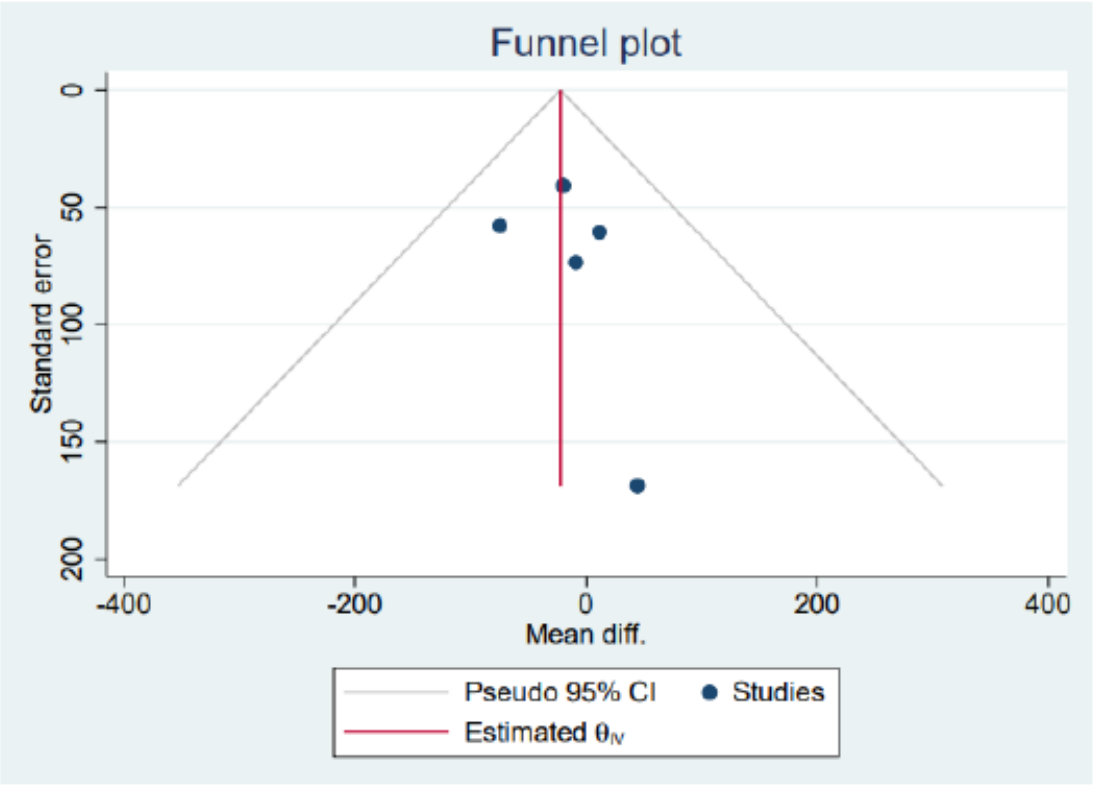
Supplemental Figure 10.1. Leave one out sensitivity analysis for acute propionate on postprandial blood insulin. n=117. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 10.2. Galbraith plot for acute interventions with propionate investigating postprandial insulin



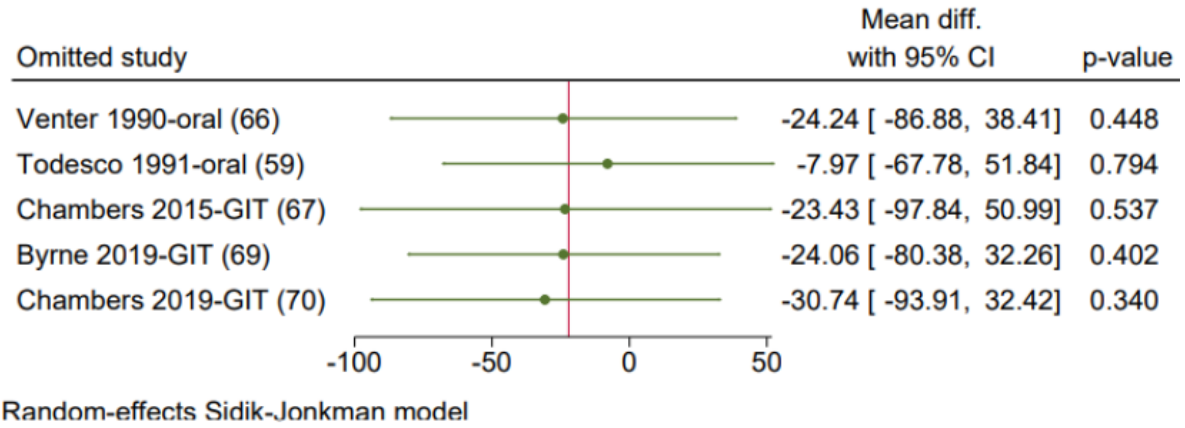
Supplemental Figure 10.2. Galbriath plot to assess for acute propionate on postprandial blood insulin. n=117. CI, confidence interval.

Supplemental Figure 11. Funnel plot for chronic interventions with propionate investigating postprandial blood glucose



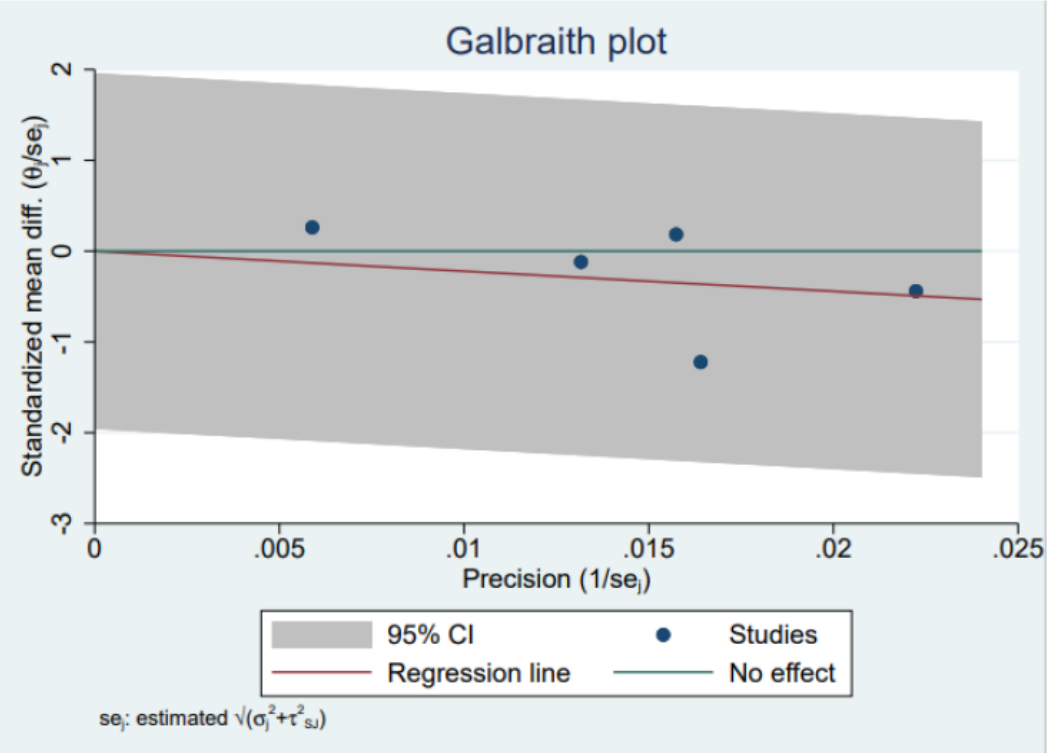
Supplemental Figure 11. Funnel plots to assess publication bias in chronic propionate on postprandial blood glucose. n=73. CI, confidence interval.

Supplemental Figure 11.1 Leave one out analysis for chronic interventions with propionate investigating postprandial blood glucose



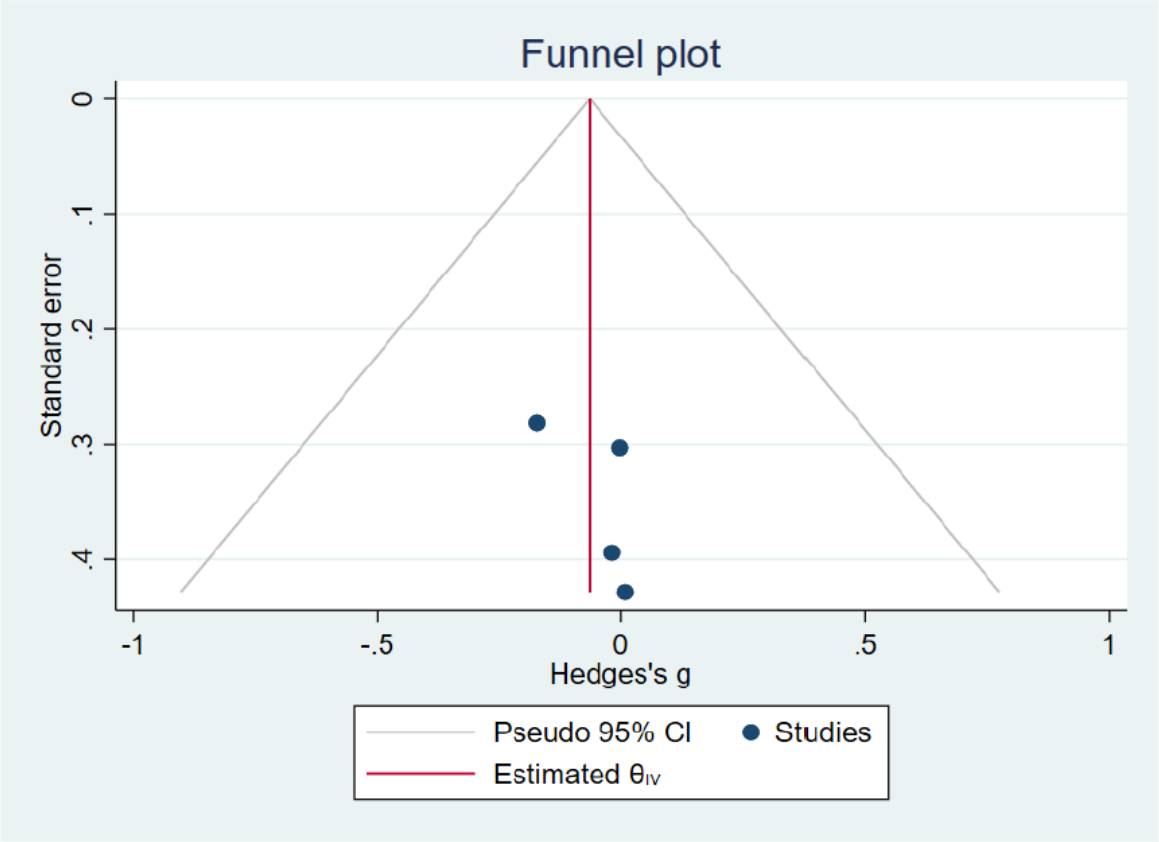
Supplemental Figure 11.1. Leave one out sensitivity analysis for chronic propionate on postprandial blood glucose. n=73.Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 11.2. Galbraith plot for chronic interventions with propionate investigating postprandial blood glucose



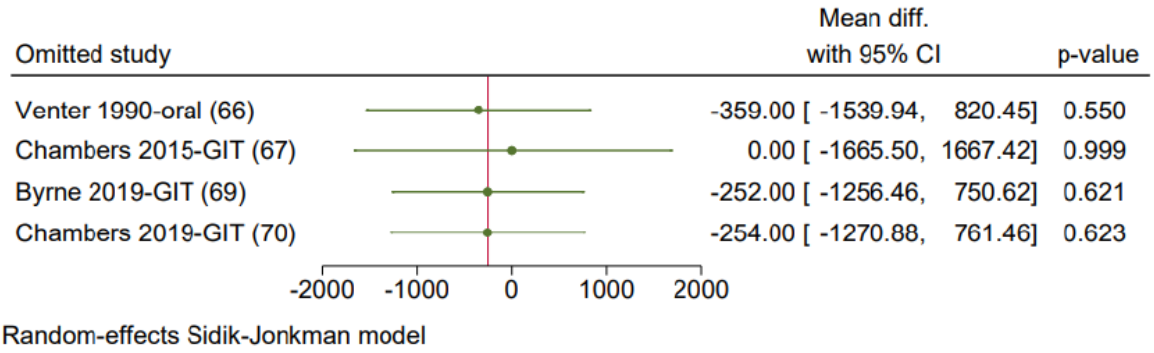
Supplemental Figure 11.2. Galbriath plot to assess for chronic propionate on postprandial blood glucose. n=73. CI, confidence interval.

