

The role of dietary lipids in glucose homeostasis

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Statement of Originality

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person. Any contribution made to the research by others is explicitly acknowledged in the thesis.

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Declaration of Contributors

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

Chapter 2

Dr Adam Buckley performed the gut hormone analysis and assisted with data collection.

Chapter 3

This study was performed with assistance from Dr Tanveer Ashraf (TA) and Dr George Lam (GL).

Chapters 4 and 5

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Abstract

Type 2 Diabetes (T2DM) is a pressing global health issue with high morbidity, mortality and socioeconomic burden. Despite pharmacologic advances, the drug accessibility remains limited especially in lower income countries. Dietary interventions, such as the Mediterranean diet, have been emerging as promising complementary therapy due to their cardiovascular benefits.

This thesis explores the utility of extra virgin olive oil (EVOO), a major component of Mediterranean diet, in glucose regulation through the action of the gut peptide, Neurotensin (NT). The first part of the thesis examined the relationship between fasting NT levels and metabolic parameters. Fasting NT concentrations were higher in individuals with T2DM and in females. However, no significant link was identified with microvascular complications of diabetes. A systematic review explored the acute effects of dietary fat on postprandial glucose in healthy adults without diabetes. The fat addition showed modest, albeit nonsignificant, blunting of glycaemia without substantial changes in insulin concentration. A second pooled analysis investigating the substitution of saturated fatty acids with monounsaturated fatty acid, in a single fat containing meal, did not demonstrate glucose lowering postprandially.

The second part of the thesis examined the dose response effect of EVOO administration on NT secretion. These pilot studies demonstrated that EVOO (10–30 mL) stimulates NT secretion, peaking at 60–90 minutes, with no additional benefit at higher dose. A subsequent cross over trial comparing EVOO to coconut oil preloads found no significant differences in glucose or insulin responses, though a trend toward lower postprandial glucose was noted. NT elevation did not differ between oils, suggesting NT-independent mechanisms or its rapid degradation.

This work contributes to growing understanding of dietary fat's role in glucose regulation highlighting its potential role in non-pharmacologic management of

T2DM. Future studies are required to clarify NT's glucoregulatory actions through its more stable precursors.

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Abbreviations

AA	Arachidonic acid
ACBP	Acyl-CoA-binding protein
ACC	Acetyl CoA carboxylase
ADP	Adenosine Diphosphate
ALA	Alpha-Linolenic Acid
ALT	Alanine Aminotransferase
AMPK	AMP-activated protein kinase (AMPK)
AP	Area Postrema
APC	Anterior Piriform Cortex
ARC	Arcuate Nucleus
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BMI	Body Mass Index
CaSR	Calcium Sensing Receptor
CCK	Cholecystokinin
CNS	Central Nervous System
CPT1	Carnitine palmitoyl transferase 1
DAG	Diacylglycerol
DHA	Docosahexaenoic acid
EECs	Enteroendocrine Cells
EPA	Eicosapentaenoic acid
EVOO	Extra Virgin Olive Oil
FAs	Fatty Acids
FFA	Free Fatty Acids
FFAR	Free Fatty Acid Receptor
FIB-4	Fibrosis-4 Index
FOSHU	Foods for Special Health Use
GFR	Glomerular filtration rate
GI	Gastrointestinal
GIP	Glucose-dependent insulintropic peptide
GLP-1	Glucagon-Like Peptide 1
GLUT	Glucose Transporter
GLUT2	Glucose Transporter 2
GPCR	G Protein Coupled Receptor
HDL	High Density Lipoprotein
HGP	Hepatic Glucose Production
HOMA- β	Homeostatic Model Assessment of β -cell function

HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
iAUC	Incremental Area Under the Curve
IDF	International Diabetes Federation
KATP	ATP-gated potassium
LA	Linoleic Acid
LCFA	Long Chain Fatty Acid
LDL	Low Density Lipoprotein
LHA	Lateral Hypothalamus Nucleus
MAG	Monoacylglycerol
MBH	Mediobasal Hypothalamus
MedDiet	Mediterranean Diet
MCFA	Medium Chain Fatty Acid
MUFA	Monounsaturated Fatty Acid
n	Number
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NMDA	N-Methyl-D-Aspartate
NT	Neurotensin
NTS	Nucleus of the Solitary Tract
NTSR1	Neurotensin Receptor 1
NTSR2	Neurotensin Receptor 2
NTSR3	Neurotensin Receptor 3
OA	Oleic Acid
OO	Olive Oil
PC	Prohormone Convertase
PKA	Phosphokinase A
PL	Phospholipids
PP	Pancreatic Polypeptide
Pro-NT	Proneurotensin
PUFA	Polyunsaturated Fatty Acid
PVN	Paraventricular Nucleus
PYY	Peptide YY
RIA	Radioimmunoassay
SCFA	Short Chain Fatty Acid
SEM	Standard Error of the Mean
SGLT	Sodium-Glucose Transporter
SGLT1	Sodium-Glucose Co-transporter 1
SMD	Standardized Mean Difference
T2DM	Type 2 Diabetes Mellitus
TAG	Triacylglycerols
T1R2/3	Sweet taste receptors

UAE	United Arab Emirates
VLCFA	Very Long Chain Fatty Acids
VMN	Ventromedial Nucleus

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Chapter 1: General Introduction

1.1 Type 2 Diabetes

Type 2 Diabetes Mellitus (T2DM) is a complex metabolic disorder that is characterised by elevated blood glucose concentrations resulting from defective insulin action and a relative insulin deficiency. In suspected cases, it is usually diagnosed if blood glucose levels are above a certain threshold in fasting state or following a standard glucose load. Glycated haemoglobin (HbA1c), a marker of glucose levels in the preceding months, is an approved alternative test for diagnosing diabetes.

1.1.1 The diabetes epidemic

T2DM is one of the commonest disabling chronic diseases worldwide with serious consequences on health-related and socioeconomic outcomes at both individual and population level. The World Health Organisation (WHO) has recognised diabetes as one of the major non-communicable diseases that requires action.

T2DM accounts for 90% of all diabetes prevalence. Recent estimates suggest that the total number of adults with diabetes could rise above 1.3 billion by the year 2050 (1). This is an alarming increase from current International Diabetes Federation (IDF) estimates of 537 million (over 10.5% of the world's adult population) in 2021 (2). Additionally, approximately 464 million people have impaired glucose tolerance and thus are at increased risk of developing diabetes (3). About 80% of those with diabetes or prediabetes are living in low- or middle-income countries. In the United Kingdom, 4.3 million people are living with a diagnosis of diabetes of which 90% have T2DM (4). The increase in diabetes prevalence seems to be driven by the obesogenic environment with urbanisation, westernised and sedentary lifestyle, food abundance and demographic shifts being important factors. Migration, an aging population,

availability and utilisation of effective measures of treatment and prevention in certain groups may influence disease distribution and outcomes (5).

Traditionally considered to be the disease of a later life, T2DM is being increasingly diagnosed in younger age groups. According to a recent analysis, the incidence of T2DM, from 1990 through 2019, increased by 56% in those aged 15 – 39 years (6). Diabetes is one of the top 10 causes of death globally. In 2021, diabetes was responsible for 6.7 million deaths worldwide (2).

Diabetes is associated with an increased risk of developing other complications such as cardiovascular disease, peripheral vascular disease, end stage renal disease, retinopathy and blindness, neuropathy and lower limb amputations. More recently, diabetes has been linked to an increased risk of dementia (7). A recent analysis of Global Burden of Disease Study 2019 showed that the age-standardised Disability-Adjusted-Life-Years (DALYs) rate, a summary measure of total health loss, was 801.5 per 100000, an increase of 27.6% since 1990 (8).

Diabetes poses a substantial burden on health economies; recent IDF analysis estimates global expenditures of \$966 billion that are projected to reach \$1054 billion by 2045 (2). UK NHS spends around £10 billion a year on diabetes which is around 10% of its entire budget (4). This is predicted to rise to £16.9 billion by the year 2035/6 (9).

Hence, in the context of enormous burden of T2DM on population health and its socio-economic implications, there is an urgent need for ongoing measures to counter this growing problem. There is increasing evidence that dietary components affect the development and progression of T2DM beyond their simple energy content (10). Nutrient compositions that can curb glucose excursions may have clinical utility.

1.1.2 Current treatments

Management of T2DM requires a multi-faceted approach and is aimed at controlling glycaemia, intensive cardiovascular risk factors modification and screening for various micro-vascular complications. The various therapeutic strategies employed are lifestyle interventions, glucose lowering drugs, and sometimes metabolic surgery, particularly when diabetes is complicated with obesity. The diabetes pharmacotherapeutics has seen considerable progress in the last two decades with introduction of new classes of drugs that not only address hyperglycaemia but are also effective at improving the cardiovascular and renal outcomes (11, 12). In addition, metabolic surgery and dietary interventions such as very low calorie diets have now made diabetes remission a possibility (13, 14).

1.1.3 Diabetes in the United Arab Emirates

The United Arab Emirates (UAE), a federation of seven emirates on the Arab peninsula, has witnessed significant economic growth since the 1960s due to oil discovery. This prosperity led to an influx of expatriates and a population surge. Despite variations amongst emirates, overall life expectancy has improved from 50 years in the 1960s to 80 years now, comparable to Europe and North America (15). The rapid economic transformation has also led to major changes in lifestyle trends with physical inactivity and increased consumption of unhealthy diets leading to increased burden of diabetes and obesity. According to the IDF, the UAE ranked 14th globally with a 16.3% age-adjusted prevalence of diabetes in 2019. However, estimating diabetes prevalence is challenging due to methodological differences, limited population representation, and exclusion of migrants in some studies. A prospective cohort study of young Emiratis aged 18 to 40 years showed a notably high age-adjusted prevalence (approximately 22%) of cardiometabolic risk factors closely linked with diabetes, such as obesity, dyslipidaemia, and hypertension (16).

Another study amongst people with diabetes attending a large teaching hospital in Abu Dhabi showed that only 19% had optimal glycaemic control (HbA1c <6.5%). The authors estimated that up to one-third of diabetes-related deaths in this cohort were attributable to inadequate glycaemic control (17). These concerning trends underscore the urgent need for comprehensive strategies to address the growing diabetes burden in the UAE. In 2021, the UAE government introduced a series of initiatives to reduce the prevalence of diabetes and its complications under their Vision 2021 for health programme (18).

1.2 Glucose Homeostasis

The regulation of blood glucose is tightly governed by complex interactions between pancreas, liver, gastrointestinal (GI) tract, brain and other related organs such as skeletal muscles and adipose tissue. This precise regulation of blood glucose is essential for long-term health, bodily functions and survival.

Insulin and Glucagon, the two principal pancreatic islet hormones, are responsible for glucose homeostasis through their mutually opposing actions. The glucose levels in circulation, at a particular time, are determined by endogenous glucose production, the glucose absorbed from GI tract post meal ingestion and the rate of glucose disappearance from the circulation (19). Liver is the primary target for insulin and glucagon action. In fasting state, the liver is responsible for most of the endogenous glucose production through glycogenolysis and gluconeogenesis. Whereas, postprandially, the liver is mostly responsible for clearance of glucose post absorption and increased glycogen synthesis. These hepatic functions are mediated by insulin action; insulin promotes glycogen synthesis, suppresses gluconeogenesis and stimulates glycolysis (20). Moreover, insulin mediates glucose uptake in other tissues such as skeletal muscle and adipose tissues. In healthy subjects, muscle glycogenesis accounts for almost 90% of insulin-stimulated whole body glucose disposal (21).

Insulin has other anabolic actions such as lipogenesis and incorporation of amino acids into proteins. Glucagon, on the other hand, maintains blood glucose in the fasting state by promoting glycogenolysis and gluconeogenesis.

1.2.1 Glycaemic dysregulation in Type 2 Diabetes

T2DM is a progressive metabolic disorder that occurs as a result of the interaction between genetic and environmental factors. It is characterised by insulin resistance and an initial compensatory rise in insulin secretion that is followed by progressive decline in β -cell function.

Insulin resistance is identified as one of the earliest abnormalities in T2DM (22). It is characterised by reduced insulin mediated glucose uptake in skeletal muscles and adipocytes, increased lipolysis in adipocytes and impaired suppression of hepatic glucose output. Central (visceral) obesity correlates strongly with insulin resistance and associated metabolic derangements observed in diabetes. The adipose tissue insulin resistance is an inflammatory state with increased recruitment of macrophages, release of fatty acids (FAs) and triacylglycerols (TAG) from macrophage induced lipolysis and release of these substrates to the circulation. The ectopic fat accumulation (beyond adipose tissue) in myocytes, hepatocytes, beta cells and vascular endothelial cells results in activation of novel protein kinase isoforms which in turn inhibit insulin signalling and action (23). In people with T2DM, the glycogen synthesis in muscles may reduce to 60% of that observed in healthy individuals (24). In liver, the abnormal flux of FAs leads to excessive accumulation of diacylglycerol (DAG) and increased de novo lipogenesis which results in defective insulin action, reduced glycogen synthase activity and impaired suppression of insulin mediated hepatic gluconeogenesis (25).

β -cell dysfunction is a major factor that determines the development and rate of progression of T2DM. In genetically predisposed individuals, environmental factors

such as nutritional excess and obesity provoke metabolic stress which then results in progressive loss of β -cells mass and function. Chronic exposure to hyperglycaemia and dyslipidaemia thus impairs insulin secretory capacity. Studies have demonstrated that the β -cell mass has already declined by 30 – 40% by the time T2DM is diagnosed (26).

Other proposed mechanisms that contribute to metabolic abnormalities are hyperglucagonaemia, diminished incretin effect, increased renal tubular absorption of glucose, central nervous system resistance to insulin and altered gut microbiome (27).

1.2.2 Role of incretins in the pathogenesis of Type 2 Diabetes

The incretin effect, a phenomenon that the insulin secretion following orally administered glucose is much greater than secretion after intravenous glucose administration, is known to scientists for almost 60 years (28). This effect is due to the action of GI derived 'incretin' hormones. These incretin hormones, when released into the circulation from the gut following nutrient ingestion, stimulate insulin producing β -cells in the pancreatic islets and enhance insulin secretory response (29). The incretin effect is thus defined as the difference between the amount of insulin secreted in response to iso-glycaemic oral and intravenous glucose administration. It is usually expressed as a percentage of the insulin secretory response to oral glucose. In healthy human subjects, incretin effect accounts for approximately 50 to 70% of the insulin released following oral glucose administration (30). The effect relies on the amount of glucose ingested with greater amounts of glucose triggering higher amount of insulin concentration. About two-thirds of the ingested glucose is thus removed from the circulation through incretin action. Thus, the incretin effect has a major role in maintaining normal glucose tolerance. Of note, the intravenous administration of nutrients doesn't increase the secretion of these incretin hormones (31).

The two main incretin hormones are Glucose-dependent insulintropic peptide (GIP) and Glucagon-like peptide 1 (GLP-1). GLP-1 is primarily secreted from the L cells of the distal GI tract whereas GIP is released from the K cells in upper GI tract especially duodenum and jejunum. In healthy humans, these hormones have synergistic actions on insulin release in a glucose-dependant manner. The incretin hormones only exert their glucose lowering action by augmenting insulin when glucose levels are above fasting levels. Consequently, high levels of these hormones do not cause hypoglycaemia. GIP seems to be responsible for the bulk of the incretin effect. Using GIP and GLP-1 receptor antagonists and measuring insulin secretion rates following oral glucose, GIP actions accounts for about 50% of the insulin response, GLP-1 for 30% and glucose itself 20% (32).

GLP-1 suppresses glucagon secretion and delays gastric emptying, thereby curtailing postprandial glycaemic excursions (33). In contrast, GIP can stimulate glucagon secretion and doesn't influence gut motility (34).

In people with T2DM, the incretin effect is reduced (35). This has been attributed to defective actions of GLP-1 and GIP and impairment of β -cell function. This is despite near normal secretion of both hormones. In T2DM, GIP loses most of its acute stimulatory action on insulin secretion whereas GLP-1 activity is only slightly impaired. The infusion of high doses of GLP-1 above physiological levels improves and restores insulin secretion to normal levels (36). This has not been observed with GIP infusions (37). The insulin secretory response of β -cells to GLP-1 administration depends on the residual β -cell function and may not be adequate to normalise glucose levels in those with advanced T2DM with very high fasting hyperglycaemia (38).

Impaired incretin effect is considered to be a consequence than the cause of T2DM. This is supported by the reports of intact incretin effect in those with chronic pancreatitis (and normal glucose tolerance) and in first-degree relatives of patients with T2DM (39, 40). Reduction of β -cell mass to a critical level is thought to be major determinant of diminished incretin effect in T2DM. β -cell function also correlates with

the defective incretin action (41). In animal models, the GLP-1 analogues have been shown to improve β -cell function and preservation by inhibiting apoptosis, inducing β -cell proliferation and expansion of β -cell mass (42, 43).

1.3 Nutrient sensing

Nutrient sensing is a mechanism by which a cell detects a specific nutrient component and responds by often changing its metabolic processes or signalling to other cells and organs to allow them to make appropriate metabolic preparations. Impairment of nutrient sensing mechanisms and consequent disruption of metabolic signalling have been implicated in the pathogenesis of metabolic disorders such as diabetes and obesity (44). Compared to glucose-lowering drugs, dietary modification may be easier to implement at a population level, especially among those with non-diabetic hyperglycaemia. The incretins discussed above are examples of responses to gut nutrient sensing. Therefore, utilizing dietary modifications and exploiting these nutrient sensing mechanisms could translate into potential therapies for managing these disorders.

1.3.1 Peripheral nutrient sensing

The GI tract is a major site of peripheral nutrient sensing. Enteroendocrine cells (EECs) are specialised chemosensory cells that are sparsely distributed throughout the gut epithelium constituting less than 1% of the total gut epithelial population. At least 15 subtypes of EECs have been identified that produce more than 20 different hormones (45). Traditionally cells were defined by the peptides that they produced. However, the one cell one hormone theory is no longer thought to be correct, and it is recognised that individual EECs can produce multiple, often functionally related, peptide hormones (46). These peptide hormones play an important role in regulating many postprandial functions like GI motility and secretion, energy expenditure, appetite

regulation and glucose homeostasis. EECs not only interact with the lumen but also respond to the nutrients that have been absorbed through the gut epithelium (47, 48). These GI peptides exert their actions in paracrine or endocrine fashion: by acting on neighbouring neurons of the enteric nervous system or vagal afferents; or by entering circulation and affecting distant targets. These chemosensing-induced signals are integrated with others such as those instigated by mechanical distension of the gut to allow for a co-ordinated response to food ingestion and digestion.

1.3.2 Central nutrient sensing

The neural and humoral communication between the GI tract and central nervous system (CNS) centres constitute the gut-brain axis (49). However, the CNS also detects nutrients and regulates appetite and feeding behaviours. Nutrient sensing in the brain acts in concert with the signals received from the peripheral nutrient sensors. Specific neuronal populations in the hypothalamus and brainstem are particularly involved in the processing of neural and humoral signals and integration of this information to maintain energy balance. Brain nutrient sensing nuclei (such as arcuate nucleus ARC, ventromedial nucleus VMN, lateral hypothalamic nucleus LHA) are typically located in or close to the circumventricular regions and can sense nutrients including amino acids, glucose, FAs and other fuel-related stimuli (49). The area postrema (AP) and the nucleus of the solitary tract (NTS) in the brainstem are two adjacent structures that receive and process information from circulation and vagal afferents (50). The fenestrated nature of the capillaries and the lack of tight junctions between endothelial cells at the AP, NTS and at the ARC nuclei allows a swift transport of nutrients and peptides to the energy sensing nuclei (51).

1.3.3 Carbohydrate Sensing

Peripheral sensing of carbohydrates occurs in the GI tract, liver, pancreas and portal vein. Moreover, the gustatory system in the oral epithelium has 'sweet' taste receptors which detect carbohydrate presence and inform the gut and brain of the anticipated nutrients. The digestion of complex carbohydrates by pancreatic amylases and beta glucosidase converts them into monosaccharides (glucose, galactose, and fructose) which are sensed by a variety of receptors and transporters such as sodium-glucose transporters (SGLTs), glucose transporters (GLUTs) and sweet taste receptors (T1R2/3). SGLT1 mediates the absorption of glucose and galactose from the intestinal lumen and is vital for incretin secretion (52). GLUT2 is a bidirectional membrane protein distributed in intestine, liver and pancreas and facilitates glucose uptake and release depending upon glucose abundance (53). The T1R2/3 taste receptors located on the surface of various EECs mediate glucose sensing, SGLT1 upregulation, translocation of GLUT2 and secretion of regulatory peptides including Glucagon-like peptides (GLP-1 and GLP-2) and Peptide YY(PYY) (54, 55).

Centrally, glucose sensing neurons have been identified in the hypothalamus and the brainstem, and have connections to the amygdala, paraventricular thalamus, hippocampus and prefrontal cortex (56). These are broadly divided into glucose-excited (mainly in ARC, VMN and Paraventricular nucleus (PVN)) and glucose-inhibited neurons (primarily in median ARC, LHA, PVN) (57). Glucose-excited neurons perceive changes in glycaemia by detection of ATP/ADP ratio, SGLT 1/3, and sweet taste receptors (56, 58, 59). The glucose-inhibited neurons use similar mechanisms and also tandem-pore potassium channels which do not require glucose entry into the cells (60). Many neuronal glucose sensing mechanisms are analogous to those in pancreatic beta cells, namely glucokinase, ATP-gated potassium (KATP) channels and GLUT2. Glucokinase, a low affinity hexokinase, acts as an intracellular glucose sensor controlling systemic glucose homeostasis. Neural projections from

these brain areas influence secretion of glucose regulating hormones such as insulin, glucagon, catecholamines, cortisol and growth hormone (56).

1.3.4 Protein Sensing

Proteins are vital components of human diet and are crucial to building cell structure, tissue repair, growth, and energy provision. Dietary protein also has an have an important role in satiety through the sensing of specific amino acids (AAs). The digestive actions of gastric enzyme pepsin, pancreatic proteases and intestinal enterokinases converts ingested proteins into AAs (61). Sensing of these AAs by the EECs trigger the release of appetite and glucose regulating hormones including GLP-1, Cholecystokinin (CCK) and PYY (62). The Calcium-sensing receptor (CaSR) is a G protein coupled receptor (GPCR) which detects L AAs. It is highly expressed in gastrin producing G cells, somatostatin producing D and CCK producing I cells and thus affects gastric motility, secretion and protein digestion (63, 64). GPCR6A is another GPCR activated by AA (including L lysine, L arginine, L ornithine). Binding of L ornithine drives GLP-1 secretion from intestinal L cells (65). However, GPCR6A does not appear necessary for the effects of a high protein diet on food intake and body weight (66). TIR1-TIR3 taste receptor responds to glutamate and aspartate and modulates AA induced CCK release (67).

AA sensing in CNS occurs in the NTS, Mediobasal hypothalamus (MBH), Lateral Hypothalamus (LHA) and Anterior Piriform Cortex (APC). Central amino acid sensing plays an important role in regulating feeding behaviour and appetite (68). AAs such as L-tyrosine and L-tryptophan are utilised in the synthesis of catecholamines and serotonin, respectively. However certain nonessential AA can directly function as neurotransmitters. Appetite regulation has been shown to utilise these neurotransmitters (69, 70). Food intake reduces considerably following high protein ingestion and this doesn't seem to be the result of peripheral AA sensing and vagal signalling alone (71, 72). APC has been shown to modulate the hedonic response

to the protein diets, particularly being responsible for the taste aversion observed in response to imbalanced diets lacking essential AA and the preference for those diets containing essential AAs (73).

1.4 Dietary fat digestion and absorption

Dietary fat is one of the most important energy sources of all the nutrients. The dietary fats include TAG, phospholipids (PLs), and sterols (such as cholesterol). TAGs constitute 90 – 95% of the total energy derived from the dietary fat. Due to their hydrophobic nature, TAGs cannot cross the cell membrane. Thus, they are first hydrolysed in the intestinal lumen to FAs and monoacylglycerol (MAG) which in turn are taken up by the enterocytes, repackaged into TAGs and transported in the form of Apolipoprotein B (ApoB) containing chylomicrons. Some medium-chain fatty acids (MCFAs) may be directly absorbed into the circulation, rather than via the lymphatics (74).

1.4.1 Digestion

Dietary fat breakdown begins in the mouth through the action of lingual lipase which hydrolyzes medium and long-chain TAGs into partial glycerides and free fatty acids (FFA) (75). The digestion continues further in the stomach where gastric lipase, through similar action, produces more DAG and FFA. The lingual and gastric lipases are acidic enzymes and work at optimum pH of 3.0 – 6.0. These carry out up to 30% of total fat hydrolysis. Moreover, gastric peristalsis facilitates fat emulsification, a process by which complex large fat molecules are converted into smaller droplets thereby increasing their surface area which makes them more accessible to digestive enzymes (76).

Most of the dietary fat digestion occurs in the small intestine. Bile and pancreatic juices are essential for digestion and absorption of lipids. The partially hydrolysed lipid

emulsion in the upper duodenum stimulates the secretion of CCK and secretin hormones from the enteroendocrine cells of the duodenum and jejunum. CCK stimulates release of pancreatic juice (rich in digestive enzymes), bile production and its delivery into the duodenum. Secretin primarily regulates the pH of the duodenum by inhibiting gastric acid production and promoting bicarbonate secretion from the pancreatic duct cells. The resultant alkaline pH is necessary for optimum function of pancreatic lipases. Pancreatic lipase converts TAGs and DAGs into MAGs, FFA and glycerol (77). Small aggregates of mixed lipids (FAs, MAGs, other fat-soluble substances such as Vitamin E and cholesterol) and bile salts constitute mixed micelles; these are water soluble and deliver the lipid digestion products to the apical surface of enterocytes at the small intestinal brush membranes.

1.4.2 Absorption

Uptake of FAs and MAGs by enterocytes occurs through diffusion and protein mediated transport (78). FAT/CD36 receptor, abundantly expressed in the proximal small intestine, plays a key role in absorption. Inside enterocytes, the TAG products are transported through FA binding proteins (liver FABP and intestinal FABP) to the endoplasmic reticulum (ER). These hydrolysis products are resynthesized into TAGs. This occurs through acylation of MAG and FAs into DAGs and then further acylation into TAGs. The esterification of acylglycerols, cholesterol and retinols occur through acyltransferases on the ER membranes. These esterified products are then incorporated into Apo-B48 containing chylomicrons which are then later transported to golgi apparatus for further processing and then released into the lymph through the basolateral membrane of the enterocytes (79).

1.4.3 Chylomicron transport

The newly formed chylomicrons are released into the lymphatic system and subsequently enter the bloodstream through the thoracic duct. Once in circulation, High density lipoprotein (HDL) delivers apolipoprotein E and CII to these chylomicrons. This facilitates the hydrolysis of TAGs in chylomicrons through the action of lipoprotein lipase, an enzyme found on the endothelial cells. FAs, thus released, are either utilised by muscles for energy production or can be stored in the adipose tissue as triglycerides (80). The chylomicron remnants, which are now depleted of a significant portion of TAG content, are recycled back to the liver.

1.5 Lipid Sensing

1.5.1 Peripheral lipid sensing

Stimulation of free fatty acid receptors (FFARs) on the surface of EECs drive the secretion of a variety of gut hormones including GLP-1, CCK, GIP, and PYY. FFAR1 (also called GPR40) and FFAR4 (also known as GPR120) are involved in sensing both saturated and unsaturated medium and long chain FAs (LCFAs). Like most other intestinal nutrient sensors, these are GPCRs which on FA binding cause phospholipase C stimulation, subsequent phosphokinase A (PKA) activation and the release of intracellular calcium stores. FFAR2 and FFAR3 detect the short-chain fatty acids (SCFAs) namely acetate, propionate and butyrate. SCFAs are products of carbohydrate fermentation, and are primarily located on colonic L cells. Other receptors identified are GPR84, which is activated by MCFAs, and GPR119, which detects MAG. GPR119 activation is thought to trigger GLP-1, GIP and insulin release, lowering blood glucose (81). CD36, a membrane protein expressed on the taste buds and brush border membranes of intestinal villi, plays an important role in GI lipid sensing. FA binding to the CD36 in lingual taste buds triggers calcium mediated

release of serotonin that transmits signals from the taste buds to the brain. In the intestine, FA interaction with CD36 stimulates the release of secretin and CCK which aid in fat digestion and absorption (82).

Once absorbed, LCFAs undergo esterification by acyl CoA synthetase. This is followed by protein kinase C activation and CCK release from duodenal I cells (83). Similarly, GLP-1 and PYY from L cells and GIP from K cells are released but the intracellular signalling mechanisms are unclear (84-86). LCFAs have a role in delaying gastric emptying, increasing satiety and insulin release (87).

1.5.2 Central lipid sensing

Hypothalamic FA sensing neurons, particularly in the ARC and VMN, have been shown to regulate food intake, energy balance, insulin secretion, and glucose homeostasis (88). The brain also utilises acylated lipids to generate phospholipids for cell membranes (89). Fatty acid transport proteins (FATP1 and FATP4) expressed at the blood brain barrier and albumin may have role in translocating FAs into the brain. In addition, FA translocase and CD36 transporters are important in mediating FA sensing in brain vascular endothelial cells (90). FFAR4 (GPR120) may also have a role. However, the mechanisms governing neuronal uptake of FA are not clearly understood. Astrocytic glial cells surrounding neurons are also involved in lipid sensing, and express the transporters discussed above (91). After uptake, LCFAs are esterified by acyl-CoA-binding protein (ACBP). AMP kinase (AMPK) serves as an intracellular energy sensor which regulates the activity of acetyl CoA carboxylase (ACC). ACC converts Acetyl CoA (from glucose metabolism) to Malonyl CoA which inhibits Carnitine palmitoyl transferase 1 (CPT1, an enzyme on surface of mitochondria) and LCFA oxidation (92). Inhibition of CPT1 leads to LCFA CoA accumulation. This in turn leads to inhibition of hepatic glucose production through central circuit signalling via the hepatic vagal branch (93).

1.5.3 Regulation of glucose homeostasis: Role of fat and gut peptides

Glucose homeostasis is governed by the tightly coordinated control of endogenous glucose output and glucose clearance. Though FFAs can directly stimulate beta cells, particularly through FFAR1 in pancreatic beta cells (94). A number of studies support the role of GI tract in regulating glucose metabolism through a gut-brain-liver axis (95). The hypothalamus and hindbrain have been recognised as important sites for gluco-regulation. Autonomic nerve fibres from the hypothalamus and brainstem relay signals to the liver and pancreas respectively (96). Sympathetic neuronal activity increases hepatic glucose production (HGP) whereas parasympathetic signals inhibit hepatic glucose output and facilitate glucose storage (97). After nutrient sensing, gut regulatory peptides can exert indirect glucoregulatory actions by targeting specific receptors located on the vagal afferent nerve fibres. Peptide receptors for many hormones such as CCK, GLP-1, and PYY are expressed at these nerve endings (98).

In the upper small intestine, LCFA sensing potently stimulates the production of CCK which then stimulates gallbladder contraction and pancreatic enzyme secretion, and delays gastric emptying through vagal signalling, thus facilitating further fat digestion (99). CCK also activates CCK-1 receptors on neighbouring vagal afferents that innervate the nucleus tractus solitarius (NTS) in the brainstem. N-methyl-D-aspartate (NMDA) receptors in the NTS mediate gut signals initiated by CCK. Pharmacological blockade of these receptors in the rodent NTS seems to lower HGP and food intake (100). LCFAs that reach the distal gut after incomplete absorption trigger the release of GLP-1 and PYY secretion. These hormones reduce gastric emptying and thus provide an 'ileal brake' to help digestion and further absorption of nutrients (101). Besides its well-known incretin effect, GLP-1 acts on its receptors on gut vagal afferent fibres to lower HGP through a gut-brain-liver axis (102). GIP, another known incretin, is often co-expressed with GLP-1 in EECs. In healthy individuals, both saturated and unsaturated FAs have been shown to stimulate GIP secretion, which in turn promotes insulin release (103). In FFAR4 and FFAR1 double knockout mice, oil induced GIP

secretion was significantly reduced (104). A recent study showed little expression of GIP receptors in mouse vagal afferents and thus it has been suggested that GIP exerts its actions directly on the pancreas rather than through vagal afferents (105).