Supplemental Figure 12. Funnel plot for chronic interventions with propionate investigating postprandial insulin



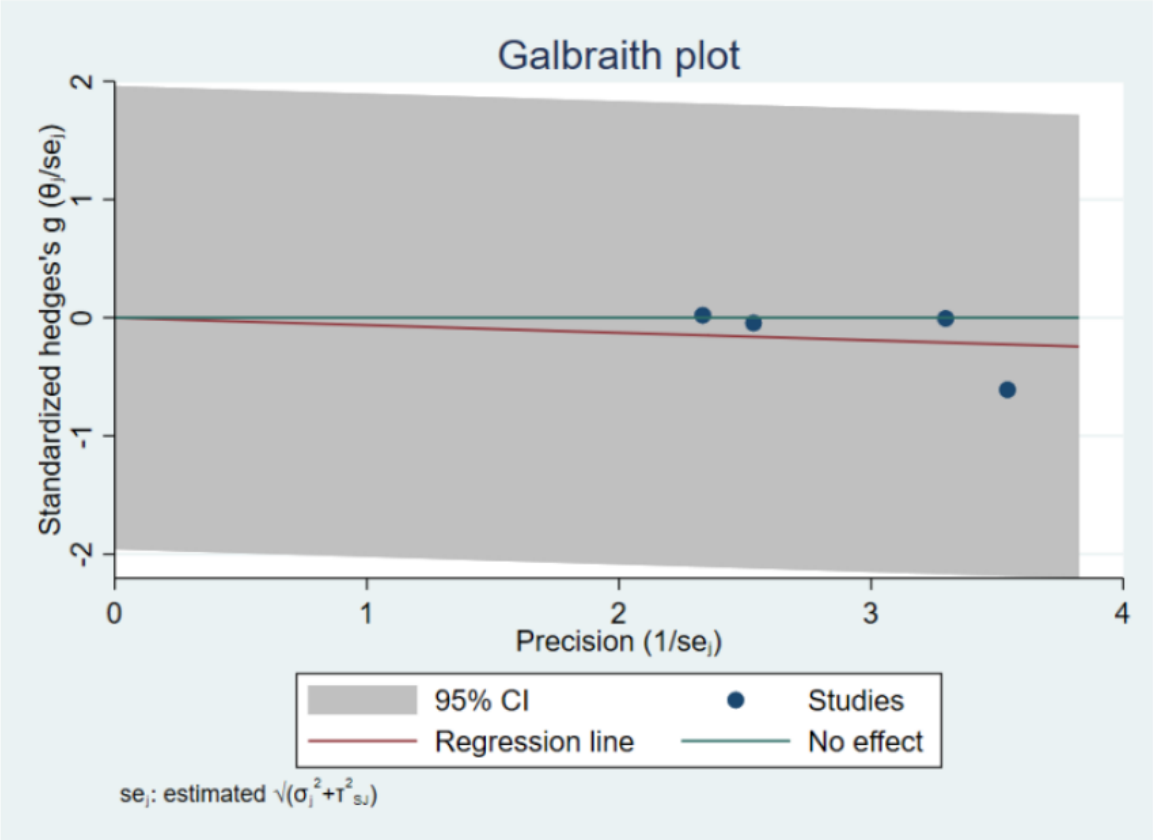
Supplemental Figure 12. Funnel plots to assess publication bias in chronic propionate on postprandial blood insulin. n=117. CI, confidence interval.

Supplemental Figure 12.1 Leave one out analysis for chronic interventions with propionate investigating postprandial insulin



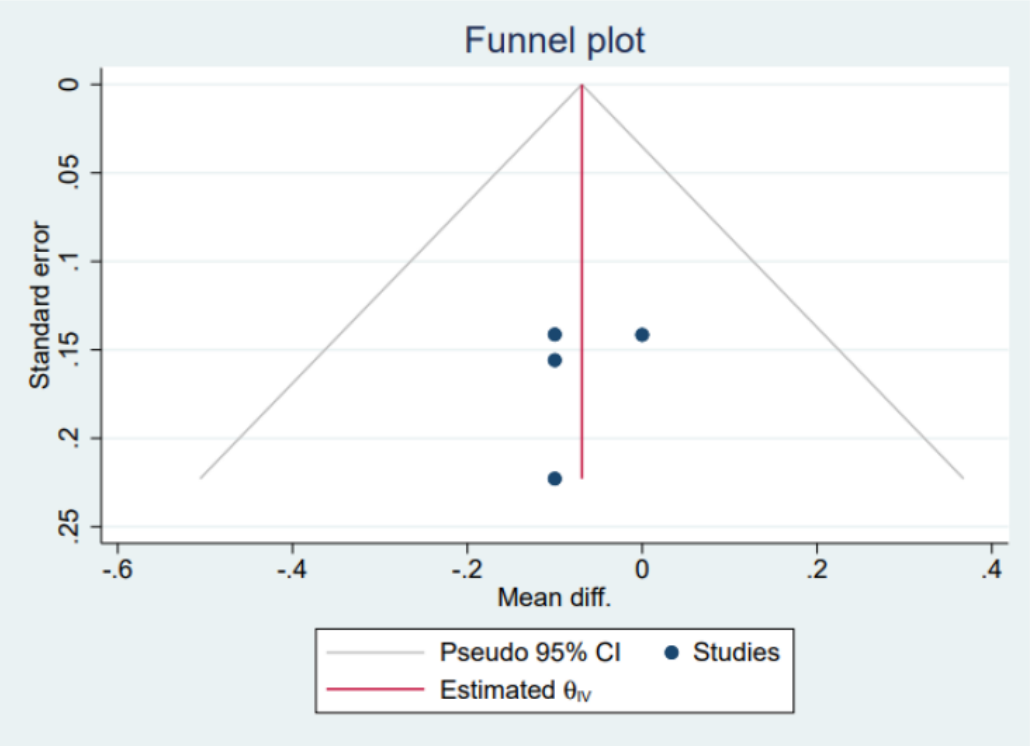
Supplemental Figure 12.1. Leave one out sensitivity analysis for chronic propionate on postprandial blood insulin. n=117. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval, GIT, gastrointestinal tract

Supplemental Figure 12.2. Galbraith plot for chronic interventions with propionate investigating postprandial insulin



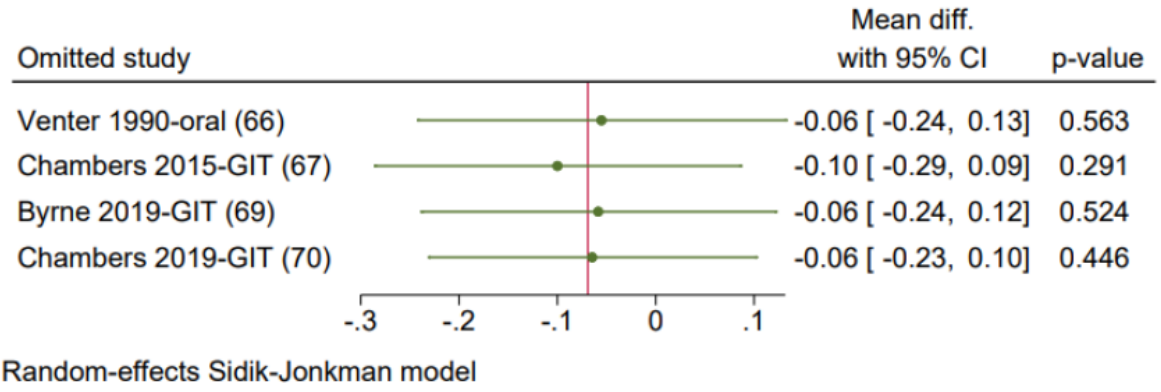
Supplemental Figure 12.2. Galbriath plot to assess for chronic propionate on postprandial blood insulin. n=117. CI, confidence interval.

Supplemental Figure 13. Funnel plot for chronic interventions with propionate investigating fasting blood glucose



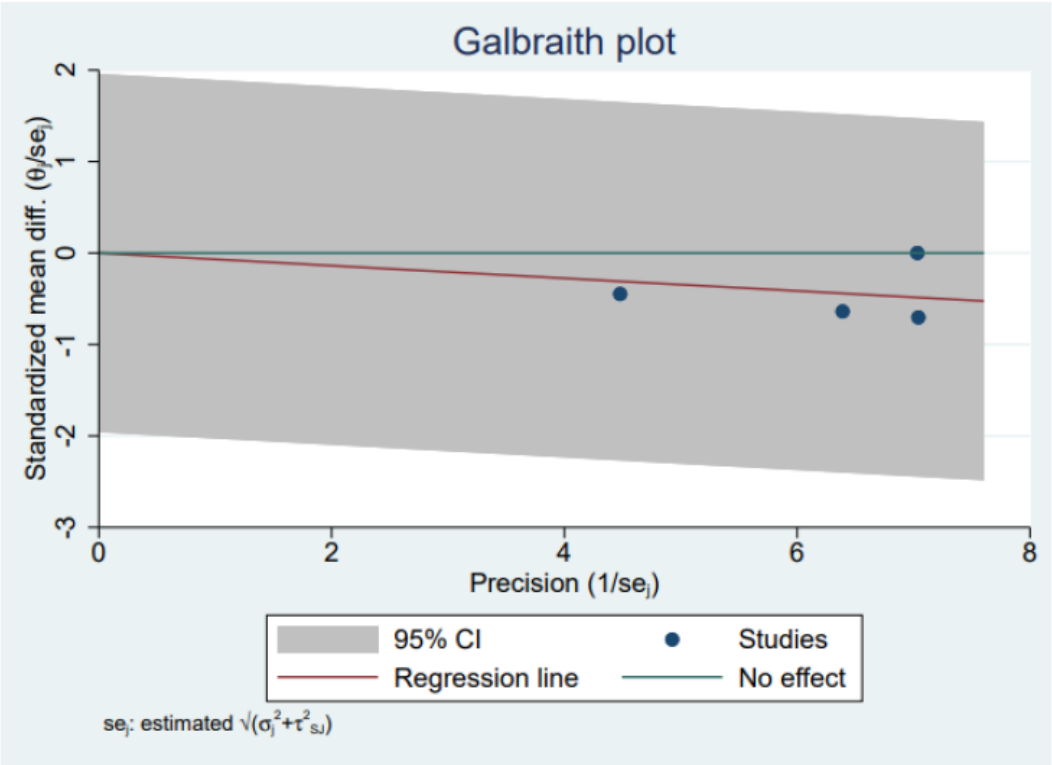
Supplemental Figure 13. Funnel plots to assess publication bias in chronic propionate on fasting blood glucose. n=67. CI, confidence interval.

Supplemental Figure 13.1 Leave one out analysis for chronic interventions with propionate investigating fasting blood glucose



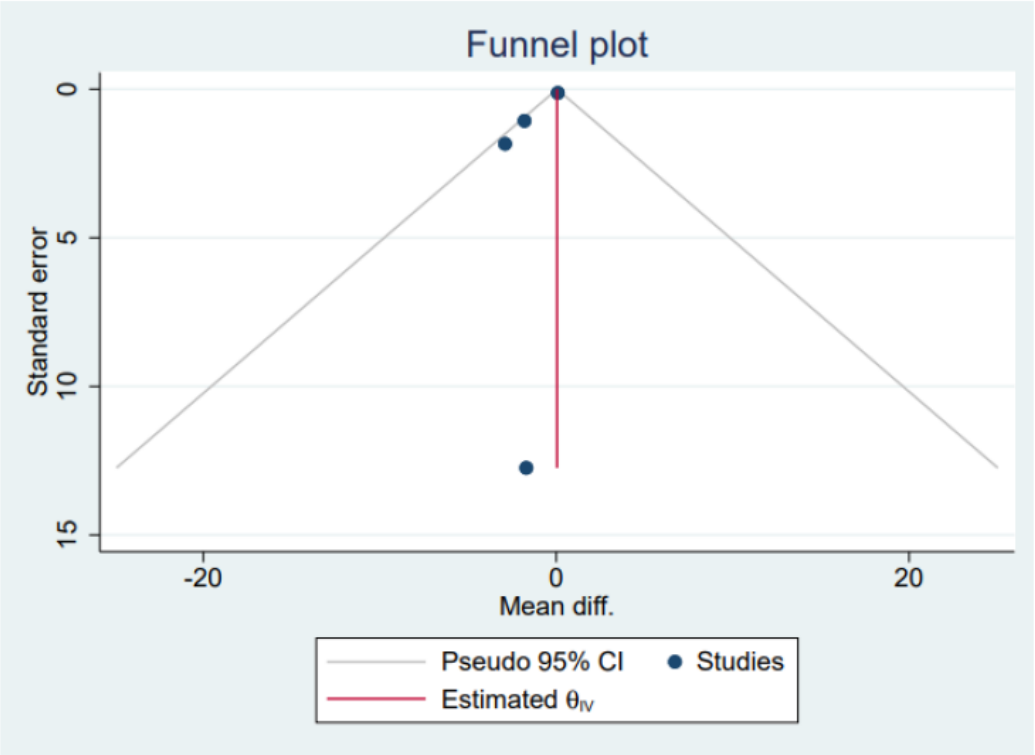
Supplemental Figure 13.1. Leave one out sensitivity analysis for chronic propionate on fasting blood glucose. n=67. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval.

Supplemental Figure 13.2. Galbraith plot for chronic interventions with propionate investigating fasting blood glucose



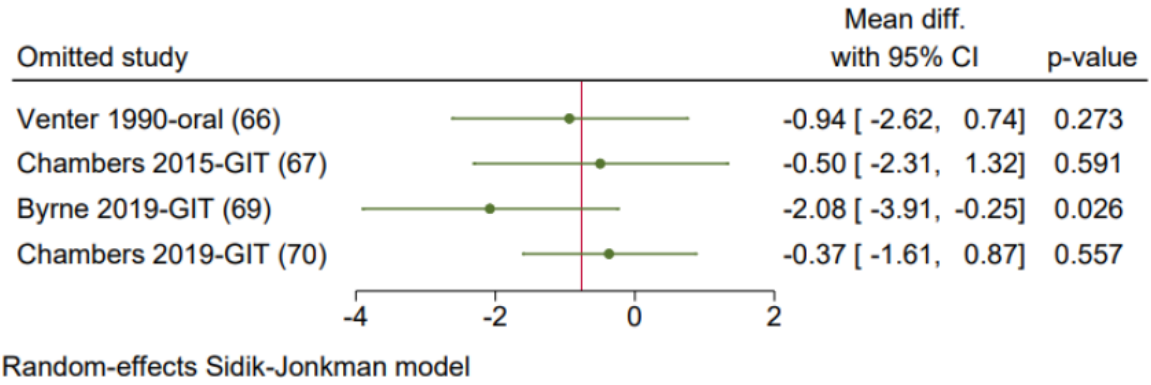
Supplemental Figure 13.2. Galbriath plot to assess for chronic propionate on fasting blood glucose. n=67. CI, confidence interval.

Supplemental Figure 14. Funnel plot for chronic interventions with propionate investigating fasting insulin



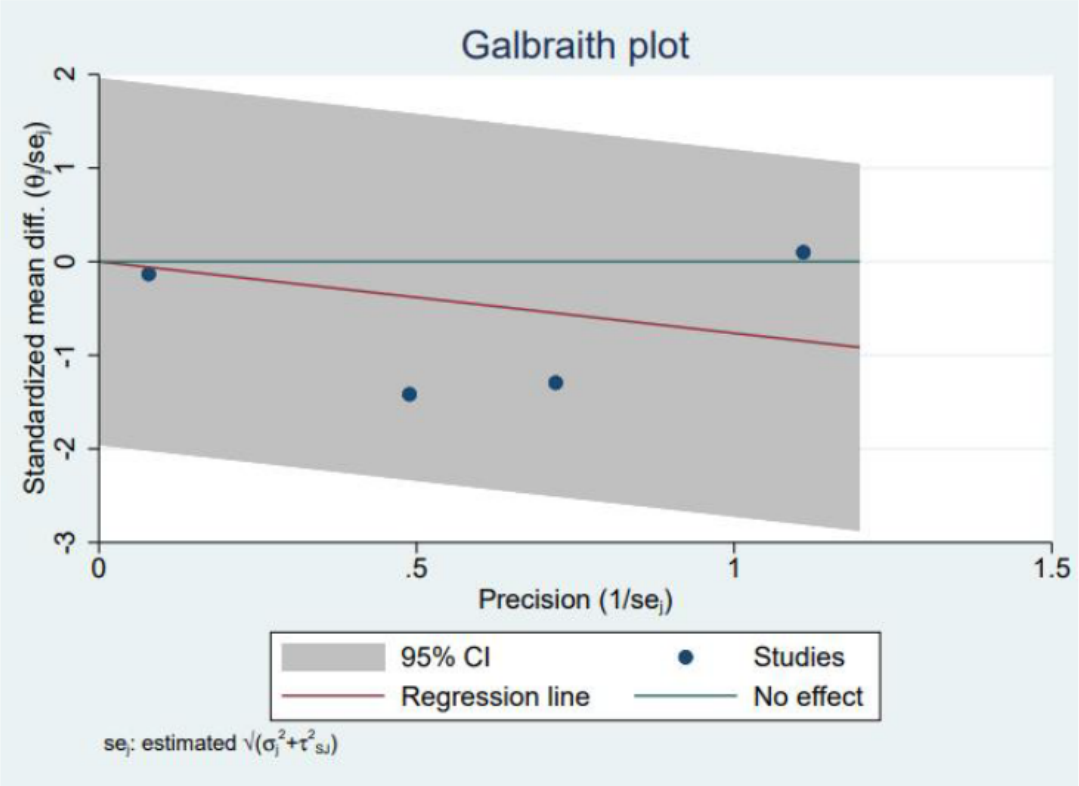
Supplemental Figure 14. Funnel plots to assess publication bias in chronic propionate on fasting blood insulin. n=67. CI, confidence interval.

Supplemental Figure 14.1. Leave one out analysis for chronic interventions with propionate investigating fasting insulin



Supplemental Figure 14.1. Leave one out sensitivity analysis for chronic propionate on fasting blood insulin. n=67. Random-effects Sidik-Johnkman model. P-value≤0.05 was considered statistically significant. CI, confidence interval, GIT, gastrointestinal tract.

Supplemental Figure 14.2. Galbraith plot for chronic interventions with propionate investigating fasting insulin



Supplemental Figure 14.2. Galbriath plot to assess for chronic propionate on fasting blood insulin. n=67. CI, confidence interval.

APPENDIX CHAPTER # 4

Impact of inulin propionate ester on weight and glucose regulation in overweight young adults, A multi-centre, double-blind, randomised, parallel-group study.

Supplement 4.1. Pre-screening eligibility questionnaire

Supplement 4.2. Participant consent form

Supplement 4.3 Participant Case report form

Supplement 4.4. Participant information sheet

Supplement 4.5. Baseline characteristics and randomisation stratifiers of those who withdrew from the study and those who completed the study

Supplement 4.6. Data availability for clinical and self-reported weight by visit and study arm

Supplement 4.7. Comparability of baseline characteristics within each arm between those with principal weight whether was self-reported or clinic measured

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

Supplement Table 4.10. IPAQ summary statistics over time and by study arm

Supplement Table 4.11. Smoking, vaping, drinking and recreational drug status over time and by study arm

Supplement Table 4.12. Summary of adverse events by arm

Pre-screening eligibility questionnaire

Age	
Weight	
Height	
Ethnicity	
Have you gained weight in the last 12 months? If Yes, approximately how much weight did you gain?	
How would you describe your exercise / physical activity levels? (none/low/medium/high)	
Do you eat less than 2 servings of fruit or vegetable per day?	
Do you drink more than 1 sugary drink per day?	
Do you take any regular medication?	
Do you currently suffer from any long term illness? (If Yes, please specify)	
Do you have any of the following? Coeliac disease Inflammatory bowel disease Irritable bowel syndrome Previous bowel reconstruction	
Are you pregnant, breast feeding, planning to get pregnant or your partner is planning to get pregnant, in the next 12 months?	
Have you used any antibiotics in the past 3 months?	
Are you known to be anaemic and vitamin B12 deficient too?	
Are you taking part in a weight loss program or consuming a weight loss product?	

Participant consent form

iPREVENT

Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults

Chief Investigator: Professor Gary Frost

Study Protocol Number: 18HH4903

IRAS ID: 229300

Participant Trial ID Number:	
Site Number/Name:	
Name of Researcher:	

Participant Consent Form

Please initial each box if you agree with the following:

☐ I confirm I have read and understood the Participant Information Sheet, Version ___ dated _ _ /_ _ /_ _ for the above study, have had enough time to review the information and have been able to ask questions which have been answered fully.