1.6 Free Fatty Acids

FAs serve important biological functions in human body. They provide essential sources of energy and offer structural integrity to cells as membrane constituents. Beyond these roles, they have vital actions such as regulation of intracellular signalling, and the modulation of gene transcription and immune function (106). In an unesterified state, FAs are described as free fatty acids (FFAs). Essential FAs (alpha-linolenic acid and linoleic acid) cannot be synthesized in the body and therefore must be obtained from the diet. Nonessential FAs can be generated from acetyl CoA (a product of carbohydrate metabolism) through the action of fatty acid synthetases.

Fatty acids can be classified in many ways: by chain length, by number of carbon-to-carbon double bonds, by linear vs branched and by even vs odd carbon content.

1.6.1 Short, medium and long chain fatty acids

FFAs are categorized into three groups based on their carbon chain length: SCFAs (C1 - C6), medium-chain fatty acids; MCFAs (C7 - C12), and LCFAs (> C12).

SCFAs: Acetic acid, propionic acid, and butyric acid are the three most common SCFAs produced in humans. They are metabolites generated by microbial action in the large intestine through anaerobic fermentation and may have a role in energy metabolism, inflammation and regulation of immunity (107).

MCFAs: These include caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0). The triglycerides of MCFAs are found in selective natural sources such as coconut and kernel oils and bovine milk (108). These can be rapidly absorbed from the gut without

requiring bile salts or energy. Thus, they are often used as nutritional sources in malabsorptive and malnourished states (109).

LCFAs: Oleic, linoleic, palmitic, docosahexaenoic acid, and eicosapentaenoic acid are noteworthy LCFAs with implications on human health and disease. LCFAs not only play essential roles as cell membrane constituents and in intracellular signalling but also modulate inflammation and oxidative stress. Very long chain fatty acids (VLCFAs) are a subclass with > 22 carbon atoms (108).

1.6.2 Saturated and Unsaturated fatty acids

The presence of double bond classifies fatty acids into saturated (SFA) and unsaturated FAs.

SFAs: SFAs lack a double bond between carbon atoms. They tend to be solid at room temperature. Common SFAs include myristic, palmitic, and stearic acids. Although they can be synthesized in the body, the major sources of SFAs are dairy products, red meat and poultry. Tropical plant oils such as coconut and palm oil contain a high amount of SFAs. The consumption of SFAs has been linked to oxidative stress, inflammation, insulin resistance and cardiovascular disease (109). However, more recent appraisals of the evidence have raised questions on the deleterious effects of SFA on cardiovascular health (110).

Unsaturated FAs: These contain one (monounsaturated; MUFA) or more (polyunsaturated; PUFA) double bonds between carbon atoms. Oleic acid (OA) is the most abundant MUFA, found at high levels in Mediterranean diet (MedDiet). The position of double bond at the carbon atom from the methyl end of FA defines omega 3 and 6 FAs. Omega 3 and Omega 6 are commonly known PUFAs which have been shown to lower the risk of cardiovascular disease; these are essential FAs and need to be obtained from the diet (111, 112). Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are omega 3 FAs found in plant oils and fish

oil supplements (113). Linoleic acid (LA) and Arachidonic acid (AA) are commonly known Omega 6 FAs.

1.7 Functional foods

A functional food is a food that has an additional action on metabolism beyond its nutritional value. The term was first coined in Japan in 1980s where there is a formal policy for approving these as Foods for Specific Health Use (FOSHU) (114). In EU legislation there is no clear definition for functional foods, though they are regulated by existing legislation.

The European Food Safety Authority defines functional foods as those that beneficially affect one or more target functions in the body beyond providing the basic nutrients. They confer their health benefits either through improving the state of health and well-being and/or reduction of risk of disease (115). The UK follows independent but similar regulatory procedures for approving nutrition and health claims on foods (116).

1.7.1 Role of functional foods in diabetes management

Functional foods are being increasingly employed in the management and prevention of T2DM owing to their presumed antioxidant, anti-inflammatory, and insulin sensitisation effects. Polyphenols and flavonoids as found in various fruits and vegetables (especially berries) may improve glucose homeostasis through a variety of mechanisms such as alpha glucosidase inhibition, enhanced glucose uptake in peripheral tissues, reduced gluconeogenesis and enhanced insulin secretion (117, 118). Fermented dairy products, especially those enriched with probiotics, have been shown to improve glycaemia through altering gut microbiota, reducing appetite, decreasing insulin resistance and improving glucose tolerance (119). Dietary polysaccharides in herbs, fruits and vegetables may have glucose lowering effects by

reducing glucose absorption, SCFA formation and enhancing insulin sensitivity (120). Increasing fibre intake by 15 to 35g per day has been shown to improve glycaemic control and can reduce HbA1c by up to 2% (121). MUFAs and PUFAs such as those found in fish, fish oils and plant oils have insulin sensitising, anti-inflammatory and favourable cardiovascular effects (122).

However, the adoption of functional foods in the management of type 2 diabetes face several challenges. The awareness, albeit increasing, of incorporating functional foods as a glucose lowering measure remains low. Their cost and availability may also limit their use particularly in low-income communities. People may also find the taste and palatability of certain foods unappealing. Finally, the lack of standardization and regulation may cause variation in the quality of food products.

1.7.2 Olive oil and its role in glycaemic management

Olive oil (OO) is the principal source of dietary lipids in the MedDiet and is directly extracted by pressing olive fruit. EVOO is extracted by cold-pressing without using heat or chemical treatment. It has a more favourable flavour and minimal free acidity. Since it's not refined, the FA composition of EVOO, largely incorporated in TAGs, includes Oleic acid (55–83%), Linoleic acid (3.5–21%), Palmitic acid (7.5–20%), Stearic acid (0.5–5%) and Linolenic acid (0.1–1.5%) (123). Other important components of EVOO that may have a role in glycaemic management include plant sterols, polyphenols and carotenoids.

OO has been investigated in detail in the management and prevention of diabetes. A systematic review of four cohort studies and 29 trials including nearly 16000 patients reported that OO supplementation led to a more pronounced reduction in HbA1c by about 0.27% and a lower fasting glucose by 0.44 mmol/l, in comparison to respective control groups (124). The same analysis reported a 16% reduction in risk of developing T2DM (RR: 0.84; 95% CI: 0.77, 0.92) in those taking the highest doses of OO. Some of

the mechanisms that have been thought to be responsible for glycaemic improvement are proposed to be increased insulin sensitivity, improved beta cell function and better glucose handling (123). A recent re-analysis of the PREDIMED trial, one of the largest trials of MedDiet using EVOO supplementation in approximately 2500 patients, found an approximate 50% reduction in the risk of incident diabetes over a median follow-up of 4 years (adjusted HR 0.47, CI 0.23 – 0.97) (125, 126). Other prospective trials evaluating the efficacy of EVOO in improving postprandial glycaemia have reported noticeable benefits (127-129). These glucose lowering effects of EVOO have been attributed to enhanced incretin secretion and delayed gastric emptying.

1.8 Neurotensin

1.8.1 Introduction

Neurotensin (NT) is a 13 amino acid regulatory peptide found in both the central nervous system and the GI tract. It was first isolated from bovine hypothalamic extracts in 1973 and then later from small intestine (130, 131). Prohormone convertases (PC) cleave and process Proneurotensin/Neuromedin N, a precursor molecule, in a tissue specific manner (132). PC1 enzyme is responsible for this cleavage in the GI tract whereas PC2 is the predominant enzyme responsible in the brain (133, 134).

The functions of NT in the brain have been extensively investigated. Central NT has been linked to appetite regulation & energy balance, opiate independent analgesia, inhibition of dopaminergic pathways, hypothermia and anterior pituitary function (135). NT also stimulates the growth of various tissues like the gut, pancreas, liver, adrenal, and breast. The endocrine, autocrine and paracrine effects of NT have been shown to promote neoplastic proliferation of certain cancers of GI tract in animal models (136, 137). Although the role of NT in mediating enteric functions has been extensively studied, its metabolic effects on glucose metabolism largely remain unexplored (138).

1.8.2 Regulation of NT release

GI NT is mainly secreted by the enteroendocrine N cells of the jejunum and ileum (139). However, studies have also shown that NT is co-expressed and co-secreted with other gut hormones such as GLP-1 and PYY from the distal small intestine (140). The distribution of the three hormones and their differential distribution along the length of mice intestinal villus-crypt axis suggests a common enteroendocrine precursor cell type (141).

The major physiological stimulus that triggers GI NT release is intraluminal fat. Ingestion of fat stimulates a dose related increase in NT plasma concentration, whereas protein or carbohydrate intake results in little or no response (142-144). Drewe and colleagues demonstrated that fat hydrolysis is a crucial step for NT release in humans and this at least in part requires CCK interaction with CCK-1 receptors. CCK appears to modulate NT secretion (probably through neural or humoral circuits) at least for the first 60 minutes post-ingestion, beyond which the effect is independent of circulating CCK levels and is perhaps due to direct exposure of NT secreting EECs to LCFAs (145).

Through intraduodenal perfusion studies, Drewe et al also showed that NT secretion is stimulated by LCFAs (C18s), but not MCFAs (C8s). Li et al investigated the effect of saturated and unsaturated FAs of various chain lengths on NT release in in vitro and in vivo models, and demonstrated that unsaturated FAs (DHA, OA and palmitoleic acid) had the greatest stimulatory effect on NT secretion (146). Using FFAR1 and FFAR4 agonists, it was shown that these receptors are involved in NT release, with FFAR4 having the more pronounced effect. Moreover, FFAR4 agonism and overexpression augmented OO mediated NT release (146).

NT secretion is also stimulated by GI fluids such as gastric acid, bile acids and pancreatic juice (147, 148).

1.8.3 NT receptors (NTSR)

There are three known receptors for NT.

NTSR1 and NTSR2 are classic seven-transmembrane GPCR, while NTSR3 is a type I receptor with a single transmembrane domain. NTSR1 has the highest affinity for NT and is mainly expressed in the CNS and GI tract (especially in the enteric nervous system), whereas NTSR2 is prominently expressed on astrocytes in the brain, brown adipocytes in the periphery, the GI tract, and the cardiovascular system. NTSR3 is identical to sortilin, a protein implicated in neurodegenerative and metabolic diseases (149).

While studies suggest the presence of all three receptors in rodent islets and cell models, these findings are not consistent (150, 151). Levocabastine, a partial agonist of NTSR2, has been shown to have similar effects on pancreatic beta cells as NT (150).

1.8.4 Physiologic effects of NT

1.8.4.1 Gastrointestinal effects

The GI functions of NT have been primarily investigated in animal studies. In response to fat, NT facilitates a variety of functions that promote absorption. NT affects GI tract motility; it slows transit time in the stomach and small intestine, thereby supporting absorption. It reduces pepsin and gastric acid output in humans (152). NT increases ileal blood flow, pancreatic amylase secretion, and hepatic bile secretion (153-155). The impact of NT on bile acid secretion is dual, with NT acting directly by stimulating gall bladder and bile duct contraction and indirectly through bile reabsorption in the ileum, thereby enhancing enterohepatic bile circulation (156, 157).

1.8.4.2 Glucose homeostasis

Previous studies have demonstrated a possible role for NT in glucose homeostasis but so far, a definitive link between NT and insulin release or diabetes has not been established. NT appears to have important trophic effects on the pancreas. NT increases pancreatic mass, DNA, RNA and protein content and also has a cytoprotective role against external agents (150, 158, 159). These actions are hypothesised to be synonymous to GLP-1's role in protecting pancreas from the oxidative stress of fat rich diet post absorption (160). Previous rodent studies have showed that NT exhibits a dual action on glucose regulation, increasing insulin and glucagon secretion at low glucose concentrations, but suppressing them at higher glucose concentrations (161, 162). Intravenous NT infusion in calves and healthy adults significantly elevated glucose stimulated insulin secretion (163, 164). In contrast, Brown and Vale found that the injection of NT induced hyperglycaemia in rats by causing a rise in glucagon and a fall in insulin (165). Similar hyperglycaemia-inducing effects of NT injections were shown in rats by Carraway and Leeman (166).

NT binding to NTSR2 receptor in the pancreatic beta cells has been reported to activate the protein kinase C pathway, and to increase calcium flux and subsequent insulin secretion (161). It has been suggested that NTSR3 potentiates this insulin secretion by working in conjunction with NTSR2-mediated insulin release by activating phospholipase C (167).

NT acts synergistically with GLP-1 and PYY in delaying gastric emptying, and this has been postulated to reduce glucose excursions. It has been suggested that the previously described hypoinsulinemic effect of NT in rodents is countered by GLP-1 mediated insulin release, which has been proposed as the main driver of its glucose lowering effect (141, 168).

Finally, Xenin, a peptide structurally related to NT, is co-secreted with GIP from K cells and appears to exert its actions through non-ganglionic cholinergic neurons

innervating islet cells. Xenin is thought to act on NT receptors at these nerve endings. It increases Pancreatic Polypeptide (PP) secretion and augments GIP mediated insulin release (169, 170).

1.8.4.3 Appetite regulation and energy balance

NT has been extensively investigated in modulating appetite and energy expenditure. Its actions are thought to be pleiotropic, that is, peripherally NT contributes to fat absorption and weight gain without affecting food intake. In contrast, in the CNS it has anorexigenic actions and suppresses hunger. Thus, it has opposing effects but the interaction between the peripheral and central NT systems remains unclear.

NT knockout mice have reduced intestinal fat absorption and are protected from weight gain, fat deposition in the liver and insulin resistance (171). Circulating levels of proneurotensin (Pro-NT) have been linked to weight gain, obesity, diabetes, hepatic steatosis and insulin resistance in cross-sectional and longitudinal studies in humans (172). Interestingly, diet induced weight loss and gastric bypass surgery was shown to be associated with significantly higher NT, Pro-NT and xenin levels in both rodents and humans (173). Moreover, a long acting pharmacological form of NT seems to synergize with liraglutide in inducing weight loss through their appetite suppressing effects (174).

In the CNS, the interaction of NT, NTSR and leptin seems to mediate the appetite suppressing effects of NT in the ventral tegmental and lateral hypothalamic areas (175). NT pathways also promote weight loss by increasing physical activity (176).

Previous work from our group has identified that acute administration of NT in both lean and diet-induced obese mice improves glucose tolerance through insulin elevation and this seems to be mediated via NTSR1 receptors expressed on the enteropancreatic neurons (unpublished data). Oral olive oil administration improved

glucose tolerance in similar fashion and this effect was abolished in the presence of NTSR1 antagonist.

In view of the potential, albeit ill-defined, actions of NT in regulating both glucose metabolism and energy expenditure, pharmacological forms of NT or therapies acting on NT receptors may have a role in management of diabetes, obesity and other insulin resistant states.

1.9 Hypothesis and aims

Gastrointestinal NT is stimulated by the presence of intraluminal fat especially MUFAs such as those present in olive oil. These can trigger insulin secretion through NT action on NTSR1 receptors expressing enteropancreatic neurons and can improve glucose tolerance acutely.

1.9.1 Hypothesis

High levels of MUFAs in olive oil stimulate NT release which, in turn, can reduce postprandial glycaemic excursions by promoting insulin release.

1.9.2 Aims

- 1) To investigate the relationship of fasting plasma NT levels to the metabolic parameters and microvascular complications of diabetes.
- 2) To conduct a systematic review and meta-analysis investigating the acute effects of lipid administration on glucose tolerance in healthy adults.
- 3) To establish an optimum dose of EVOO that maximises NT release in healthy human participants.
- 4) To determine the effect of EVOO on glucose tolerance and NT release following oral ingestion compared to a control oil (coconut oil).