☐ I understand my participation is voluntary and I am free to withdraw consent at any time, without giving any reason, without my medical care or legal rights being affected.

☐ I understand my identity will never be disclosed outside of research and any information collected will remain confidential.

☐ I understand that sections of any of my medical records and other personal data generated during the study may be examined by the NHS, by representatives of the Sponsor (Imperial College London), by people working on behalf of the Sponsor (Imperial Clinical Trials Unit), or by representatives of regulatory authorities where it is relevant to taking part in this research. I give permission for these individuals to access my records.

☐

I understand that the information collected about me may be used to support other ethically approved research in the future, which relates to weight management and/or metabolism, and may be shared anonymously with other researchers.

☐ I give permission for my GP to be informed of my participation in this study.

☐ I give permission for my GP to be contacted to get details about my medical history and/or medications I may be taking and understand that proof of this may be provided to the research team, such as a prescription list, medical letter or report

☐ I give permission for my GP to be informed about my health, in the event that something is discovered that I am unaware of.

☐ I agree to participate in this study.

☐ I agree to be contacted in the future for related research to this study.

The following is an optional aspect of the study, please initial the appropriate box:

I am in agreement to the storage and use of my unused samples for future ethically approved related research, which may or may not include genetic analysis.

☐ Yes ☐ No

☐ I understand that long term follow up data and further information about my health status may be obtained from NHS Digital (England) / NHS Scotland, by linking my health records. I give permission for this to occur.

Participant	**Person responsible for collecting the informed consent**
Date: *Signature:* *Printed Name:*	*Date:* *Signature:* *Printed Name:*

Case report form

VOLUNTEER NUMBER:
VOLUNTEER INITIALS:
HOSPITAL NUMBER:
DATE ENTERED STUDY:

BASELINE/RANDOMISATION VISIT

DATE OF VISIT	

VITAL SIGNS

WEIGHT:	**BMI:**
HEIGHT:	**PREGNANCY TEST:**
	TIME WEIGHT TAKEN **TOTAL BODY WATER** **TOTAL BODY FAT** **TOTAL FAT-FREE BODY MASS** **WAIST: CM** **HIP: CM**
BLOOD PRESSURE **PULSE:**	

BLOOD PRESURE **(1) (1) (1)** **(2) (2) (2)** **(3) (3) (3)**	

BIOCHEMISTRY

SAMPLE COLLECTED?	**YES / NO**
GLUCOSE	
LDL	
HDL	
TOTAL CHOLESTEROL	
TRIGLYCERIDES	
BLOOD SAMPLE MEASURED FOR INSULIN?	**YES / NO**

LIFESTYLE CHANGES

SMOKING STATUS: **NO CHANGE** **INCREASED** **DECREASED** **VAPING STATUS** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**	**DRINKING STATUS:** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**

COMMENTS	
RECREATIONAL DRUGS TAKEN:	**YES NO**

IPAQ

PHYSICAL ACTIVITY QUESTIONNAIRE
VIGORIOUS ACTIVITY **THINK ABOUT ACTIVITIES THAT YOU DO AT LEAST 10 MINS AT A TIME, WHICH MAKE YOU BREATHE MUCH HARDER THAN NORMAL. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO VIGOROUS PHYSICAL ACTIVITIES LIKE HEAVY LIFTING, DIGGING, FAST CYCLING, AEROBICS (DAYS PER WEEK)** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
MODERATE ACTIVITY **THINK ABOUT ONLY THOSE PHYSICAL ACTIVITIES THAT YOU DID FOR AT LEAST 10 MINUTES AT A TIME, THAT MADE YOU BREATHE SOMEWHAT HARDER. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO MODERATE PHYSICAL ACTIVITIES LIKE CARRYING LIGHT LOADS, CYCLING AT A REGULAR PACE, PLAYING TENNIS? PLEASE DO NOT INCLUDE WALKING (DAYS PER WEEK).** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED**

HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS
WALKING **DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU WALK FOR AT LEAST 10 MINUTES AT A TIME ? PLEASE DO NOT COUNT ANY WALKING YOU DID TO TRAVEL TO OR FROM WORK (DAYS PER WEEK).** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
SITTING **DURING THE LAST 7 DAYS, HOW MUCH TIME DID YOU USUALLY SPEND SITTING ON A WEEK DAY? (THIS INCLUDES LYING DOWN AWAKE.)** **…MINS**

FOOD DIARY

FOOD DIARY COMPLETED?	**YES / NO**
DATE FOOD DIARY STARTED	**/ /**
DATE FOOD DIARY ENDED	**/ /**
NO. OF DAYS FOOD DIARY COMPLETED	**1 2 3 4 5 6 7**
FOOD DIARY ANALYSED BY DIET PLAN?	**YES / NO**
COMMENTS	

INCLUSION

ADULT AGED 20-35 YEARS	**YES / NO**
BODY MASS INDEX (BMI) OF 24-27 KG/M^2 IF OF SOUTH ASIAN ETHNICITY OR 25-30 KG/M^2 IF NON-SOUTH ASIAN	**YES / NO**
ATLEAST ANSWER ONE OF THE QUESTIONS YES	
TOTAL WEIGHT GAIN OF 2KG OR MORE OVER THE LAST 12 MONTHS	**YES** **TOTAL WEIGHT GAIN OVER THE LAST 12 MONTHS: KG** **NO**
LOW SELF REPORTED PHYSICAL ACTIVITY ('LOW AS PER IPAQ)	**YES / NO**
LOW SELF REPORTED FRUIT AND VEGETABLE INTAKE (<2 SERVINGS OF FRUIT OR VEGETABLES PER DAY)	**YES** **NUMBER OF PORTIONS PER DAY: (PORTIONS)** **NO**
SELF REPORTED HIGH INTAKE OF SUGAR SWEETENED BEVERAGES (>1 SERVING PER DAY)	**YES** **NUMBER OF SERVINGS PER DAY: (SERVINGS)** **NO**
ON STABLE MEDICATION (IF TAKING ANY) AT POINT OF SCREENING	**YES / NO / NOT APPLICABLE**
WRITTEN INFORMED CONSENT OBTAINED	**YES / NO**
SUBJECT IS NOT PREGNANT OR PLANNING A PREGNANCY	**YES / NO / NOT APPLICABLE**

EXCLUSION

DIAGNOSED CHRONIC DISEASE: TYPE I AND II DIABETES, CANCER, RENAL FAILURE, HEART DISEASE, ORGANIC ACIDAEMIA (PROPIONIC	**YES / NO**

ACIDAEMIA, METHYL MALONIC ACIDAEMIA)	
DIAGNOSED GASTROINTESTINAL CONDITION: COELIAC DISEASE, INFLAMMATORY BOWEL DISEASE AND IRRITABLE BOWEL SYNDROME	YES / NO
PREVIOUS BOWEL RECONSTRUCTION SURGERY	YES / NO
PREGNANCY OR LACTATION	YES / NO / NOT APPLICABLE
USE OF ANTIBIOTICS AT ANY TIME IN THE PAST 3 MONTHS	YES / NO
ANAEMIC AND VITAMIN B12 DEFICIENT (< 160 NG/L)	YES / NO
TAKING PART IN A WEIGHT LOSS PROGRAM OR CONSUMING A WEIGHT LOSS PRODUCT	YES / NO
HAS LOST 3KG OR MORE IN THE LAST 3 MONTHS	YES / NO
ANY OTHER GASTROINTESTINAL UPSET (SUCH AS DIARRHOEA/CONSTIPATION IN THE LAST 2 WEEKS, ABDOMINAL CRAMPING ETC.)	YES / NO
ANY OTHER REASON IN THE OPINION OF THE INVESTIGATOR	YES / NO

ELIGABILITY

IS THE SUBJECT ELIGIBLE FOR THE TRIAL?	YES / NO

SUB STUDY

HAS THE SUBJECT CONSENTED TO TAKING PART IN THE SUB STUDY?	**YES** **DATE THE CONSENT FORM WAS SIGNED FOR THE SUB STUDY: / /** **NO**

RANDOMISATION

WAS RANDOMISATION OUTSIDE OF INFORM COMPLETED SUCCESSFULLY?	**YES / NO**

BASELINE STUDY TREATMENT

STUDY KIT CODE NO. DISPENSED	
NO. OF STUDY TREATMENT SACHETS DISPENSED	
DATE WHEN STUDY TREATMENT WAS STARTED BY PARTICIPANT, FOLLOWING THIS VISIT	**/ /**

VISIT AE CM

HAVE THERE BEEN ANY ADVERSE EVENTS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE ADVERSE EVENT FORM	**YES / NO**
HAVE THERE BEEN ANY CONCOMITANT MEDICATIONS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE CONCOMITANT MEDICATION FORM	**YES / NO**

VISIT AT 2 MONTHS

DATE OF VISIT	

VITAL SIGNS

WEIGHT:	**BMI:**
HEIGHT:	**PREGNANCY TEST:**
	TIME WEIGHT TAKEN **TOTAL BODY WATER** **TOTAL BODY FAT** **TOTAL FAT-FREE BODY MASS** **WAIST: CM** **HIP: CM**
BLOOD PRESSURE **PULSE:** **BLOOD PRESURE** **(1) (1) (1)** **(2) (2) (2)** **(3) (3) (3)**	

LIFESTYLE CHANGES

SMOKING STATUS: **NO CHANGE** **INCREASED** **DECREASED** **STOPPED** **VAPING STATUS**	**DRINKING STATUS:** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**

NO CHANGE **INCREASED** **DECREASED** **STOPPED**	
COMMENTS?	
RECREATIONAL DRUGS TAKEN:	**YES NO**

PHYSICAL ACTIVITY QUESTIONNAIRE
<u>VIGORIOUS ACTIVITY</u> **THINK ABOUT ACTIVITIES THAT YOU DO AT LEAST 10 MINS AT A TIME, WHICH MAKE YOU BREATHE MUCH HARDER THAN NORMAL. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO VIGOROUS PHYSICAL ACTIVITIES LIKE HEAVY LIFTING, DIGGING, FAST CYCLING, AEROBICS (DAYS PER WEEK)** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
<u>MODERATE ACTIVITY</u> **THINK ABOUT ONLY THOSE PHYSICAL ACTIVITIES THAT YOU DID FOR AT LEAST 10 MINUTES AT A TIME, THAT MADE YOU BREATHE SOMEWHAT HARDER. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO MODERATE PHYSICAL ACTIVITIES LIKE CARRYING LIGHT LOADS, CYCLING AT A REGULAR PACE, PLAYING TENNIS? PLEASE DO NOT INCLUDE WALKING (DAYS PER WEEK).** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
<u>WALKING</u> **DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU WALK FOR AT LEAST 10 MINUTES AT A TIME ? PLEASE DO NOT COUNT ANY WALKING YOU DID TO TRAVEL TO OR FROM WORK (DAYS PER WEEK).**

0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
SITTING **DURING THE LAST 7 DAYS, HOW MUCH TIME DID YOU USUALLY SPEND SITTING ON A WEEK DAY? (THIS INCLUDES LYING DOWN AWAKE.)** **…MINS**

FOOD DIARY

FOOD DIARY COMPLETED?	**YES / NO**
DATE FOOD DIARY STARTED	**/ /**
DATE FOOD DIARY ENDED	**/ /**
NO. OF DAYS FOOD DIARY COMPLETED	**1 2 3 4 5 6 7**
FOOD DIARY ANALYSED BY DIET PLAN?	**YES / NO**
COMMENTS	

STUDY TREATMENT & ACCOUNTABILITY

NO. OF SATCHETS OF STUDY TREATMENT RETURNED	**NO. OF UNUSED SATCHETS OF STUDY TREATMENT RETURNED:** **NO. OF USED SATCHETS OF STUDY TREATMENT RETURNED:**
DATE OF STUDY TREATMENT RETURNED	**/ /**

HOW MANY TIMES DID SUBJECT MISS A SACHET SINCE THE PREVIOUS STUDY VISIT?	