Chapter 2: Investigating the correlation between fasting plasma neurotensin levels and metabolic parameters and microvascular complications of type 2 diabetes

2.1 Introduction

Gastrointestinal NT has been implicated in lipid absorption following fat ingestion by aiding the translocation of fatty acids across the intestinal mucosa. Pro-NT is a stable precursor of NT that is secreted in equimolar amounts (177). Pro-NT has been a subject of interest in cross-sectional and longitudinal studies; it has been linked to metabolic disorders, cardiovascular diseases, and cancer occurrence.

NT appears to have opposing actions on the control of body weight in the central nervous system and the periphery. Centrally, NT has largely anorexigenic effects, but peripherally it promotes fat absorption and deposition in ectopic sites such as liver and visceral compartments (178). Studies on NT gene knock-out mice have shown that they are protected from high fat diet induced obesity, insulin resistance and hepatic steatosis (171). In the same report studying the possible role of Pro-NT in humans, the non-obese individuals with higher baseline Pro-NT levels were found to have much higher risk of developing obesity later in life.

In epidemiological studies, there is convincing evidence of association of Pro-NT with higher body mass index. However, its association with diabetes is less clear. Cross-sectionally, Pro-NT has been shown to be weakly associated with impairment of beta cell function, insulin resistance, fasting blood glucose and poorer glycaemic control (179-181). Increasing evidence supports the role of NT in predicting cardiovascular outcomes. In experimental models, genetic variations of the NT receptor NTSR3 predispose to vascular inflammation, arteriosclerosis and plaque formation (182). Moreover, NT has regulatory actions on heart rate, blood pressure, cardiac and vascular contractility (183). In large population studies, high fasting Pro-NT predicts

major adverse cardiovascular outcomes (MACE) and cardiovascular mortality (184-186). In the Framingham Heart Study Offspring cohort, Pro-NT modestly correlated with coronary calcium score and left ventricular mass (187).

These data suggest that assessing the activity of the NT system may provide insight into obesity and metabolic disease. We therefore used an existing dataset of fasting plasma NT samples from people with and without dysfunctional glucose homeostasis, and investigated correlations between NT and various metabolic parameters and diabetes associated vascular complications. We hypothesised that the fasting NT levels will positively correlate with the presence of adverse metabolic profiles and microvascular complications in those with T2DM.

2.2 Methods

2.2.1 Study setting

The study was performed at Imperial College London Diabetes Centre (ICLDC) in the Emirate of Abu Dhabi, UAE. ICLDC is a large referral centre for specialist diabetes and endocrine care which receives patients from across the country. The centre also offers primary care and other specialist services such as cardiology, nephrology, ophthalmology and radiology. Most patients (>90%) attending ICLDC are of Arab Emirati origin. Participants were recruited between 10/01/2015 and 19/12/2016.

2.2.2 Study design

A cross-sectional retrospective study design was used to evaluate the correlation of fasting plasma NT with metabolic parameters and diabetes associated vascular complications. Written informed consent was obtained from all participants for collecting venous blood samples and reviewing their electronic medical records. The ethical approval of the study was approved by the ICLDC Research and Ethics

Committee (refs: IREC 016, IREC 019). The study complied with the ethical principles of the Declaration of Helsinki 2008.

2.2.3 Sample collection and analysis

Venous blood samples for fasting NT measurement were obtained during routine phlebotomy. A six millilitre blood sample was collected in an EDTA tube and was centrifuged using an Eppendorf Centrifuge 5702R at 4000 rpm and 4°C for 20 mins at 4°C within twenty minutes of blood collection. Plasma was separated and stored at -80°C. HbA1c was measured by high-performance liquid chromatography (HPLC) using the Variant II Turbo system (Bio-Rad, Hercules, California). Urea and electrolytes, lipid profile (total cholesterol [TC], triglycerides [TG], high-density lipoprotein [HDL] and low-density lipoprotein [LDL] cholesterol), liver function tests (alanine transaminase [ALT], aspartate transaminase [AST], gamma-glutamyl-transferase [GGT], alkaline phosphatase [ALP], albumin) and serum glucose were measured using the automated Cobas® 8000 analyser (Roche, Basel, Switzerland). Glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. The CKD-EPI equation is expressed as $GFR = 141 \times \min(Scr/\kappa, 1)^\alpha \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/ κ or 1, and max indicates the maximum of Scr/ κ or 1 (188, 189).

2.2.4 NT measurement

2.2.4.1 Principles of Radioimmunoassay

Radioimmunoassays (RIAs) are *in vitro* assays used to measure the concentration of an antigen in a sample using specific antibodies. They work on the principle of competitive antagonism between the peptide (unknown antigen) of interest and a fixed amount of iodinated peptide (radio-label) that serves as a tracer. A radiolabel is prepared by using known quantities of antigen and labelling them with gamma-radioactive iodine isotopes, such as I^{125} . The antibody selective to the antigen of interest is produced through immunisation of another species (often rabbits). The antibody produced is often polyclonal, is of animal origin (usually rabbits) and acts against the peptide (ligand) of interest. The known amount of this antibody is incubated with a fixed concentration of radiolabelled antigen resulting in formation of antigen-antibody complexes. The sample containing unknown quantities of antigen is added. This unlabelled antigen of interest competes with the radiolabelled antigen for the antibody binding sites. Centrifuging the reaction mixture separates the bound antigen from unbound antigen. To separate the bound and unbound antigen, several methods are available. Charcoal separation uses the addition of a charcoal suspension. The bound antigen-antibody complex is too large to become trapped in the pores in the charcoal particles, but the unbound antigen does become trapped. A higher concentration of unknown antigen in the sample will displace and increase the amount of free labelled antigens. After separating the bound antigens-antibody complex, the radioactivity of the free antigens is measured using a gamma counter to calculate %binding. A standard curve is plotted by using increasing concentrations of known antigen. The concentration of unknown antigen is interpolated from the standard curve (190).

2.2.4.2 NT radioimmunoassay

Plasma NT remains stable in blood samples collected in EDTA tubes for at least 8 hours at room temperature (191). Plasma NT was measured using an established in-house RIA (192). The NT antibody was produced in rabbits and is specific for neurotensin with no cross-reactivity to other gut hormones. The NT iodinated labels were prepared by Dr Paul Bech (Department of Metabolism, Digestion and Reproduction, Imperial College London) using the Iodogen method and purified by high-performance liquid chromatography (HPLC) (193). The standard curve ranged from 1-100 fmol/tube. Further details of the RIA are described in Appendix 1.

2.2.5 Clinical variables and outcomes

Data on participant demographics, diabetes diagnosis and duration, previous glycaemic control, pre-existing diabetes complications, other comorbidities and medications were collected from electronic medical records. Body mass index (BMI, kg/m²) was calculated as weight in kilograms divided by height in meters squared. The homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model for insulin sensitivity (HOMA-S) and homeostasis model assessment of β -cell function (HOMA- β) were calculated as previously described (194, 195). Briefly, HOMA uses a computer model that estimates insulin sensitivity and function by incorporating fasting plasma glucose and plasma insulin (or C-peptide) concentrations. It correlates well with estimates from the euglycaemic clamp method ($r = 0.88$) (194).

Diabetes Mellitus and Prediabetes were defined according to the American Diabetes Association (ADA) Criteria 2015 (196). Most participants were diagnosed with diabetes or prediabetes on the basis of fasting glucose and HbA1c; only a few had prior oral glucose tolerance test. Prediabetes was defined as per ADA criteria of fasting plasma glucose 5.6 – 6.9 mmol/l or HbA1c 39 – 47 mmol/mol (196, 197). Where both

HbA1c and fasting glucose were available, fasting glucose cut-offs were used to classify participants into diabetes, prediabetes and normoglycaemic groups. Diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/l or HbA1c ≥ 48 mmol/mol. Diagnosis of diabetic retinopathy was established on most recent digital retinal images which were reported by ICLDC optometrists and recorded in participant's electronic medical record. In ICLDC, people with diabetes are screened at least annually if they have no known or background retinopathy, as per UK national retinal screening programme recommendations.

Diabetic kidney disease was defined as persistent proteinuria in a person with diabetes in the absence of an alternative clinical diagnosis. For the purposes of the study, persistent microalbuminuria was defined as a urinary albumin: creatinine ratio (ACR) in a spot urine sample > 2.5 mg/mmol ($17 \mu\text{g}/\text{mg}$) in males or > 3.5 mg/mmol ($25 \mu\text{g}/\text{mg}$) in females, on two separate occasions more than three months apart (198). Information on diagnosis of diabetic neuropathy was extracted from the World Health Organisation (WHO) International Classification of Diseases (ICD) records as coded in the participant's electronic health records.

For this study, the risk of non-alcoholic fatty liver disease (NAFLD) was evaluated using Fibrosis-4 (FIB-4) Index for Liver Fibrosis. This non-invasive scoring system is based on using various laboratory parameters such as ALT, AST, and platelets. This data was available from the screening blood tests of the participants; the FIB-4 score was thus calculated for each participant. The EASL-EASD-EASO guidelines recommend to routinely screen type 2 diabetes patients for the presence of NAFLD and to assess the presence of advanced fibrosis in high risk individuals (199).

2.2.6 Participants

Participants were recruited from outpatient clinics through convenience sampling. Criteria for inclusion were age greater than eighteen years and being fasted for at least

eight hours or since the previous evening meal. Exclusion criteria were insufficient fasting duration and incapacity to comply with assessment of study outcomes.

2.3 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). Prism 9.5.1 (GraphPad Software, San Diego, CA, USA) was used for data reduction of NT assays. Continuous variables are reported as the mean $\pm$ standard deviation (SD) or median (interquartile range, IQR), and categorical variables are reported as percentages. The Student t test for continuous variables and χ^2 test for categorical variables were used to compare mean values between two independent groups. Correlations between parameters were explored by Pearson (continuous variables) or Point bi-serial (categorical variables) coefficients. Multivariable logistic regression models were built to identify determinants of presence of microvascular complications (yes/no, dependent variable) in those with T2DM. Statistical significance was assessed at the level of $P < 0.05$.

2.4 Results

NT was measured in fasting venous blood samples of 802 participants. Clinical and biochemical characteristics of the study population in relation to the diagnosis of diabetes are shown in Table 2.1.

NT was marginally higher in females than in males [(Median (IQR): 36.24 (26.5 – 50.7) vs 33.03 (23.87 – 45.89) pmol/l, $P = 0.011$]. 18 participants had history of previous bariatric surgery (10 in T2DM, 4 in Prediabetes and 4 in normoglycaemic groups respectively). 71 participants were on orlistat (53 in T2DM, 13 in Prediabetes and 5 in normoglycaemic groups respectively). NT in participants on orlistat was not significantly different from those who were not (Median (IQR): 32.02 (26.22 – 50.25) vs 34.79 (25.27 – 49.30) pmol/l, $P = 0.868$).

Table 2.1: Clinical and Biochemical Characteristics of Study population				
Characteristic	NG	Prediabetes	T2DM	p-value
n	83 (10.3%)	108 (13.5%)	611 (76.2%)	
Age (years)	55.48 ± 10.3	48.9 ± 12.5	41.44 ± 11.4	<0.001
Sex (Males)	33 (39.7%)	58 (53.7%)	289 (47.3%)	0.160
Weight (kg)	76.3 ± 17.8	84.3 ± 15.8	84.5 ± 16.9	0.001 ^a
BMI (kg/m ²)	29.7 ± 6.2	31.4 ± 5.2	32.1 ± 5.9	0.002 ^b
Waist circumference (cm)	94.9 ± 13.74	102.0 ± 9.3	105.1 ± 12.1	<0.001 ^c
HbA1c (mmol/mol)	33.0 ± 4.5	38.0 ± 4.5	58.0 ± 16.2	<0.001 ^d
FBG (mmol/l)	5.3 ± 0.4	6.2 ± 2.8	8.8 ± 3.4	<0.001 ^e
FBI (pmol/L)	80.0 (54.6, 100.7)	108.6 (75.6, 147.3)	106.9 (72.5, 157.1)	<0.001 ^f
NT (pmol/L)	29.8(22.7,49.2)	29.7 (23.3, 40.0)	35.8(25.9, 50.1)	0.001 ^g
Values are presented as mean ± standard deviation unless otherwise indicated. NG Normoglycaemic individuals, T2DM Type 2 diabetes; HbA1c glycosylated haemoglobin, FBG fasting blood glucose, FBI fasting blood insulin, NT Neurotensin. ^a Comparison between Prediabetes and T2DM was not significant ($P = 0.598$). ^b Comparison between NG and T2DM was significant ($P = 0.001$). ^c Comparison between Prediabetes and T2DM was not significant ($P = 0.109$) ^{d,e} All comparisons were significant ^f Comparison between Prediabetes and T2DM was not significant ($P = 1.00$) ^g Comparison between Prediabetes and T2DM was significant ($P = 0.002$)				

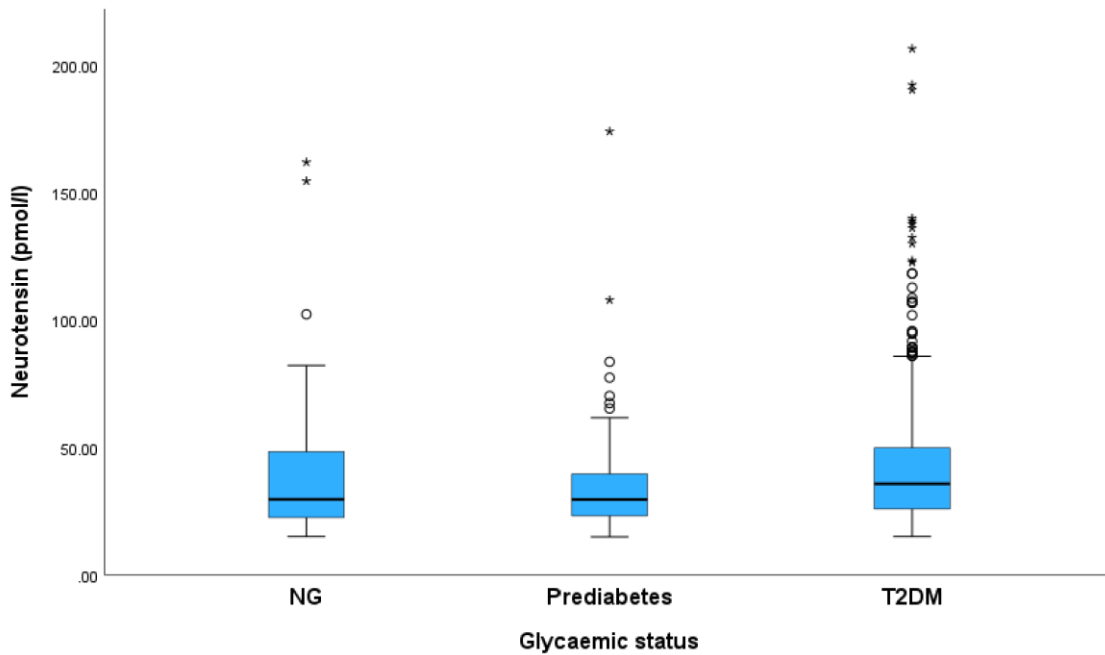


Figure 2.1: Fasting neurotensin concentration in the study population. Plots represent median and IQR, n = 802, NG, Normoglycaemic, T2DM, Type 2 Diabetes.

In the whole study population, NT correlated positively with HbA1c, FBG, TC, LDL and TG (Table 2.2). However, in participants with T2DM, this correlation remained significant with TC and TG only ($r = 0.124$, $P = 0.002$ and $r = 0.115$, $P = 0.004$ respectively).

Table 2.2: NT Bivariate Correlation analysis		
Characteristic	Correlation Coefficient (r)	P value
Age	-0.40	0.252
Weight	0.021	0.547
BMI	0.055	0.121
Waist circumference	0.054	0.177
HbA1c	0.076	0.032
FBG	0.076	0.031
FBI	0.051	0.150
TC	0.089	0.012
TG	0.112	0.002
LDL	0.05	0.156
HDL	0.01	0.784
HOMA-S	-0.035	0.327

HOMA-IR	0.009	0.798
HOMA-B	0.005	0.894
Creatinine	-0.069	0.052
eGFR	0.012	0.734
Pearson correlations unless otherwise specified. Values in bold indicate significant correlations.		
HOMA-S homeostasis model assessment for insulin sensitivity, HOMA-IR homeostasis model assessment for insulin resistance, HOMA-B homeostasis model assessment for beta-cell function, eGFR estimated glomerular filtration rate.		

No significant correlations were observed between NT and microvascular complications of diabetes (Table 2.3). In the T2DM group, NT levels correlated negatively with FIB-4 score ($r = -0.130$, $P = 0.001$).

Table 2.3: NT Point-Biserial Correlation with vascular complications of diabetes		
Characteristic	Correlation Coefficient (r)	P value
Retinopathy (yes/no)	-0.013	0.753
Neuropathy (yes/no)	0.006	0.886
Nephropathy (yes/no)	-0.025	0.539
Coronary artery disease (yes/no)	0.011	0.777
Cerebrovascular accident (yes/no)	-0.0430	0.286
Peripheral arterial disease (yes/no)	-0.034	0.399
Point bi-serial correlation unless otherwise specified.		

In univariate logistic regression, age, hypertension, dyslipidaemia, creatinine, HbA1c, and fasting glucose were significant predictors of microvascular disease. However, neither fasting NT concentration nor log-transformed NT showed a significant association. When adjusted for covariates in multivariable logistic regression, only age, creatinine, and HbA1c remained statistically significant predictors (see Appendix 2, Tables 2.4 and 2.5).

2.5 Discussion

The aim of this analysis was to investigate potential associations of fasting plasma NT levels with metabolic parameters and vascular complications of diabetes. Using an existing dataset, this work has shown that higher fasting NT levels are positively associated with HbA1c, fasting blood glucose, total cholesterol, low density lipoproteins and triglycerides. Previous cross-sectional analyses have only studied Pro-NT, a stable but non-active precursor of NT, as a predictive marker for glycaemic and metabolic parameters. In particular, the association of Pro-NT with microvascular complications of diabetes have not been investigated before. Since NT is an active form of the hormone, we opted to investigate these associations with NT.

In our cohort, NT levels were slightly higher in females. Pro-NT has been previously linked to poorer metabolic outcomes in women. Barchetta et al. observed a positive correlation of higher Pro-NT levels and female sex ($r = 0.15$, $P = 0.05$) in a cohort of obese individuals referred for bariatric surgery (181). Melander et al. observed that fasting Pro-NT was associated with a higher risk of developing diabetes, DM (HR 1.28, CI 1.09-1.50), cardiovascular disease, CVD (HR 1.17, CI 1.07 – 1.27) and cardiovascular mortality (HR 1.29, CI 1.12 – 1.49) in a Swedish cohort who were followed up over a period of 15 years. In females, these risks were much higher; DM (HR 1.41, CI 1.12-1.77), CVD (HR 1.33, CI 1.17-1.51) and cardiovascular mortality (HR 1.44, CI 1.21 – 1.71) (184). In the Malmo preventative study cohort, the increased diabetes risk was observed only in older females followed up for a period of 5.4 years (HR 1.28, CI 1.30 – 1.59) (185).

Orlistat inhibits lipase action, prevents triglyceride hydrolysis and FFA absorption (200). These actions might be expected to affect postprandial levels of NT more than the fasting levels. Indeed, the fasting NT levels, in our study, appear to be unaffected by Orlistat use. It seems likely that the postprandial NT levels would have been lower in those taking Orlistat. This effect was observed by Drewe et al. where the

intraduodenal fat absorption and the subsequent rise in neurotensin were acutely inhibited by the administration of orlistat (145).

We found weak but positive associations of fasting NT levels with HbA1c and fasting blood glucose. Previous studies have reported an association between Pro-NT and glycaemic control and diabetes incidence. Fasting Pro-NT correlated with a worsened glycaemic control at baseline in a small cohort of participants with type 1 diabetes ($n = 41$) (201). Fawad et al reported lower insulin sensitivity and disposition indices in those with higher Pro-NT (180). In a longitudinal follow-up study of overweight and obese children over almost 7 years, higher basal Pro-NT negatively correlated with beta cell function ($r = -0.19$) (179). In the population-based Malmö Diet and Cancer Study, fasting Pro-NT correlated positively with fasting blood glucose and insulin; the study also found an increased incidence of diabetes in those with higher Pro-NT levels (HR 1.28, CI 1.09 - 1.50). Conversely, in REGARDS cohort, Nicoli et al did not find an independent association between Pro-NT and incident diabetes after following up over 3500 individuals over a period of almost 10 years (202).

The positive association between fasting NT, serum triglycerides and total cholesterol is consistent with the known role of NT in promoting fat absorption. NT has also been implicated in hepatic fat accumulation in gene knock-out models (171), and Pro-NT has been shown to predict the presence and severity of histologically proven non-alcoholic fatty liver disease (NAFLD) in those with obesity (AUROC 0.836) (181). FIB-4 score was used in the current study as a predictor of presence of NAFLD (203). However, unexpectedly NT levels correlated negatively with FIB-4 scores which can be extrapolated to reduced severity of NAFLD in those with T2DM. Interestingly, Auguet et al. also found significantly lower levels of NT in women with histologically proven NAFLD in comparison to healthy controls and in those with obesity without NAFLD (204). However, a further analysis in the same cohort showed a significantly higher levels of Pro-NT in those with NAFLD and higher degree of fibrosis. It is

probable that Pro-NT degradation is relatively altered and perhaps accelerated in those with obesity and NAFLD, thus leading to lower levels of NT in these populations (205). Our findings require further evaluation in bigger cohorts with imaging-confirmed NAFLD.

To our knowledge, this is the first analysis evaluating the correlation of fasting NT with metabolic changes and vascular complications of diabetes. Although a few statistically significant correlations between fasting NT and metabolic parameters were observed in the bivariate analysis, the magnitude of these associations was uniformly weak with correlation coefficients well below thresholds generally considered biologically or clinically meaningful. This indicates that the fasting NT may account for only a small proportion of the variance in glycaemic or lipid parameters. Thus, its clinical utility as a standalone biomarker of metabolic health is likely to be limited. Nevertheless, the weak correlations with HbA1c and fasting glucose are directionally consistent with epidemiological studies using Pro-NT.

NT secretion is meal- and fat-responsive, and its biological actions are primarily postprandial; therefore, fasting concentrations may represent a biologically “blunted” signal that underestimates the functional relevance of the NT system. Moreover, the rapid degradation of NT in plasma poses a major limitation to detecting any meaningful associations. From a biomarker perspective, these factors suggest that fasting NT is unlikely to function as a reliable marker of metabolic dysfunction in observational settings.

Another limitation of this study is that, although ICLDC receives patients from across the country, the study was carried out in people presenting in a single specialist diabetes centre and may not be representative of the general population. Finally, in view of the cross-sectional study design, the causal relationship between NT and dependent outcomes cannot be established.

In summary, this analysis indicates that the fasting serum NT levels are associated with poorer glycaemic control, elevated triglycerides but lower FIB-4 scores. Future studies should investigate postprandial NT dynamics and direct comparisons with Pro-NT to better define whether NT system activity has prognostic or mechanistic relevance beyond traditional metabolic markers.

Chapter 3: The effect of fat load on acute glucose tolerance in adults without diabetes: a systematic review and meta-analysis

3.1 Introduction

An unhealthy diet is an established risk factor for the development of non-communicable diseases. The modern Western diet is caloric dense, often highly processed and is rich in refined carbohydrates and unhealthy fats. The adoption of such diet has been linked to increasing incidence of obesity, T2DM and cardiovascular disease (206).

Indeed, one of the cornerstones of T2DM management is medical nutrition therapy and maintaining a healthy diet. Whilst ADA does not specifically recommend a certain amount of daily caloric intake from dietary fat, reducing the saturated fatty acids (SFA) to less than 10% of the daily energy intake is proposed; this is primarily due to its cholesterol lowering effects, thereby reducing cardiovascular risk (207). However, the MedDiet, which is typically rich in MUFA, has been implicated in improving components of metabolic syndrome such as reducing weight and waist circumference, fasting glucose, incidence of T2DM and adverse cardiovascular events (208). Besides these functional effects of fatty acid components of a diet, the addition of fat to a carbohydrate meal may also lower postprandial glucose excursions; this has been studied for many years (209, 210). Although fat ingestion may stimulate insulin secretion directly, the incretin effect of fat has been considered an important mechanism responsible for the glucose lowering action of dietary fat (211, 212). However, the relationship between dietary fat and glucose metabolism remains unclear. In animal models, high fat dietary interventions are frequently used to study mechanisms of insulin resistance (213). Similarly, in humans, high fat diets have been shown to decrease insulin sensitivity (214, 215).

Given the inconclusive evidence on the effect of dietary fat type or its addition to a carbohydrate meal on the postprandial glucose, I conducted a systematic review and meta-analysis to examine the effects of fat administration on glucose and insulin.

3.2 Methods

3.2.1 Registration

This systematic review and meta-analysis was registered at PROSPERO (registration number: CRD42024446123). It was reported as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (216).

3.2.2 Eligibility criteria

Population: Randomised crossover or cohort studies in healthy adults (≥ 18 years) of any BMI. Participants that were pregnant, currently taking medication that might interfere with glucose or insulin concentrations, NAFLD or non-alcoholic steatohepatitis (NASH), impaired glucose metabolism, or had a history of chronic disease, including type 1 and type 2 diabetes, were excluded.

Intervention: Acute fat administration (≤ 24 hours). When comparing SFA with MUFA, a difference of 5% energy intake was considered adequate for comparison.

Comparator: When comparing SFA with MUFA, only dietary interventions that were isocaloric (approximately) with no other significant difference besides the fat type. When comparing addition of fat into a meal, the control group was the meal without fat with no significant difference in other macronutrient composition.

Outcome: Studies measuring glucose and/or insulin concentrations. Incremental area under the curve (iAUC) was considered as the primary outcome. Alternatively, the change in the outcomes measured at 120 minute post meal intervention was used for

comparison. The 120 minutes as a time point was deemed appropriate as it is widely used for diagnosing diabetes or impaired glucose tolerance and thus provides a standardized reference across different studies. An elevated 2 hour postprandial glucose has also been linked to cardiovascular risk, future risk of diabetes and mortality (217).

Studies which were not written in the English language, or included a clamp procedure (hypoglycaemic or hyperinsulinaemic euglycaemic clamp) during the study period were excluded.

3.2.3 Search strategy

The online databases Pubmed, Medline, Embase, CINAHL, Embase, Scopus and Web of Science databases were searched from inception to September 2022.

Full details of the search strategy are provided in Appendix 3. In addition, a manual search of reference lists of reviews on the topic was performed, to identify additional relevant articles. When necessary, the authors were contacted to obtain data of interest. Studies were excluded if authors did not respond.

3.2.4 Study Selection

The study selection was performed using the online Covidence systematic review software (Veritas Health Innovation; www.covidence.org). Remaining duplicate studies were removed using Endnote version x9 (Clarivate Analytics, Philadelphia, USA). All articles identified by the search strategy were screened by title and abstract by 2 reviewers independently (MS, GL or TA). After screening, full texts deemed to be potentially relevant were assessed for eligibility against the defined inclusion and exclusion criteria independently.

3.2.5 Data collection and synthesis

Data were extracted by two authors independently (MS and TA) into an electronic spreadsheet (Excel 2016, Microsoft Corporation, USA). Study characteristics included author and year of publication; study design, sample size, participant's characteristics (age, gender, body weight and body mass index), intervention characteristics and outcomes (pre- and post-intervention concentrations of glucose and/or insulin concentrations); and corresponding concentrations of glucose and/or insulin concentrations in the comparator arm.

When individual participant data or summary results for postprandial glucose and insulin were not available in the published text or supplementary material, graphical data were extracted using WebPlotDigitizer Version 5.2 (<https://automeris.io> , Ankit Rohatgi, USA). The values were then used to calculate the incremental area under the curve (iAUC_{0-120 mins}). All extracted data were checked for accuracy by a second author (TA).

Glucose was converted to mmol/l when presented in mg/dl (divided by 18) and insulin to pmol/l when available in mIU/l or uIU/ml (multiplied by 6.95). Standard errors and confidence intervals were converted to a standard deviation. When within-participant correlation coefficients were not available, a correlation coefficient of 0.5 was assumed to calculate standard deviation, as recommended in the Cochrane handbook (218, 219). iAUC was calculated by the trapezoidal rule using data from available time points, in Graphpad Prism (version 10.4.1, GraphPad, La Jolla, CA, USA). The random-effects meta-regression was performed for continuous covariates including age, body mass index, % of male participants and amount of fat (in grams) used in the study.

Eligible studies comparing SFA vs MUFA had to be isocaloric (approximately), with no major difference in other macronutrients. If not all error bars were presented, homoscedasticity (constant variance) was assumed and the variance from the nearest timepoint within the same experimental arm was imputed (220). Some records had

more than 2 intervention arms per test intervention. Selection of the interventions against the control was based on the highest dose used. Thus, not all comparisons were meta-analysed.

3.2.6 Statistical analysis

Descriptive data is reported as mean $\pm$ SD unless stated otherwise. The results are presented as standardized mean difference (SMD) with their respective 95% confidence interval (CI). These were calculated using a DerSimonian-Laird random-effects model (221). The random-effects model assumes that the differences in effect across studies reflect both true effects of the diet as well as sampling variation. Heterogeneity was assessed using the I^2 statistic and Chi-square (χ^2) statistic. I^2 values above 25%, 50%, and 75% indicate low, moderate, and high heterogeneity, respectively (222). Statistical significance was determined at a P value ≤ 0.05 . Review Manager Software v5.4.1 (The Cochrane collaboration, 2020) was used for meta-analyses. Meta-regression was performed using the open source programme JASP v0.95.4 (Apple Silicon, University of Amsterdam, Amsterdam, The Netherlands).

3.2.7 Assessment of risk of bias

The risk of bias was evaluated using the Revised Cochrane Risk of Bias Tool for Randomized trials (RoB 2.0) with additional considerations for cross-over trials (223). The RoB2.0 tool is composed of five distinct domains: randomization process, deviations from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Each domain is characterized as “low risk,” “some concerns,” or “high” risk. The additional considerations for crossover trials include carryover and period effects.

3.3 Results

3.3.1 Study selection

Database search retrieved a total of 61029 potentially eligible papers. Following removal of duplicates, 12517 studies underwent title and abstract screening. After screening, 11055 records were excluded. A total of 1462 studies were then subjected to full text review against the PICO criteria. The review of full texts identified 21 papers which were considered eligible to be included in this review. Out of these 21 articles, 13 were meta-analysed to evaluate the effect of addition of fat into a carbohydrate meal on glucose and insulin and 12 were meta-analysed to study the effect of substituting SFA with MUFA on glucose tolerance and insulin concentration. 4 studies overlapped and were included in both meta-analyses. This is summarised in Figure 3.1.

3.3.2 Effect of addition of fat to a carbohydrate meal

3.3.2.1 Characteristics of studies

Thirteen trials (Table 3.1) were included evaluating the effect of addition of fat to carbohydrate. The total number of participants was 168, mean age was 34.2 (SD: 6.2) years, 61.8% were male, mean weight was 71.8 (SD: 10.4) kg and mean BMI was 23.9 (SD: 2.8) kg/m². Mean baseline fasting glucose was 4.74 (SD: 0.39) mmol/L.

7 studies were deemed at low risk of bias, 3 had some concerns and 3 at high risk of bias (Table 3.2).