REASON(S) FOR MISSING DOSE(S)	**FORGETFULNESS** **TASTE** **SIDE EFFECT** **ILLNESS** **TRAVEL** **OTHER**
COMMENTS	
HAS THE SUPPLEMENT DIARY BEEN COMPLETED?	**YES / NO**
DATE STUDY TREATMENT DISPENSED	**/ /**
NO. OF LEFTOVER SACHETS REDISPENSED FROM PREVIOUS KIT CODE	
DATE WHEN THE STUDY TREATMENT WAS STARTED BY THE SUBJECT, FOLLOWING THIS VISIT	**/ /**
STUDY TREATMENT	
STUDY KIT CODE NO. DISPENSED	
NO. OF SACHETS OF STUDY TREATMENT DISPENSED	
DATE OF STUDY TREATMENT DISPENSED IF DIFFERENT FROME ABOVE	**/ /**

VISIT AE CM

HAVE THERE BEEN ANY ADVERSE EVENTS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE ADVERSE EVENT FORM	**YES / NO**
HAVE THERE BEEN ANY CONCOMITANT MEDICATIONS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE CONCOMITANT MEDICATION FORM	**YES / NO**

VISIT AT 6 MONTHS

DATE OF VISIT	

VITAL SIGNS

WEIGHT:	**BMI:**
HEIGHT:	**PREGNANCY TEST:**
	TIME WEIGHT TAKEN **TOTAL BODY WATER** **TOTAL BODY FAT** **TOTAL FAT-FREE BODY MASS** **WAIST: CM** **HIP: CM**
BLOOD PRESSURE **PULSE:** **BLOOD PRESURE** **(1) (1) (1)** **(2) (2) (2)** **(3) (3) (3)**	

BIOCHEMISTRY

SAMPLE COLLECTED?	**YES / NO**
GLUCOSE	

LDL	
HDL	
TOTAL CHOLESTEROL	
TRIGLYCERIDES	
BLOOD SAMPLE MEASURED FOR INSULIN?	**YES / NO**

LIFESTYLE CHANGES

SMOKING STATUS: **NO CHANGE** **INCREASED** **DECREASED** **STOPPED** **VAPING STATUS** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**	**DRINKING STATUS:** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**
COMMENTS	
RECREATIONAL DRUGS TAKEN:	**YES NO**

IPAQ

PHYSICAL ACTIVITY QUESTIONNAIRE
VIGORIOUS ACTIVITY THINK ABOUT ACTIVITIES THAT YOU DO AT LEAST 10 MINS AT A TIME, WHICH MAKE YOU BREATHE MUCH HARDER THAN NORMAL. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO VIGOROUS PHYSICAL ACTIVITIES LIKE HEAVY LIFTING, DIGGING, FAST CYCLING, AEROBICS (DAYS PER WEEK) 0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS
MODERATE ACTIVITY THINK ABOUT ONLY THOSE PHYSICAL ACTIVITIES THAT YOU DID FOR AT LEAST 10 MINUTES AT A TIME, THAT MADE YOU BREATHE SOMEWHAT HARDER. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO MODERATE PHYSICAL ACTIVITIES LIKE CARRYING LIGHT LOADS, CYCLING AT A REGULAR PACE, PLAYING TENNIS? PLEASE DO NOT INCLUDE WALKING (DAYS PER WEEK). 0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS
WALKING DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU WALK FOR AT LEAST 10 MINUTES AT A TIME ? PLEASE DO NOT COUNT ANY WALKING YOU DID TO TRAVEL TO OR FROM WORK (DAYS PER WEEK). 0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS
SITTING DURING THE LAST 7 DAYS, HOW MUCH TIME DID YOU USUALLY SPEND SITTING ON A WEEK DAY? (THIS INCLUDES LYING DOWN AWAKE.) …MINS

FOOD DIARY

FOOD DIARY COMPLETED?	YES / NO

DATE FOOD DIARY STARTED	**/ /**
DATE FOOD DIARY ENDED	**/ /**
NO. OF DAYS FOOD DIARY COMPLETED	**1 2 3 4 5 6 7**
FOOD DIARY ANALYSED BY DIET PLAN?	**YES / NO**
COMMENTS	

STUDY TREATMENT AND ACCOUNTABILITY

NO. OF SATCHETS OF STUDY TREATMENT RETURNED	**NO. OF UNUSED SATCHETS OF STUDY TREATMENT RETURNED:** **NO. OF USED SATCHETS OF STUDY TREATMENT RETURNED:**
DATE OF STUDY TREATMENT RETURNED	**/ /**
HOW MANY TIMES DID SUBJECT MISS A SACHET SINCE THE PREVIOUS STUDY VISIT?	
REASON(S) FOR MISSING DOSE(S)	**FORGETFULNESS** **TASTE** **SIDE EFFECT** **ILLNESS** **TRAVEL** **OTHER**
COMMENTS	

HAS THE SUPPLEMENT DIARY BEEN COMPLETED?	**YES / NO**
DATE STUDY TREATMENT DISPENSED	**/ /**
NO. OF LEFTOVER SACHETS REDISPENSED FROM PREVIOUS KIT CODE	
DATE WHEN THE STUDY TREATMENT WAS STARTED BY THE SUBJECT, FOLLOWING THIS VISIT	**/ /**
STUDY TREATMENT	[redacted]
STUDY KIT CODE NO. DISPENSED	
NO. OF SACHETS OF STUDY TREATMENT DISPENSED	
DATE OF STUDY TREATMENT DISPENSED IF DIFFERENT FROME ABOVE	**/ /**

VISIT AE CM

HAVE THERE BEEN ANY ADVERSE EVENTS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE ADVERSE EVENT FORM	**YES / NO**
HAVE THERE BEEN ANY CONCOMITANT MEDICATIONS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE CONCOMITANT MEDICATION FORM	**YES / NO**

VISIT AT 12 MONTHS

DATE OF VISIT	

VITAL SIGNS

WEIGHT:	**BMI:**
HEIGHT:	**PREGNANCY TEST:**
	TIME WEIGHT TAKEN **TOTAL BODY WATER** **TOTAL BODY FAT** **TOTAL FAT-FREE BODY MASS** **WAIST: CM** **HIP: CM**
BLOOD PRESSURE **PULSE:** **BLOOD PRESURE** **(1) (1) (1)** **(2) (2) (2)** **(3) (3) (3)**	

BIOCHEMISTRY

SAMPLE COLLECTED?	**YES / NO**
GLUCOSE	
LDL	
HDL	
TOTAL CHOLESTEROL	
TRIGLYCERIDES	
BLOOD SAMPLE MEASURED FOR INSULIN?	**YES / NO**

LIFESTYLE CHANGES

SMOKING STATUS: **NO CHANGE** **INCREASED** **DECREASED** **STOPPED** **VAPING STATUS** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**	**DRINKING STATUS:** **NO CHANGE** **INCREASED** **DECREASED** **STOPPED**
COMMENTS?	
RECREATIONAL DRUGS TAKEN:	**YES NO**

IPAQ

PHYSICAL ACTIVITY QUESTIONNAIRE
VIGORIOUS ACTIVITY **THINK ABOUT ACTIVITIES THAT YOU DO AT LEAST 10 MINS AT A TIME, WHICH MAKE YOU BREATHE MUCH HARDER THAN NORMAL. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO VIGOROUS PHYSICAL ACTIVITIES LIKE HEAVY LIFTING, DIGGING, FAST CYCLING, AEROBICS (DAYS PER WEEK)** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED**

HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS
MODERATE ACTIVITY **THINK ABOUT ONLY THOSE PHYSICAL ACTIVITIES THAT YOU DID FOR AT LEAST 10 MINUTES AT A TIME, THAT MADE YOU BREATHE SOMEWHAT HARDER. DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU DO MODERATE PHYSICAL ACTIVITIES LIKE CARRYING LIGHT LOADS, CYCLING AT A REGULAR PACE, PLAYING TENNIS? PLEASE DO NOT INCLUDE WALKING (DAYS PER WEEK).** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
WALKING **DURING THE LAST 7 DAYS, ON HOW MANY DAYS DID YOU WALK FOR AT LEAST 10 MINUTES AT A TIME ? PLEASE DO NOT COUNT ANY WALKING YOU DID TO TRAVEL TO OR FROM WORK (DAYS PER WEEK).** **0 1 2 3 4 5 6 7 8 9 10 UNSURE, REFUSED** **HOW MUCH TIME DID YOU USUALLY SPEND ON ONE OF THOSE DAYS DOING VIGOROUS PHYSICAL ACTIVITIES? …MINS**
SITTING **DURING THE LAST 7 DAYS, HOW MUCH TIME DID YOU USUALLY SPEND SITTING ON A WEEK DAY? (THIS INCLUDES LYING DOWN AWAKE.)** **…MINS**

FOOD DIARY

FOOD DIARY COMPLETED?	**YES / NO**
DATE FOOD DIARY STARTED	**/ /**
DATE FOOD DIARY ENDED	**/ /**
NO. OF DAYS FOOD DIARY COMPLETED	**1 2 3 4 5 6 7**

FOOD DIARY ANALYSED BY DIET PLAN?	**YES / NO**
COMMENTS	

STUDY TREATMENT AND ACCOUNTABILITY

NO. OF SATCHETS OF STUDY TREATMENT RETURNED	**NO. OF UNUSED SATCHETS OF STUDY TREATMENT RETURNED:** **NO. OF USED SATCHETS OF STUDY TREATMENT RETURNED:**
DATE OF STUDY TREATMENT RETURNED	**/ /**
HOW MANY TIMES DID SUBJECT MISS A SACHET SINCE THE PREVIOUS STUDY VISIT?	
REASON(S) FOR MISSING DOSE(S)	**FORGETFULNESS** **TASTE** **SIDE EFFECT** **ILLNESS** **TRAVEL** **OTHER**
COMMENTS	
HAS THE SUPPLEMENT DIARY BEEN COMPLETED?	**YES / NO**
DATE STUDY TREATMENT DISPENSED	**/ /**
NO. OF LEFTOVER SACHETS REDISPENSED FROM PREVIOUS KIT CODE	
DATE WHEN THE STUDY TREATMENT WAS STARTED BY THE SUBJECT, FOLLOWING THIS VISIT	**/ /**

STUDY TREATMENT	[redacted]
STUDY KIT CODE NO. DISPENSED	
NO. OF SACHETS OF STUDY TREATMENT DISPENSED	
DATE OF STUDY TREATMENT DISPENSED IF DIFFERENT FROME ABOVE	/ /

VISIT AE CM

HAVE THERE BEEN ANY ADVERSE EVENTS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE ADVERSE EVENT FORM	**YES / NO**
HAVE THERE BEEN ANY CONCOMITANT MEDICATIONS? IF ANSWERED YES PLEASE ENSURE DETAILS ARE ENTERED ON THE CONCOMITANT MEDICATION FORM	**YES / NO**

iPREVENT

Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults

Chief Investigator: Professor Gary Frost

Study Protocol Number: 18HH4903

IRAS ID: 229300

Participant Information Sheet

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

Part 1 tells you about the purpose of this study and what will happen if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you would wish to take part. Thank you for reading this.