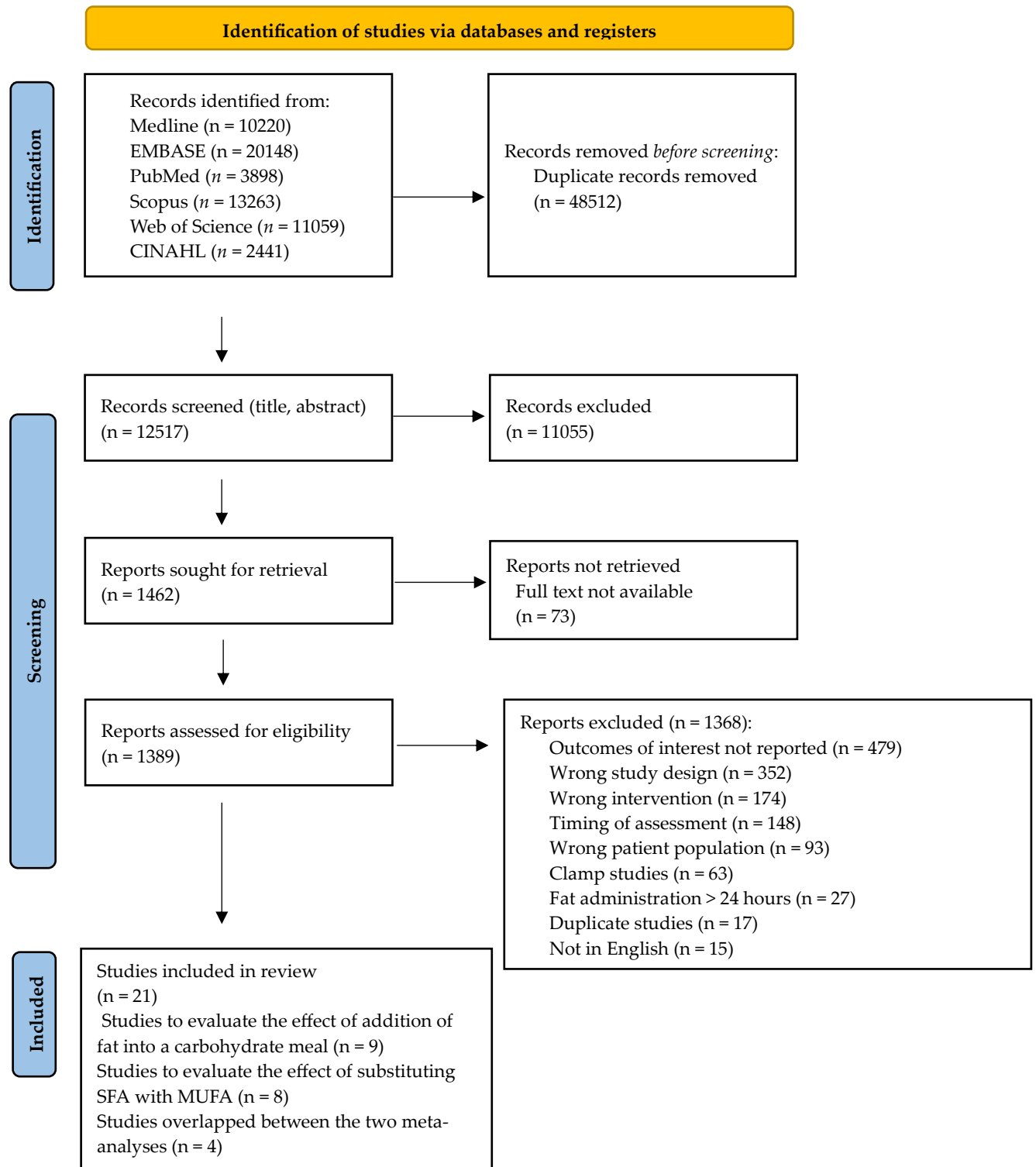


Figure 3.1: Study selection. The PRISMA 2020 flow diagram was adapted from Page et al. (216).

Reference	Study design	Sample size	Age, years Mean $\pm$ SD	Sex, Males	Weight, kg Mean $\pm$ SD	BMI, kg/m ² Mean $\pm$ SD	Glucose, mmol/l Mean $\pm$ SD	Fat added to Carbohydrate	Carbohydrate control	Amount of fat, grams
Gannon et al. 2013, USA (210)	RCT, XO	10	29 $\pm$ 2	10	NR	24 $\pm$ 2.3	5.2 $\pm$ 0.1	Butter added to starch	Starch	50
Henry et al. 2008, UK (224)	RCT, XO	15	30.8 $\pm$ 12.1	7	66.9 $\pm$ 9.6	23.4 $\pm$ 4.2	4.55 $\pm$ 0.35	Olive oil added to white bread	White bread	24.8
Meng et al. 2017, USA (225)	RCT, XO	20	64 $\pm$ 9	10	NR	29.3 $\pm$ 3	5.2 $\pm$ 0.6	Butter added to white bread	White bread	22.2
Owen et al. 2003, Canada (226)	RCT, XO	12	28.6 $\pm$ 3.5	5	NR	21.4 $\pm$ 0.6	NR	Margarine added to white bread	White bread	40
Sørensen et al. 2021, Denmark (227)	RCT, XO	9	54 $\pm$ 8	4	105 $\pm$ 21	34.4 $\pm$ 4.2	5.3 $\pm$ 0.4	Hydrolysed pine nut oil and olive oil added to glucose	Glucose	6
Tan et al. 2017, Singapore (228)	RCT, XO	16	27 $\pm$ 6	16	65.5 $\pm$ 5.5	21.9 $\pm$ 1.7	4.5 $\pm$ 0.5	Coconut oil added to carbohydrate meal	Carbohydrate meal	22.25
VanOostrom et al. 2004, Netherlands (229)	RCT, XO	6	23 $\pm$ 2	6	72 $\pm$ 6	21.2 $\pm$ 1.3	4.1 $\pm$ 0.2	Fat cream added to glucose	Glucose	50
Yamaguchi et al. 2018, Japan (230)	RCT, XO	8	29 $\pm$ 1	4	NR	21.1 $\pm$ 0.4	5.1 $\pm$ 0.05	Butter added to glucose	Glucose	34
Zibella et al. 2017, Italy (231)	RCT, XO	10	27.4 $\pm$ 7	3	NR	NR	NR	EVOO added to bread	Bread	40
Violi et al. 2015, Italy (127)	RCT, XO	25	35.9 $\pm$ 2.1	12	NR	23.1 $\pm$ 3.4	4.78 $\pm$ 0.1	Olive oil added to mixed meal	Mixed meal	10
Lau et al. 2016, Singapore (232)	RCT, XO	15	25.3 $\pm$ 4.64	15	63.5 $\pm$ 5.8	21.2 $\pm$ 1.2	4.16 $\pm$ 0.47	Olive oil added to bread	Bread	25.3
Radulescu et al. 2009, USA (233)	RCT, XO	9-12	25 $\pm$ 5	5	NR	23 $\pm$ 2.5	NR	Olive oil added to potato	Potato	25
Thomsen et al. 1999, Germany (234)	RCT, XO	10	23 $\pm$ 2	5	NR	21.4 $\pm$ 1.7	4.4 $\pm$ 0.4	Olive oil added to bread with soup	Bread with soup	80

Table 3.1: Summary of studies evaluating addition of fat to a carbohydrate. RCT, Randomised Controlled Trial, XO, Cross over, NR, Not reported.

Study ID	D1	DS	D2	D3	D4	D5	Overall
Gannon et al. 2013, USA (210)	!	-	+	+	+	+	-
Henry et al. 2009, UK (224)	+	!	+	+	+	+	!
Meng et al. 2017, USA (225)	+	!	+	+	+	+	!
Owen et al. 2003, Canada (226)	+	!	+	+	+	+	!
Sørensen et al. 2021, Denmark (227)	+	+	+	+	+	+	+
Tan et al. 2017, Singapore (228)	+	+	+	+	+	+	+
VanOostrom et al. 2004, Netherlands (229)	+	+	+	+	+	+	+
Yamaguchi et al. 2018, Japan (230)	+	-	+	+	+	+	-
Zibella et al. 2017, Italy (231)	!	-	+	+	+	+	-
Violi et al. 2015, Italy (127)	+	+	+	+	+	+	+
Lau et al. 2016, Singapore (232)	+	+	+	+	+	+	+
Radulescu et al. 2009, USA (234)	+	!	!	+	+	+	+
Thomsen et al. 1999, Germany (234)	+	+	+	+	+	+	+

Table 3.2: Risk of bias assessment using Cochrane ROB 2.0 tool

	Low risk	D1 Randomisation process
	Some concerns	DS Bias arising from period and carryover effects
	High risk	D2 Deviations from intended interventions
		D3 Missing outcome data
		D4 Measurement of the outcome
		D5 Selection of the reported result

3.3.2.2 Outcomes

3.3.2.2.1 Glucose

Addition of fat non-significantly reduced iAUC of glucose measured over 120 minutes compared to carbohydrate alone (SMD: -0.39, 95% CI: -0.84 – 0.05, $P = 0.08$; $n = 120$; Figure 3.2). $I^2 = 63\%$ and $Q (24.53, df = 9, P = 0.004)$ suggest moderate but significant heterogeneity across studies. However, Tan et al. had a considerable effect on the pooled effect (SMD 0.84) and on the heterogeneity (228). The leave one out sensitivity analysis led to a more significant pooled estimate (SMD -0.54, 95% CI -0.91 – -0.17, $P = 0.004$, $n = 104$) and a reduced heterogeneity ($I^2 = 39\%$ and Q statistic of 13.02 ($df = 8, P = 0.11$)) (Appendix 5, Figure 3.7A).

There was no significant difference in change in glucose concentration at 120 minutes between the intervention and control groups (SMD = 0.03, 95% CI: -0.53 to 0.59; $P = 0.93$; $n = 157$; Figure 3.3). Substantial heterogeneity was observed across studies, as indicated by an I^2 of 82% and a Q statistic of 60.34 ($df = 11, P < 0.00001$).

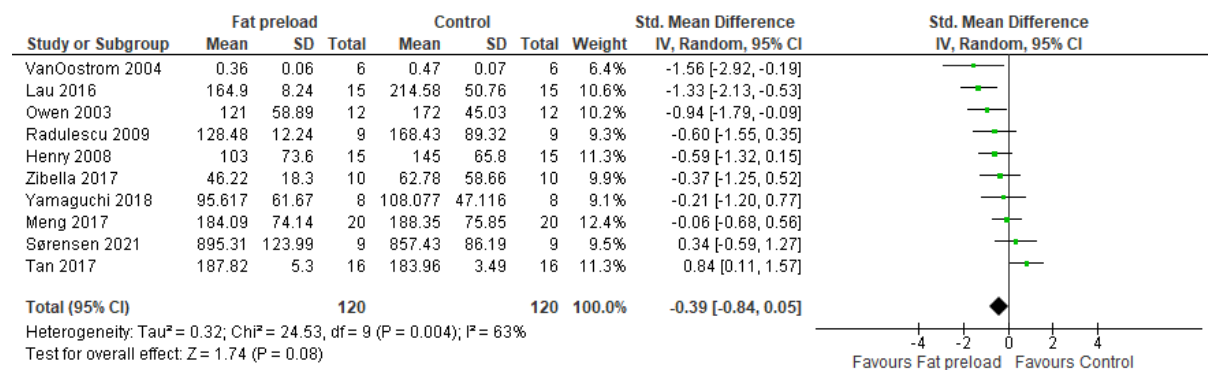


Figure 3.2: Forest plot comparing the iAUC_{0-120 mins} for glucose between fat added to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

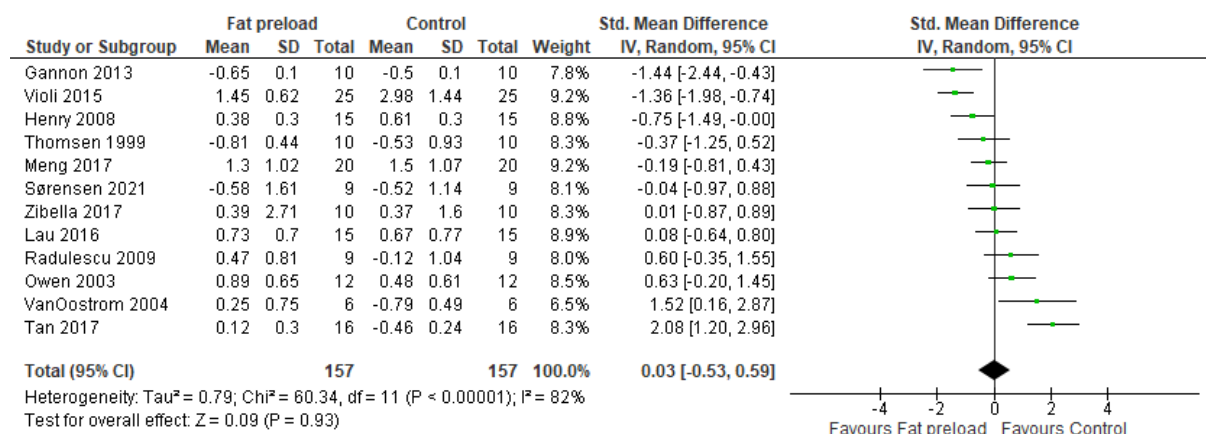


Figure 3.3: Forest plot comparing the change in glucose concentration at 120 minutes on addition of fat to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

Meta-regression revealed that the iAUC of glucose over 120 minutes was also moderated by fat dose (coefficient: -0.037, 95% CI: -0.073 to -1.238×10^{-4} , $P = 0.049$) (Appendix 6). Inclusion of fat dose reduced residual heterogeneity ($\tau^2 = 0.18$; $Q_e(8) = 15.30$, $p = 0.054$), indicating that variation in fat dose accounted for a substantial proportion of between-study heterogeneity. After adjustment for fat dose, the pooled effect of fat addition was statistically significant (SMD = -0.42, 95% CI -0.81 to -0.03; $p = 0.037$)

The funnel plot for studies assessing glucose iAUC_{0-120 mins} appeared mostly symmetrical, suggesting a low likelihood of publication bias (Appendix 4, Figure 3.6A). Similarly, the funnel plot for studies evaluating the change in glucose at 120 minutes showed a generally symmetrical distribution of effect sizes around the pooled estimate (Appendix 4, Figure 3.6B).

3.3.2.2.2 Insulin

Addition of fat to carbohydrate did not affect iAUC of insulin measured over 120 minutes compared to carbohydrate alone (SMD: -0.27, 95% CI: -1.75 – 1.22, $P = 0.73$; n

= 87; Figure 3.4). $I^2 = 94\%$ and $Q (93.15, df = 6, P < 0.00001)$ suggests high heterogeneity across studies.

There was a significant difference in change in insulin concentration at 120 minutes between the intervention and control groups (SMD = 0.96; 95% CI: 0.16 – 1.75; $P = 0.02$ $n = 120$; Figure 3.5). Substantial heterogeneity was observed across studies, as indicated by an I^2 of 87% and a Q statistic of 59.80 ($df = 8, P < 0.00001$). The results were affected by the disproportionately large effect seen in Tan et al. (SMD 3.71) which may have influenced the pooled estimates (228). Leave one out sensitivity analysis by removing this study reduced the pooled effect (SMD = 0.64, CI -0.03 – 1.32; $P = 0.06$, $n = 104$) and the heterogeneity ($I^2 = 80\%$ and Q statistic = 35.33 ($df = 7, P < 0.00001$)) (Appendix 5, Figure 3.7B). Random-effects meta-regression showed no significant moderator effects of continuous covariates.

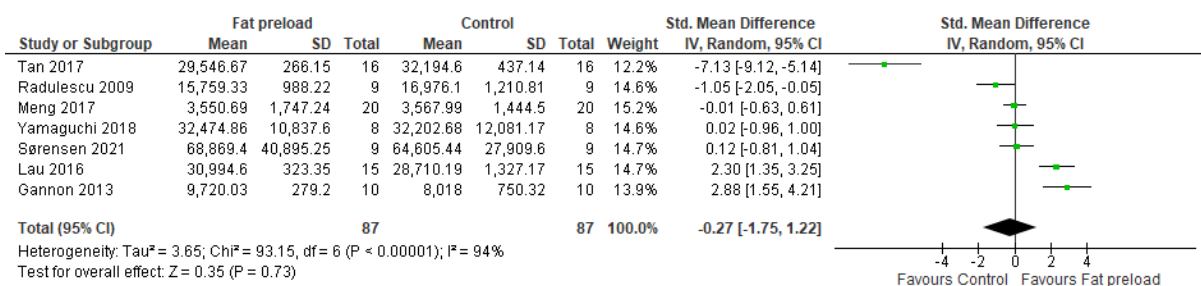


Figure 3.4: Forest plot comparing the $iAUC_{0-120 \text{ mins}}$ for insulin between fat added to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

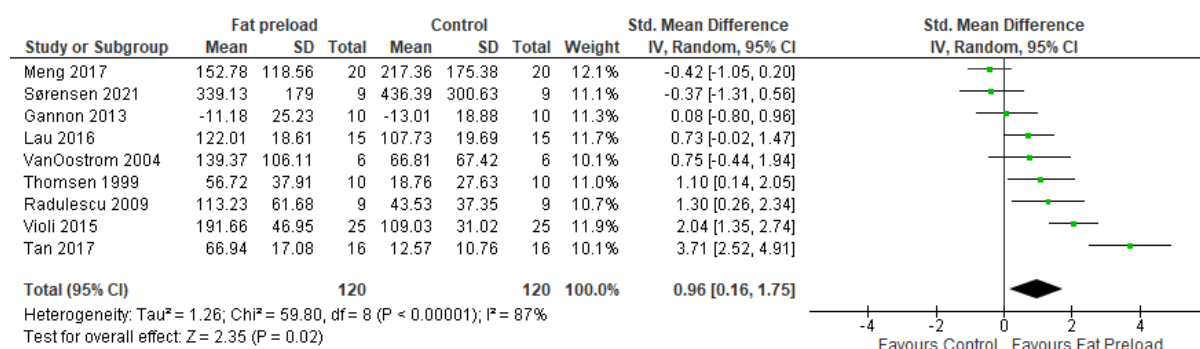


Figure 3.5: Forest plot comparing the change in insulin concentration at 120 minutes on addition of fat to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

Visual inspection of the funnel plot for studies assessing insulin iAUC_{0–120} minutes appeared asymmetrical, with one study (Tan et al. 2017) showing a substantially large negative effect size (228). This suggests potential publication bias; however, the limited number of studies included in funnel plot require cautious interpretation (Appendix 4, Figure 3.6C). Similarly, funnel plot for change in insulin concentration at 120 minutes showed asymmetry, indicative of risk of publication bias (Appendix 4, Figure 3.6D).

3.3.3 Effect of substituting SFA with MUFA on glycaemia

3.3.3.1 Characteristics of studies

Twelve trials (Table 3.3) with 221 participants were included evaluating the effect of SFA to MUFA on glycaemic control. After excluding studies (with no reported data), the mean age of the participants was 31.2 (SD: 6.5) years, 63.8 % were males, mean weight was 74.0 (SD: 9.1) kg and mean BMI was 24.9 (SD: 3.3) kg/m². Mean fasting glucose was 5.03 (SD: 0.4) mmol/L.

Reference	Study design	Sample size	Age, years Mean $\pm$ SD	Sex, Males (n)	Weight, Mean $\pm$ SD	BMI, kg/m ² Mean $\pm$ SD	Glucose, mmol/l Mean $\pm$ SD	Main source of fat		Amount of fat, grams
								SFA	MUFA	
Chang et al. 2018, Malaysia (235)	RCT, XO	30	33.8 $\pm$ 1.7	15	80.3 $\pm$ 2.6	30.9 $\pm$ 0.8	5.7 $\pm$ 0.1	Palm olein	High oleic sunflower oil blended with high linoleic sunflower oil (95:5 ratio)	50.9
Filippou et al. 2014, UK (236)	RCT, XO	50	24.8 $\pm$ 5.5	25	70.9 $\pm$ 11.1	NR	NR	Lard	High oleic sunflower oil	50
Fawad et al. 2020, Sweden (237)	RCT, XO	22	31 $\pm$ 9.5	10	NR	NR	5.2 $\pm$ 0.5	Cream	Olive oil	54
Henry et al. 2008, UK (224)	RCT, XO	15	30.8 $\pm$ 12.1	7	66.9 $\pm$ 9.6	23.4 $\pm$ 4.2	4.55 $\pm$ 0.3	Butter	Olive oil	30 g butter, 24.8g Olive oil
Jenkins et al. 2006, Canada (238)	RCT, XO	15	26.3 $\pm$ 8.6	7	NR	23.4 $\pm$ 3.4	NR	Cheese and butter	Almonds	68g Cheese + 14 g butter, 60g almonds
Lau et al. 2016, Singapore (232)	RCT, XO	15	25.3 $\pm$ 4.6	15	63.5 $\pm$ 5.8	21.2 $\pm$ 1.16	4.16 $\pm$ 0.5	Butter	Olive oil	23.2
Metin et al. 2022, Turkey (239)	RCT, XO	10	33.5 $\pm$ 3.9	10	76 $\pm$ 5.8	24.1 $\pm$ 0.7	4.77 $\pm$ 0.4	Coconut oil	Olive oil	25
Teng et al. 2011, Malaysia (240)	RCT, XO	10	21.9 $\pm$ 0.7	10	NR	21 $\pm$ 1.6	5.2 $\pm$ 0.5	Palm olein	Olive oil	50
Piers et al. 2002, Australia (241)	RCT, XO	14	38 $\pm$ 9	14	89 $\pm$ 13.2	27.8 $\pm$ 3.2	NR	Cream	Olive oil	57 g cream, 20g olive oil
Radulescu et al. 2009, USA (233)	RCT, XO	9-12	25 $\pm$ 5	5	NR	23 $\pm$ 2.5	NR	Lard	Olive oil	25
Thomsen et al. 1999, Germany (234)	RCT, XO	10	23 $\pm$ 2	5	NR	21.4 $\pm$ 1.7	4.4 $\pm$ 0.4	Butter	Olive oil	80
Van Dijk et al. 2012, Netherlands (242)	RCT, XO	18	61.8 $\pm$ 5.9	18	NR	23.8 $\pm$ 0.8	5.2 $\pm$ 0.4	Palm oil	High oleic sunflower oil	95

Table 3.3: Summary of studies evaluating the effect of SFA vs MUFA on glycaemic control. RCT, Randomised Controlled Trial, XO, Cross over, NR, Not reported.

<u>Study ID</u>	<u>D1</u>	<u>DS</u>	<u>D2</u>	<u>D3</u>	<u>D4</u>	<u>D5</u>	<u>Overall</u>
Chang et al. 2018, Malaysia	+	-	+	+	+	+	-
Filippou et al. 2014, UK	+	+	+	+	+	+	+
Fawad et al. 2020, Sweden	!	+	+	+	+	+	!
Henry et al. 2008, UK	+	!	+	+	+	+	!
Jenkins et al. 2006, Canada	+	+	+	+	+	+	+
Lau et al. 2016, Singapore	+	+	+	+	+	+	+
Metin et al. 2022, Turkey	+	+	+	+	+	+	+
Teng et al. 2011, Malaysia	+	!	+	+	+	+	!
Piers et al. 2002, Australia	+	+	+	+	+	+	+
Radulescu et al. 2009, USA	+	!	!	+	+	+	+
Thomsen et al. 1999, Germany	+	+	+	+	+	+	+
Van Dijk et al. 2012, Netherlands	+	+	+	+	+	+	+

Table 3.4: Risk of bias assessment using Cochrane ROB 2.0 tool

+	Low risk	D1 Randomisation process
!	Some concerns	DS Bias arising from period and carryover effects
-	High risk	D2 Deviations from intended interventions
		D3 Missing outcome data
		D4 Measurement of the outcome
		D5 Selection of the reported result

8 studies were deemed at low risk of bias, 3 had some concerns and 1 at high risk of bias (Table 3.4).

3.3.3.2 Outcomes

3.3.3.2.1 Glucose

The pooled analysis comparing glucose iAUC over 120 minutes between MUFA and SFA did not demonstrate a significant difference between interventions (SMD = 0.42, 95% CI: -0.41 – 1.25; $P = 0.32$; $n = 154$; Figure 3.6). There was considerable heterogeneity among studies ($I^2 = 90\%$, $\chi^2 = 67.71$, $df = 7$, $P < 0.00001$).

There was no significant difference in change in glucose concentration at 120 minutes between the two fat types, SFA vs MUFA (SMD = -0.08, 95% CI: -0.26 – 0.11; $P = 0.43$; $n = 218$ in SFA, 221 in MUFA; Figure 3.7). The risk of heterogeneity for glucose change at 120 minutes was low ($I^2 = 0\%$, $\chi^2 = 3.43$, $df = 11$, $P = 0.98$).

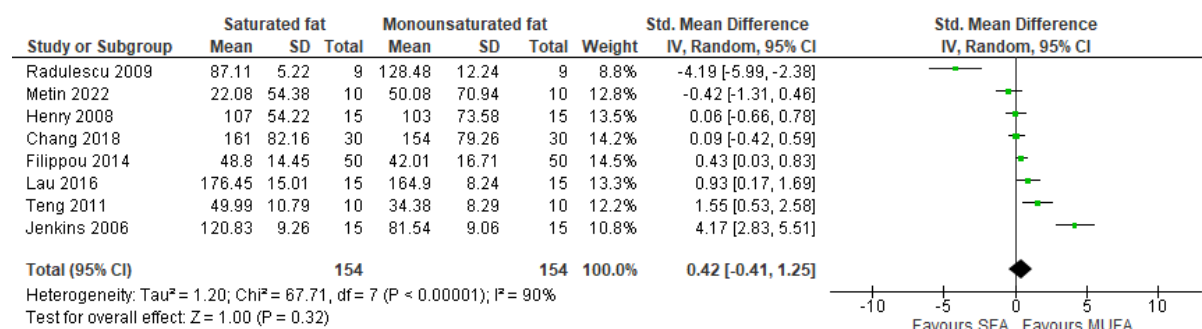


Figure 3.6: Forest plot comparing the iAUC_{0-120 mins} for glucose between SFA and MUFA. Data are presented as a standardized mean difference with 95% confidence intervals. SFA, Saturated fat, MUFA, Monounsaturated Fat.

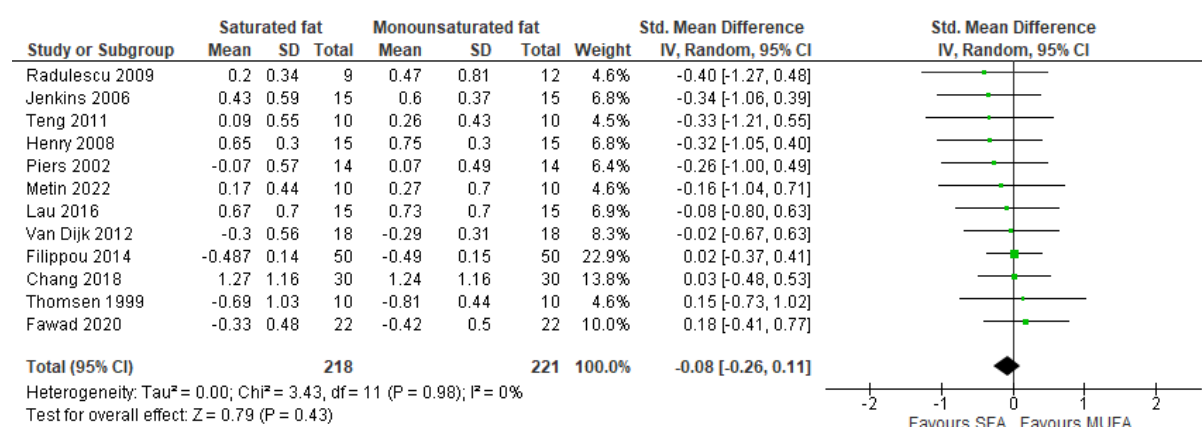


Figure 3.7: Forest plot comparing the change in glucose concentration at 120 minutes

between SFA and MUFA. Data are presented as a standardized mean difference with 95% confidence intervals. SFA, Saturated fat, MUFA, Monounsaturated Fat.

The funnel plot for studies assessing glucose $iAUC_{0-120 \text{ mins}}$ showed asymmetry, with one extreme outlier and an imbalance in the distribution of smaller studies; this may suggest publication bias (Appendix 4, Figure 3.6E). However, the funnel plot for studies evaluating change in glucose at 120 minutes showed a symmetrical distribution of effect sizes around the overall mean asymmetry, suggesting a low risk of publication bias (Appendix 4, Figure 3.6F).

3.3.3.2.2 Insulin

The pooled analysis of studies comparing the effect of SFA against MUFA on $iAUC$ of insulin measured over 120 minutes showed a significantly higher response with SFA (SMD: 1.95, 95% CI: 0.60 – 3.31, $P = 0.005$; $n = 139$ in SFA, 142 in MUFA; Figure 3.8). However, there was considerable heterogeneity among studies ($I^2 = 95\%$ and $\chi^2 = 109.74$, $df = 6$, $P < 0.00001$). Jenkins et al. had a disproportionate influence on the effect estimates owing to its very large effect size. Leave one out sensitivity analysis resulted in a nonsignificant SMD of 0.99 (95% CI: -0.08 – 2.06, $P = 0.07$) and a lowered heterogeneity ($I^2 = 92\%$ and $\chi^2 = 62.95$, $df = 5$, $P < 0.00001$) (Appendix 5, figure 3.7C).

There was no significant difference in change in insulin concentration at 120 minutes between the SFA and MUFA groups (SMD = 0.03, 95% CI: -0.33 – 0.39]; $P = 0.87$; $n = 181$ in SFA and 184 in MUFA; Figure 3.9). Moderate heterogeneity was observed across studies, as indicated by an I^2 of 62% and $\chi^2 = 23.94$ ($df = 9$, $P = 0.004$).

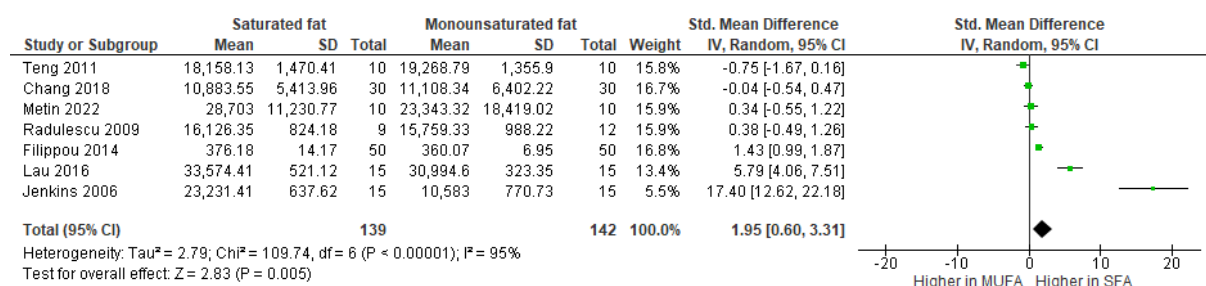


Figure 3.8: Forest plot comparing the iAUC_{0-120 mins} for insulin between SFA and MUFA. Data are presented as a standardized mean difference with 95% confidence intervals. SFA, Saturated fat, MUFA, Monounsaturated Fat.