PART 1

What is the purpose of the study?

Overweight and obesity affects over 60% of the UK population. In adults, most weight gain occurs when people are in their 20s and 30s and causes many adults to become obese by the time they are middle-aged, leading to increased risk of developing diabetes, heart disease and cancer. There is increasing evidence that diet plays an important role in preventing obesity. The amount of fibre (found in edible plant foods such as cereals, fruits, vegetables, dried peas, nuts, lentils and grains) eaten in the diet has been linked to body weight. People who eat low amounts of dietary fibre are more likely to gain weight. Dietary fibre is the major food for the bacteria that live in our gut. We know that dietary

fibre keeps the bacteria in our gut healthy. These bacteria break down dietary fibre to short chain fatty acids (smaller pieces of fibre) which can reduce appetite and body fat.

We have focused on one short chain fatty acid called Propionate because it appears to be the most powerful at reducing appetite. Propionate by itself would be broken down rapidly in the body so we have created a new food supplement called Inulin Propionate Ester (IPE) which is Propionate chemically bound to a fibre called Inulin. Inulin is a type of fibre normally found in garlic, Jerusalem artichoke, chicory and onion. The IPE simply delivers the Propionate to the right region of the gut where we think it is most effective.

This is a randomised controlled study to investigate the effects of IPE on prevention of weight gain in young adults aged 20-40 years at high risk of gaining weight, across a period of 12 months.

Who is involved in the study?

We are planning to study 270 participants in total, across two clinical research facilities - one in London, part of Imperial College Healthcare NHS Trust and one in Glasgow, part of NHS Research Scotland.

Why am I being invited?

You are being asked to take part in this study as you are aged between 20 to 40 years and have a Body Mass Index (BMI) of either 25-30kg/m^2 if you are non-South Asian, or 24-27kg/m^2 if you are of South Asian origin BMI is a value calculated from your height and weight, which estimates body fat. One or more of the following will also apply to you: you have gained 2kg or more over the past year, the level of physical activity you do is low, you eat less than two portions a day of fruit and vegetables, or you drink more than one sugary drink per day. You should also be on stable medication if you are taking any.

You are not suitable if:

- You have a diagnosed chronic disease; Type I and II diabetes, cancer, kidney failure, heart disease, organic acidaemia (propionic acidaemia, methyl malonic acidaemia)
- You have a gut condition including coeliac disease, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)
- You have had previous bowel (intestine) reconstruction surgery
- You are pregnant or breastfeeding
- You have used antibiotics at any time in the past 3 months
- You are anaemic and have low Vitamin B12 levels in your blood
- You have anything else which upsets your gut such as diarrhoea/constipation in the last 2 weeks, abdominal cramping
- You are currently taking part in a weight loss programme or taking something to lose weight
- You have lost 3kg or more of weight, in the last 3 months
- Any other reason that the research doctor thinks may interfere with your taking part in the study

Do I have to take part?

It is entirely up to you whether or not to take part. If you do we will ask you to sign a consent form. You are free to withdraw at any time and you do not have to give a reason. A decision not to take part or to withdraw from the study at any time, will not affect the standard of care you receive or your legal rights.

Will I get paid for participating?

You will be paid £50 per study visit and there will be a total of 4 study visits, but there is also an initial visit to check if you are eligible to take part, which isn't paid for. You are able to claim back travel expenses for all study visits and also the initial eligibility visit.

What is the study investigating?

As mentioned, we are investigating the effects of a dietary supplement called Inulin Propionate Ester (IPE), on prevention of weight gain in young adults aged 20-40 years at high risk of gaining weight, across a period of 12 months.

What will happen if I take part?

If you are eligible and still want to participate, you will be enrolled in the study for 12 months. You will first meet one of our researchers who will ask you some questions. You will then undergo a series of measurements and tests at various study visits described below.

Eligibility visit

You will be asked to complete a consent form before any study procedures are carried out. You will be given an IPAQ questionnaire to complete, this is a questionnaire which asks about your physical activity levels. You will have a standard full blood test (approx. 3mls) to check general health and if you are female you will also have a pregnancy test done by providing a urine sample.

You will be asked about your medical history and any medications you are taking. You will also be asked several questions about your lifestyle, and have your height, weight, waist, hips, body composition (proportion of body fat) measured.

You will have your blood pressure measured, and something called an Electrocardiogram (ECG) will be done. This is a test which measures the rhythm of your heart to show whether or not it is working normally and this is the only visit it will be checked. The test involves attaching a number of small, sticky sensors called 'electrodes' to your arms, legs and chest which are connected by wires to a machine. The test only lasts a few minutes. Before the electrodes are attached, you'll need to remove your upper clothing, and your chest may need to be shaved or cleaned. Once the electrodes are in place, you will be offered a hospital gown to cover yourself if you wish.

If your blood test results shows that you are anaemic, you will be asked to come in for another very short visit to take another blood sample (5mls) for checking what your Vitamin B12 levels are.

If you meet all the study eligibility criteria, you will be asked to consider taking part in the trial. If you agree to take part, you will be given a food diary to complete for 7 days before your baseline visit (first study visit).



You will be asked to change into a pair of 'scrubs' to have your measurements taken



We have special machines that you stand on, to measure your weight and body fat



A standard blood test

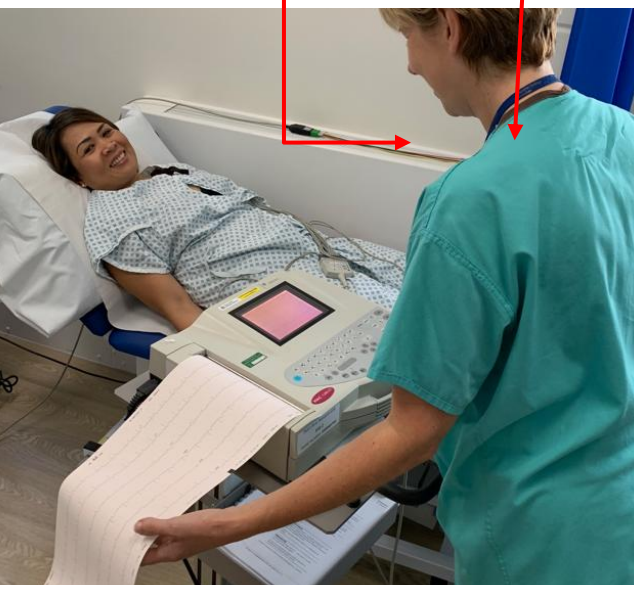


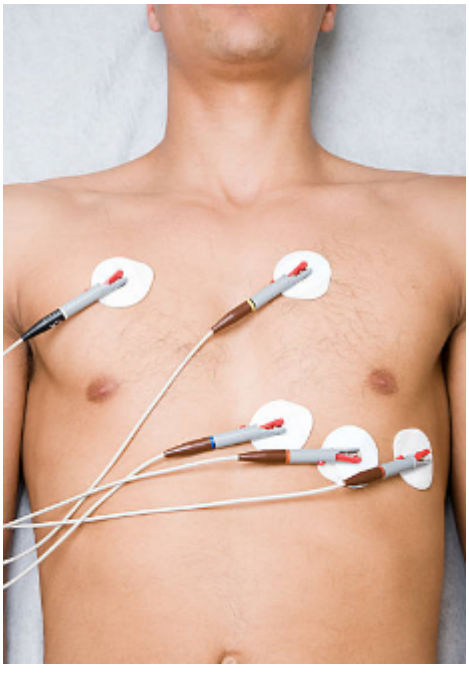
Hip and waist measurements will be taken with a measuring

Blood pressure will be measured using a cuff wrapped around your upper arm, linked to a monitor.



For an ECG, you will lay on a bed and electrodes (sticky pads that detect your heart rhythm) will be put on your chest, and connected to a screen /printer which show the results.





Baseline visit (first study visit)

You will have the same measurements that were done during your eligibility visit repeated again except for an ECG, and this time you will have fasting blood samples taken (3 tubes - total of 12mls) to measure your blood lipids (fat and cholesterol), glucose and insulin levels. A fasting blood test means you must not eat anything and can only drink water for 8 hours before your test.

Your 7 day food diary completed before this visit will also be collected.

We need to compare two different treatments. To find out which is better, we put people into two groups and give each group one of the two treatments. The results are compared to see if one is better. To try to make sure the groups are the same to start with, each participant is put into a group by chance (randomly).

You will be randomised to either the group taking the Inulin Propionate Ester (IPE) or the 'control' group taking Inulin (fibre) alone, for 12 months. These are all natural constituents of vegetables such as onions, chicory and Jerusalem artichokes.

You and your researcher will not know which food supplement you are taking, to avoid any bias.

You will be given a supply of supplement sachets to last until the next study visit and be asked to take one sachet daily. Each sachet contains 10 grams of supplement. This will just be in addition to your normal diet and is best taken with cold food or drink (i.e. sprinkled or mixed in). The supplement has very little or no taste, depending on how sensitive your taste is. You are not being asked to change your diet or lifestyle in any way. You will be given a food diary to complete for 7 days before your next study visit and you will be asked to keep and return all used and unused sachets of supplement at your next study visit.

2 months and 6 months visits

You will have a similar series of body measurements taken as the previous visits: weight, waist, hip, body composition and blood pressure. You will also be asked to complete the IPAQ questionnaire about your physical activities again, be asked for an update on lifestyle, medical history and any medications you have taken or stopped. If you are female you will have a pregnancy test. You will only have a fasting blood test taken to measure your blood lipid, glucose and insulin levels at the 6 month visit (not the 2 month visit).Your 7 day food diary completed before these visits will be collected and you will be given a food diary to complete for 7 days before your next study visit. Your unused and used sachets of food supplement will be collected and counted and you will be asked if you missed or forgot to take the supplement on any days, and why. A further supply of supplement will be given to you and once again you will be asked to keep and return all used and unused sachets of supplement at your next study visit. You will also be asked if you have had any medical events, symptoms or side effects since the last study visit and these will be recorded, even if they are not related to the study.

12 months visit

This is your final study visit. You will have the same body measurements taken as the previous visit and have all the same questions asked. . Your 7 day food diary completed before this visit and your unused and used sachets of food supplement will be collected. No further supply of food supplement will be given as you have now completed the study.

What happens to my blood/urine samples?

Your blood samples will be transferred to the hospital laboratories immediately for analysis, except for one tube from the fasting blood test, which will be stored and analysed for Insulin at the end of the study.

Any remainders of samples sent to the hospital laboratories will be safely thrown away.

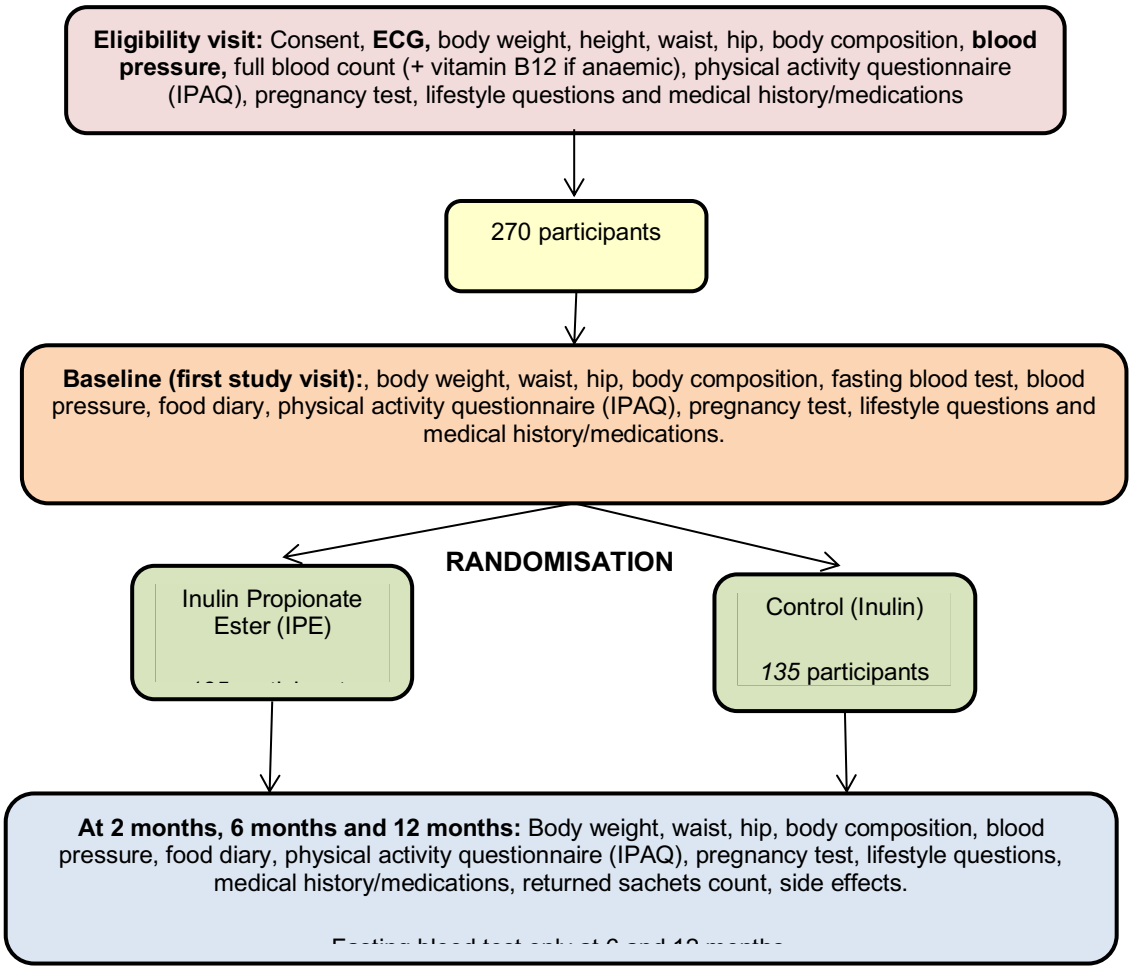
If you are female, your urine sample for the pregnancy test will also be safely thrown away.

Any unused/remaining samples for Insulin measurement would be transferred to the Imperial College Healthcare Tissue Bank (licensed by the Human Tissue Authority) and stored here indefinitely for use in future research projects that have ethical approval, but only if you consent to this.

All your test results will be provided to the research team who can also tell you your results.

The results data will be collected for the study. Your full blood count results will ensure that you are healthy enough to take part in the study and pregnancy tests will be done if you are female, for your safety throughout the study. Your fasting blood test results will help us look at health benefits that may occur from taking the food supplement.

Study schedule flowchart



What are the side effects of any treatment received when taking part?

There are no major side effects associated with taking the IPE supplement. Some participants may find the taste unpleasant (slightly metallic) or may experience mild nausea, abdominal bloating or other gut symptoms initially, however these should settle down after a few days.

What are the possible benefits of continuing to take part?

You may not benefit directly from this study but the results may help doctors in the future treat people who are overweight and at risk of gaining weight, in preventing weight gain. You may find that the IPE supplement helps prevent you gain weight, however these effects may only be short lived and at the present time the IPE supplement is not commercially available.

What are the possible disadvantages and risks of taking part?

Most of the procedures in this study, such as the recording of your weight, height, hip, waist, body composition and blood pressure present no risk to you. Other procedures, such as taking blood samples, can cause mild discomfort. The risks of taking a blood sample include: slight discomfort when the needle is inserted and possible bruising or a localised infection. These procedures will only be carried out by an experienced health professional under sterile conditions to minimise all these risks.

What if you discover something else about my health?

In the event that we discover something about your health that you were unaware of, for example if you have diabetes, we would immediately inform you of this and with your consent we will inform your GP so that you can be referred to an appropriate specialist. If you require more urgent assessment we would arrange this for you immediately within the hospital.

What if I am pregnant, plan on getting pregnant or plan on getting my partner pregnant?

You will not be able to take part in the trial if you are already pregnant or breast feeding and we advise that you do not take part if you/your partner plan on getting pregnant over the next 12 months.

You will be asked for you and/or your partner to use adequate contraception whilst taking part in the trial to prevent pregnancy or for male participants to prevent pregnancy in a female partner.

A pregnancy test will be performed at all trial visits for all female participants regardless of whether they are considered to be using adequate contraception.

If you are found to be pregnant at any point during the trial, this will be recorded and the food supplement will be withdrawn for safety as we currently do not have enough information on how this may affect pregnancy, but we would like to follow you up until the end of trial, unless you decide to withdraw your consent.

If it is your partner that becomes pregnant we will not follow them up for outcome of the pregnancy, although they are welcome to call the study team should they have any study related concerns at any point during or after the study.

PART 2

What if new information becomes available?

Sometimes during the course of a research study, new information becomes available about the supplement that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time and you do not need to give a reason. Data collected up until that point may still be used. Any stored blood samples that can still be identified as yours will be destroyed if you wish.

Can the study be stopped for other reasons?

You may be withdrawn from the treatment if the research doctor thinks that your health will be compromised due to any side effects or illness that develop while you are in the study. In this case you will no longer take the supplement but if you agree, you will still be followed up at study visits as your data is still important to collect.

An Ethics Committee has approved the study and a Trial Steering Committee is overseeing the study and may stop the study if there are any serious issues.

What if I lose capacity to consent, at some point during the study?

You would be withdrawn from the study. Identifiable data or tissue already collected with your initial consent would be retained and used in the study. However, no further data or tissue would be collected or any other research procedures carried out on or in relation to you.

What if something goes wrong?

Imperial College London is the Sponsor for this study and holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you will be eligible to

claim compensation without having to prove that Imperial College London is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action.

Please contact the study investigator or the joint research and compliance office (details below) if you have any concern.

Study Investigators Contact details

Study Investigator: Prof Gary Frost
Study Nurse: Not Applicable
Day time Telephone:
Emergency Telephone:

Joint Research and Compliance Office details

Name: Becky Ward
Role: Research and Governance Manager
Email: becky.ward@imperial.ac.uk / jrco@imperial.ac.uk
Telephone: +44 207 594 9459

Will my taking part in this study be kept confidential?

With your consent, your GP will be informed of your participation in this study.

What will happen to my information?

If you give us permission to do so, information from your health records may be shared with us by NHS Digital (England) or NHS Scotland (who have access to national health and social care data), after the end of the study. We do this by 'linking' to these records using your details (e.g. name, date of birth, NHS number, address/post code) through a secure transfer system, so that NHS Digital can correctly identify you.

Before we receive the relevant information from your health records, all your personal details will be removed and replaced with your participant trial ID number which would make the information pseudonymous (partially anonymous). We will not know who is who but we can use the participant trial ID numbers to join pieces of information together. The data would be stored on a secure server at Imperial College London, which meets the latest data protection requirements. The data may be

securely transferred between the two universities, as these are where our research teams are based and where the data would be analysed.

The reason we would like to get information from your health records after the end of the study is to see how your health may have changed since then and up to 10 years afterwards (weight, metabolism and related health changes or conditions etc.). It would be a 'follow-up' study.

We would not share any of your individual study information with NHS Digital or any other government departments or agencies. Agreeing to this however, is optional.

What will happen to the results of the research study?

The results of this study will likely be presented at medical meetings and published in scientific journals, six months following the end of the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. Only group information and no personal information will be presented. We also hope to publish the results in the press and media.

At the end of the study, the results of the study can be made available to you and/or your GP should you wish.

Who is organising and funding the research?

This study is being organised by doctors and scientists at Imperial College London. It is funded by the National Institute for Health Research (Efficacy and Mechanism Evaluation programme) and sponsored by Imperial College London.

Who has reviewed the study?

All research in the NHS is reviewed by an independent group of people called a Research Ethics Committee (REC). This study has been reviewed and approved by the London Hampstead REC.

Who can I contact for independent research information?

If you have any questions about being in a research study, you can contact the Trust's Patient Advice Liaison Service (PALS). They will give you advice about who you can talk to for independent advice.

Further information

Thank you in advance for considering participation in this study. If you have any questions about this research, the study staff will be more than happy to answer them.

Study Investigators contact details

Please see previous page for contact details.

PARTICIPANT PRIVACY INFORMATION

Research Study Title: Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults

IRAS ID: 229300

Imperial College London is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Imperial College London will keep identifiable information about you for:

- 10 years after the study has finished in relation to data subject consent forms.
- 10 years after the study has completed in relation to primary research data.

Further information on Imperial College London's retention periods may be found at https://www.imperial.ac.uk/media/imperial-college/administration-and-support-services/records-and-archives/public/RetentionSchedule.pdf.

A link to Imperial College London's data protection webpage may be found at https://www.imperial.ac.uk/admin-services/legal-services-office/data-protection/ but this is the notice most applicable to the information provided by participants and therefore takes precedence for all purposes described hereunder.

YOUR RIGHTS

Your usual statutory rights to access, change or move your information are limited, because of exceptions applicable to some types of research, and also because we need to manage your information in specific, lawful ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

LEGAL BASIS

As a university we use personally-identifiable information to conduct research to improve health, care and services. As a publicly-funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study.

Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research.

INTERNATIONAL TRANSFERS

There may be a requirement to transfer information to countries outside the European Economic Area (for example, to a research partner). Where this information contains your personal data, Imperial College London will ensure that it is transferred in accordance with data protection legislation. If the data is transferred to a country which is not subject to a European Commission (EC) adequacy decision in respect of its data protection standards, Imperial College London will enter into a data sharing

agreement with the recipient organisation that incorporates EC approved standard contractual clauses that safeguard how your personal data is processed.

CONTACT US

If you wish to raise a complaint on how we have handled your personal data or if you want to find out more about how we use your information, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ.

If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO). The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

Who will have access to your information?

Imperial College NHS Trust will collect information from you and your medical records for this research study in accordance with our instructions.

Imperial College NHS Trust will use your name, NHS number, date of birth and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Imperial College London and regulatory organisations may look at your medical and research records to check the accuracy of the research study.

Imperial College NHS Trust will pass these details to Imperial College London along with the information collected from you and/or your medical records. The only people in Imperial College London who will have access to information that identifies you will be people who need to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number, date of birth or contact details.

Imperial College NHS Trust will keep identifiable information about you from this study for 10 years after the study has finished.

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research (https://www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/uk-policy-framework-health-social-care-research/).

Your information could be used for research in any aspect of health or care, and could be combined with information about you from other sources held by researchers, the NHS or government.

Where this information could identify you, the information will be held securely with strict arrangements about who can access the information. The information will only be used for the

purpose of health and care research, or to contact you about future opportunities to participate in research. It will not be used to make decisions about future services available to you, such as insurance.

Where there is a risk that you can be identified your data will only be used in research that has been independently reviewed by an Ethics committee.

Supplement Table 4.1: Baseline characteristics and randomisation stratifiers of those who withdrew from the study and those who completed the study.

	Withdrew from study		Completed study	
	Con	**IPE**	**Con**	**IPE**
	N=20	N=22	N=115	N=113
Age (Mean [SD])	29.1 (4.4)	29.0 (5.6)	30.2 (5.8)	30.5 (5.1)
Sex (Female)	50% (10)	73% (16)	66% (76)	62% (70)
Site (London)	60% (12)	50% (11)	63% (72)	65% (74)
In sub-study	20% (4)	9% (2)	20% (23)	20% (23)
BMI within ethnicity				
Non-South Asian, BMI category: 25.00-27.49 kg/m²	50% (11)	50% (10)	41% (46)	41% (47)
Non-South Asian, BMI category: 27.50- 30.00 kg/m²	41% (9)	40% (8)	46% (52)	45% (52)
South Asian, BMI category: 24.00-25.49 kg/m²	5% (1)	0% (0)	4% (4)	4% (5)
South Asian, BMI category: 25.50-27.00 kg/m²	5% (1)	10% (2)	10% (11)	10% (11)
Baseline weight	81.6 (10.1)	77.0 (11.6)	78.7 (10.6)	80.1 (10.7)

BMI, body mass index; Con, inulin control; IPE, inulin-propionate ester; SD, standard deviation

Supplement Table 4.2 Data availability for clinical and self-reported weight by visit and study arm.

	Baseline			2 months			6 months			12 months		
	Con (%)	**IPE (%)**	**Total (%)**	**Con (%)**	**IPE (%)**	**Total (%)**	**Con (%)**	**IPE (%)**	**Total (%)**	**Con (%)**	**IPE (%)**	**Total (%)**
Clinic weight	135	135	270	89	100	189	74	78	152	80	87	167
Self-reported weight	3	5	8	35	28	63	33	30	63	36	30	66
Clinic weight **only**	132	130	262	86	97*	183	73	78*	151*	78	82	160
Self-reported weight **only**	0	0	0	32	25	57	32	30	62	33	24*	57
Both*	3	5	8	3	3	6	1	0	1	2	5	7
Principal weight	135 (100)	135 (100)	270 (100)	121 (90)	125 (93)	246 (91)	106 (79)	108 (80)	214 (79)	114 (84)	112* (83)	226* (84)
Clinic weight was used as principal weight	135	135	270	89	100	189	74	78	152	80	87	167
Neither	0	0	0	14	9	23	29	26	55	22	23	45

*Self-reported weight was collected in addition to clinic weight in a small number of participants.

Con, inulin control; CI, confidence intervals; IPE, inulin-propionate ester

Supplement 4.3: Comparability of baseline characteristics within each arm between those with principal weight whether was self-reported or clinic measured.

	Control 84% (114/135) complete		**IPE** 83% (112/135) complete	
	Clinic weight used at 12m	**Self-reported**	**Clinic** weight used at 12m	**Self-reported**

	N=80 59% (80/135)	weight used at 12m N=34 25% (34/135)	N=87 65% (87/135)	weight used at 12m N=25 19% (25/135)
Age	30.2 (5.9)	30.4 (5.7)	30.9 (4.9)	29.2 (5.6)
Sex (Female)	66% (53)	65% (22)	63% (87)	56% (14)
Site (London)	54% (43)	82% (28)	60% (52)	84% (21)
BMI within ethnicity: Non-South Asian, BMI: 25.00 - 27.49 kg/m²	43% (34)	38% (13)	41 % (36)	40% (10)
Non-South Asian, BMI: 27.50 - 30.00 kg/m²	49% (39)	38% (13)	47 % (41)	44% (11)
South Asian, BMI: 24.00 - 25.49 kg/m²	4% (3)	6% (2)	3 % (3)	4% (1)
South Asian, BMI: 25.50 - 27.00 kg/m²	5% (4)	18% (6)	8% (7)	12% (3)
Weight	79.5 (9.7)	77.1 (12.5)	79.7 (10.3)	81.6 (12.3)
Change in clinic weight to 2 months	0.03 (2.49) N=64	0.41 (1.67) N=14	0.23 (2.12) N=70	0.29 (1.84) N=20
Change in clinic weight to 6 months	0.28 (3.86) N=59	-0.97 (4.61) N=9	0.36 (4.06) N=68	1.35 (4.39) N=8
Waist	92.4 (7.5)	94.3 (10.0)	93.5 (8.4)	95.8 (10.3)
BMI	27.5 (1.6)	27.2 (1.5)	27.6 (1.5)	27.7 (1.7)
Waist/hip ratio	0.86 (0.07)	0.86 (0.07)	0.87 (0.08)	0.88 (0.09)

Supplement Table 4.4: Means (SD) of the different physical activity measurements.

	Baseline		12m follow-up		Change from baseline		Difference (95% CI) between arms of the change from baseline
	IPE Mean (SD) N=16	Con Mean (SD) N=7	IPE Mean (SD) N=16	Con Mean (SD) N=7	IPE Mean (SD) N=16	Con 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+	*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

Supplement Table 4.5: IPAQ summary statistics over time and by study arm

Physical activity level	Baseline		2m		6m		12m	
	Con % (n)	IPE % (n)	Con % (n)	IPE % (n)	Con % (n)	IPE % (n)	Con % (n)	IPE % (n)
High	17 (23)	18 (24)	27 (32)	17 (21)	24 (25)	28 (31)	24 (23)	24 (24)
Moderate	40 (54)	38 (51)	31 (36)	36 (44)	38 (39)	42 (46)	38 (36)	41 (41)
Low	43 (58)	44 (60)	42 (49)	46 (56)	38 (39)	29 (32)	39 (37)	34 (34)
Total *n*	135	135	117	121	103	109	96	99
No. missing	0	0	13 (18)	10 (14)	24 (32)	19 (26)	29 (39)	27 (36)

Con, inulin control; IPE, inulin-propionate ester.

Supplement Table 4.6: Smoking, vaping, drinking and recreational drug status over time and by study arm.

	Baseline		2m		6m		12m	
	Con	**IPE**	**Con**	**IPE**	**Con**	**IPE**	**Con**	**IPE**
	N=135	N=135	N=135	N=135	N=135	N=135	N=135	N=135
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Smoking								
Current	14 (19)	19 (25)	17 (20)	17 (21)	13 (14)	15 (16)	11 (13)	14 (16)
Ex-smoker	19 (26)	19 (25)	18 (22)	21 (26)	21 (22)	20 (22)	22 (25)	22 (25)
Never	67 (90)	63 (85)	65 (79)	63 (79)	66 (69)	65 (72)	67 (77)	63 (71)
No. missing	0	0	17 (14)	7 (9)	22 (30)	19 (25)	15 (20)	17 (23)
Vaping								
Current	9 (12)	6 (8)	8 (10)	6 (8)	9 (9)	7 (8)	9 (10)	7 (8)
Ex-vaper	2 (3)	3 (4)	4 (5)	3 (4)	4 (4)	3 (3)	5 (6)	4 (4)
Never	89 (120)	91 (123)	88 (106)	90 (114)	88 (92)	90 (99)	86 (99)	89 (100)
No. missing	0	0	10 (14)	7 (9)	22 (30)	19 (25)	15 (20)	17 (23)
Drinking								
Current	81 (110)	79 (106)	82 (99)	78 (98)	82 (86)	80 (87)	82 (94)	79 (88)
Ex-drinker	7 (9)	9 (12)	5 (6)	10 (12)	5 (5)	9 (10)	6 (7)	10 (11)
Never	12 (16)	12 (16)	13 (16)	12 (15)	13 (14)	11 (12)	12 (14)	11 (12)
No. missing	0	1 (1)	10 (14)	7 (10)	22 (30)	19 (26)	15 (20)	18 (24)
Recreational drugs								
No	95 (128)	96 (129)	93 (112)	95 (121)	92 (99)	94 (104)	92 (107)	94 (107)
Yes	5 (7)	4 (6)	7 (9)	5 (6)	8 (9)	6 (7)	8 (9)	6 (7)
No. missing	0	0	10 (14)	6 (8)	20 (27)	18 (24)	14 (19)	16 (21)

Con, inulin control; IPE, inulin-propionate ester.

Supplement Table 4.7: Summary of adverse events by arm .

	IPE N=135 N (%)	**Con** N=135 N (%)	Difference in proportions (95% CI) (IPE – Control)
Participants reporting ≥1 adverse event	75 (56)	76 (56)	-0.7% (-12.6 to 11.1%)
Participants reporting a serious adverse event	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 at least one AE in Gastrointestinal disorders SOC	30 (22)	42 (31)	-8.9% (-19.4% to 1.6%)
Participants reporting at least one AE in infection and manifestations of SOC	22 (16)	25 (19)	-2.0% (-11.3% to 6.8%)
Participants reporting at least one AE in Food sup taste/texture SOC[ł]	9 (6)	6 (4)	2.2% (-3.2% to 7.7%)
No. participants discontinuation from study due to AE[ł]	4 (3)[*]	1 (1)	2.2% (-1.6% to 6.7%)

AE, adverse event; CI, confidence intervals; Con, inulin control; IPE, inulin-propionate ester; SOC, system organ class.
[ł]there were 3 participants who mentioned the reason for stopping the supplement as “Did not like taking supplement”.
[*]One of these was the ectopic pregnancy at randomisation.