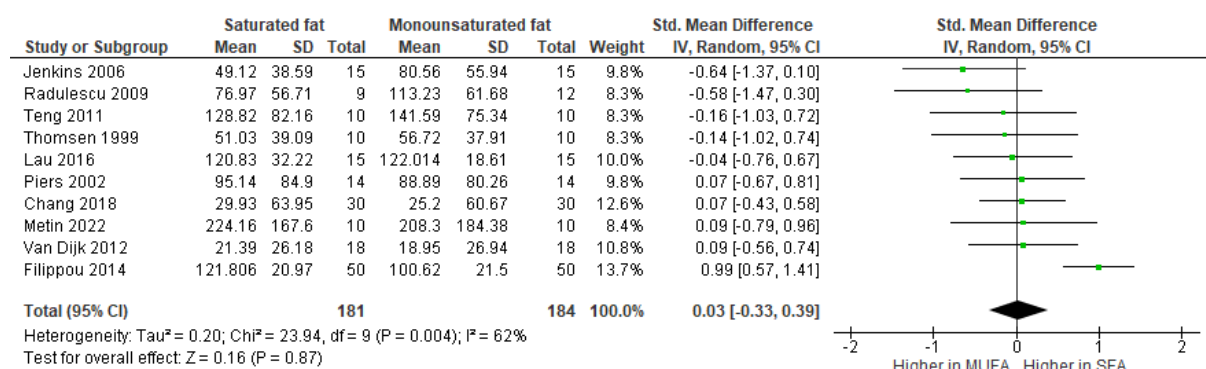


Figure 3.9: Forest plot comparing the change in insulin concentration at 120 minutes between SFA and MUFA. Data are presented as a standardized mean difference with 95% confidence intervals. SFA, Saturated fat, MUFA, Monounsaturated Fat.

Visual inspection of the funnel plot for studies assessing insulin iAUC₀₋₁₂₀ minutes appeared asymmetrical, with multiple small studies showing extreme effect sizes. This suggests potential publication bias; however, the limited number of included studies requires cautious interpretation (Appendix 4, Figure 3.6G). Similarly, the funnel plot for change in insulin concentration at 120 minutes showed asymmetry, indicative of risk of publication bias (Appendix 4, Figure 3.6H).

3.4 Discussion

This systematic review and meta-analysis was aimed at evaluating the acute effects of dietary fat on glycaemic control in healthy adults without diabetes. Two distinct comparisons were explored: (1) the effect of adding fat to a carbohydrate meal, and (2) the impact of substituting SFA with MUFA. Although neither comparison showed a significant effect on postprandial glucose or insulin responses in pooled analyses, there was a trend towards a reduction in glucose excursion over the 2 hours postprandially following fat addition. Furthermore, SFA was shown to induce a notably higher incremental insulin response than MUFA; this should be interpreted with caution given the high degree of heterogeneity across studies.

Our pooled analysis demonstrated a small to moderate but potentially meaningful effect of fat addition to the carbohydrate on postprandial glycaemia over 120 minutes (SMD: -0.39, 95% CI: -0.84 – 0.05). After accounting for fat dose, fat addition was associated with a small-to-moderate reduction in glucose iAUC compared with carbohydrate alone. The glucose lowering observed with fat addition aligns with established mechanisms. Dietary fat slows gastric emptying, thereby delaying the absorption of glucose in the bloodstream (243). Although adjustment for fat dose substantially reduced heterogeneity, residual variability remained, possibly reflecting differences in carbohydrate type, meal form, and study population. The insulin concentration at 120 minutes following fat addition was much higher than for carbohydrate alone, which may reflect the sustained but blunted response to fat administration as observed in previous studies. The blunted postprandial glucose response is often accompanied by a reduced but more sustained insulin response (31). It can also stimulate release of incretin hormones and thus may help potentiate the incretin effect. Dietary fat triggers a longer lasting elevation in the main incretins, GLP-1 and GIP, as compared to carbohydrate (244). In healthy men, Carr et al. demonstrated a significant rise in both incretins, with GIP elevation sustained for

almost 5 hours. This was associated with a mild and delayed insulin rise but without any notable associated change in glucose concentration (211). The insulin response observed in studies in this review was particularly variable; this could be due to differences in fat type, meal composition and baseline insulin sensitivity among participants. Although the difference between the two comparison arms was the fat addition alone, some of the included studies used complex carbohydrate meals which could independently affect glucose and insulin responses. In the study by Tan et al., coconut oil led to a lower postprandial insulin response compared to the carbohydrate control (228). Coconut oil has been linked to a more profound delay in gastric emptying, resulting in slower glucose absorption and thus reduced insulin demand. In addition, the high content of medium-chain triglycerides in coconut oil, which are rapidly absorbed and metabolized differently to long-chain fats, may also blunt insulin secretion (245).

Substituting SFA with MUFA did not significantly affect postprandial glucose (iAUC or 120-minute values), suggesting that this does not acutely alter glucose homeostasis in healthy individuals. Interestingly, SFA intake was associated with a higher postprandial insulin response compared to MUFA, although this finding was primarily driven by one study (Jenkins et al.) with a disproportionately large effect size (238). Removal of this study in sensitivity analysis abolished the difference. The rise in insulin observed post fat intervention followed the rise in glucose in Jenkins and Teng et al. only (238, 240). Jenkins et al used cream and butter as saturated fat mixed with mashed potato as the carbohydrate meal. The presence of two separate saturated fat sources may have influenced insulin resistance. The exaggerated insulin response following SFA may reflect an acute reduction in insulin sensitivity, requiring greater insulin secretion to maintain euglycaemia (103, 246). A single saturated-fat-rich meal has been shown to induce acute insulin resistance, with compensatory insulin secretion to maintain glucose levels (234, 247, 248). The absence of a glucose lowering effect in the pooled analysis despite differing insulin responses may reflect

subtle differences in glucose metabolism that are not readily detectable in normoglycaemic individuals over a short time frame. Other potential explanations for the lack of observed effect could be acute nature of interventions, shorter observation period, relatively small participant numbers in the included studies and variability in the type of fat intervention across studies.

Previous studies have demonstrated a favourable effect of MUFA on metabolic parameters and glucose but this evidence comes from chronic intervention studies or epidemiological data. Qian et al. in their systematic review of 24 studies, in people with T2DM, observed that MUFA intake was associated with a significant reduction in fasting plasma glucose (weighted mean difference -0.57 mmol/L; 95% CI -0.76 , -0.39) following a median intervention duration of six weeks (249). A more recent systematic review by Lytrivi et al, examining the replacement of SFA with MUFA found no significant effects on insulin sensitivity or β -cell function (using disposition index), following a median duration dietary intervention of 4 weeks. There was insufficient data to evaluate the effect of these interventions on glucose tolerance (250). Our review, which focused on acute glucose responses in healthy adults in a much larger number of studies, confirms that short-term substitutions are unlikely to yield large changes in glucose homeostasis.

The main strengths of this systematic review include well defined inclusion criteria for both meta-analyses. Only isocaloric studies with minimal differences in macronutrients were included for fat addition comparisons. While previous analyses have examined the impact of medium to long term dietary fat interventions, this review has investigated the acute effects of a single fat meal on glucose homeostasis.

Our review has some important limitations. Despite extensive search, most included studies were small and may have been underpowered to detect subtle metabolic effects. Variability in fat types, meal composition, and participant characteristics contributed to substantial between-study heterogeneity. Our review has not included the incretin changes which have important mechanistic relevance. Finally, delayed

effects of dietary fat on glycaemia and insulin may not have been captured in this analysis.

In conclusion, addition of fat to a carbohydrate meal may modestly attenuate postprandial glycaemia over the first two hours. Thus, modification of diet to incorporate dietary fat may have therapeutic implications in glucose management in people with glucose intolerance and in early T2DM. Moreover, despite established cardiovascular and metabolic benefits in the long term, MUFA may not confer immediate glucose-lowering benefits in healthy individuals. Future trials with larger sample sizes, robust design and longer observation periods are warranted to ascertain the effect of dietary fat types on glucose homeostasis.

Chapter 4: Investigating the effect of Extra Virgin Olive Oil preload on acute glucose tolerance in healthy volunteers

4.1 Introduction

T2DM is a complex metabolic disorder characterised by insulin resistance and relative insulin deficiency. An early metabolic change in T2DM is the elevation of the postprandial glucose concentrations (261, 262). Elevated postprandial glucose has been linked to increased cardiovascular risk even in individuals without diagnosed diabetes (217, 263). Recently, dietary interventions manipulating macronutrient composition have gained attention for their potential to curb appetite and attenuate postprandial glucose excursions.

Olive oil (OO), a primary source of dietary fat in MedDiet, is notably rich in MUFA, particularly oleic acid. It is widely regarded as a healthier alternative to saturated fats, offering cardiovascular and metabolic health benefits (264, 265). A 2017 systematic review including approximately 16000 individuals with T2DM reported its favourable effects in prevention and management of T2DM; the OO supplementation led to substantial improvements, including reduction of approximately 0.5 mmol/l in fasting glucose levels, a 0.27% decrease in HbA1c and 16% lower risk of T2DM incidence (124). Conversely, Coconut oil, abundant in medium chain saturated fatty acids, has been associated with increased plasma glucose through mechanisms involving reduced insulin sensitivity (245). A recent analysis of three large prospective cohorts examining nearly 150,000 participants demonstrated a risk reduction in future development of type 2 diabetes in those taking MUFA from plant sources (HRs 0.87, 95% CI 0.81, 0.94) but an increased risk of incident diabetes in those using MUFA from animal sources (HR 1.34, 95% CI 1.23, 1.45) (266).

To date, most studies have investigated the effect of chronic administration of these oils on glycaemic control. The acute effect of addition of OO, or its inclusion as a meal preload, on glucose homeostasis remains underexplored. The glucose lowering effect

of dietary fat added to a carbohydrate meal may partly be mediated via its incretin action. In fact, similar postprandial GLP-1 concentrations were reported after isocaloric intake of glucose or dietary fat (267). OO has been shown to induce greater GLP-1 and GIP responses compared to butter. Interestingly, despite differential incretin responses in this study, the insulin and glucose concentrations following both fats were similar (234). In contrast, Gatti et al observed substantially lower postprandial glucose levels with olive and corn oils compared to butter consumption, without changes in insulin concentrations in healthy volunteers (268).

NT, an upper GI peptide, is secreted in response to the dietary fat and promotes fat absorption in the small intestine. Among various nutrients, oleic acid strongly stimulates NT secretion (145). We hypothesised that an OO preload would lower glucose tolerance by triggering NT secretion, and thus augmenting insulin secretion. This glucose-lowering effect of OO was anticipated to be superior to that of a coconut oil preload, which predominantly contains saturated fatty acids and thus may be less effective.

4.2 Aims and objectives

Before examining the effect of EVOO preload on acute glucose tolerance, it was necessary to establish an EVOO dose that reliably stimulates NT secretion while remaining tolerable. Thus, a pilot dose-finding study was undertaken to inform the design of the subsequent intervention study. The aim of the intervention study was to investigate the effect of extra virgin olive oil (EVOO) preload on acute glucose tolerance in healthy volunteers.

4.3 Study Methods

4.3.1 Study setting

The study was performed at Imperial College London Diabetes Centre (ICLDC) in the Emirate of Abu Dhabi, UAE. For pilot study, the participants were recruited between 07/03/2021 and 13/06/2021. For intervention study, participants were recruited between 21/01/2022 and 23/10/2023. Further details on study setting have been previously described in chapter 2 (section 2.2.4).

4.3.2 Study design

The pilot dose titration study was carried out using a cross-over design investigating the effects of increasing doses of EVOO on plasma NT in healthy volunteers.

The main intervention study was also a crossover design involving two visits. The ethical approval of the study was granted by the ICLDC Research and Ethics Committee (ref: IREC 058, substantial amendment approved on 17/06/2022). Written informed consent was obtained from all study participants. The study complied with the ethical principles of the Declaration of Helsinki 2008.

4.3.2.1 Study participants

Study volunteers were recruited from ICLDC by convenience sampling. These were members of staff or their acquaintances. Participants who expressed an interest in taking part in the study were provided with the Participant Information Sheet and consent form (Appendix 7).

Following consent, volunteers were screened using a questionnaire (Appendix 8) on their previous medical history and current health; their weight and height were measured to calculate BMI and a blood sample was obtained for full blood count,

fasting glucose, liver function test, urea and electrolytes, lipid profile and HbA1c. Volunteers were also asked screening questions on their current olive oil intake and supplement use. People consuming ≥ 4 tablespoons of olive oil in a given day were considered to be taking a substantial amount and were thus excluded from the study. This was based on the control and intervention arms of PREDIMED trial (269). The information collected from the study visit was reviewed by me to either approve or disapprove eligibility for participation.

4.3.2.2 Inclusion criteria

- Sex: male or female
- Age: >18 years
- BMI $>18\text{kg/m}^2$ who are otherwise healthy, based on a pre-study assessment
- Ability to give written informed consent
- No previous history of diabetes, fasting glucose < 6.1 mmol/l and HbA1c < 42 mmol/mol

4.3.2.3 Exclusion criteria

- Previous bariatric or metabolic surgery
- Any form of Diabetes
- Intolerance to edible oils
- Current pregnancy or breast feeding
- Participants on regular supplement that contains olive oil (or consuming ≥ 4 tablespoon of olive oil daily)
- Significant intercurrent disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease
- History of cancers
- Regular intake of over-the-counter medication

4.3.2.4 Withdrawal criteria

- Significant deviation from the approved protocol
- Participant no longer satisfying the inclusion and exclusion criteria set out by the approved protocol
- A positive pregnancy test result
- Participant withdrawing consent

4.3.3 Study visit outline

4.3.3a) Pilot Dose-Finding Study to inform EVOO dose selection

After an overnight fast (no food after 10 pm), subjects arrived at the clinical research unit at 8 am on the following morning and were provided with an antecubital vein catheter. After taking baseline blood sample, a pre-specified dose of commercially available EVOO (Carrefour Bio Huile d'olive, Tunisia) was ingested rapidly by the participant. The EVOO dose was 10mls to 40mls (in 10 mls increment) on different study days. Further blood samples (10 mls) were obtained throughout a subsequent 120-min study period (figure 4.1). At each study time point, an additional 0.6µl volume blood sample was obtained to measure blood glucose using an Accu-Chek Performa glucometer (Roche, Mannheim, Germany). This was to ensure participant safety by allowing rapid detection and treatment in the event of hypoglycaemia. Throughout the study, the participants remained resting (sitting or lying down) and were asked not to eat anything during the experiment but were able to drink water. There was an interval of at least 7 days between study days in a given participant. A schematic representation of the study day is shown in Figure 4.1:

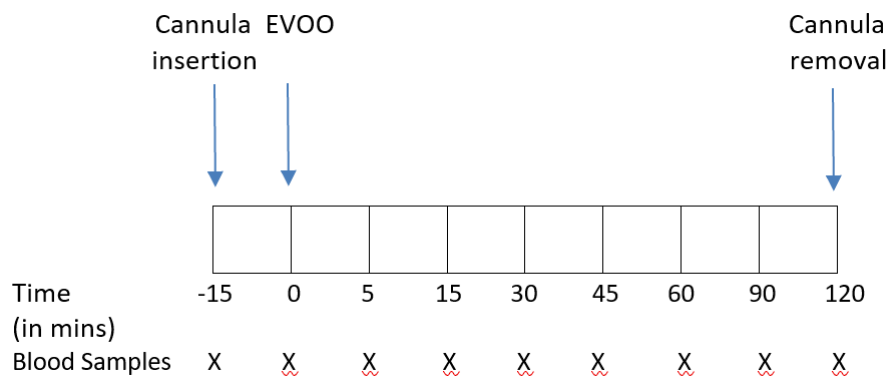


Figure 4.1: Schematic representation of the study day

4.3.3b) Main intervention Study

Study volunteers visited on two study days, separated by at least a 1-week washout period to minimize any carryover effects. After an overnight fast (no food after 10 pm), participants attended the clinical research unit at 8 am on the following morning. An antecubital vein catheter was inserted and a baseline blood sample (time – 15 min) was obtained. Following this, 20 millilitres (ml) of commercially available EVOO (Carrefour Bio Huile d'olive, Tunisia) or Coconut oil (Parachute Pure, India) was ingested by the participant. Participants were asked to take either 75 gram glucose orally (Trutol®, Thermo Fisher Scientific™, USA) or infused intravenous glucose 300mg/kg body weight (Gulf inject LLC, Dubai, UAE) as per study protocol. Further blood samples were obtained throughout a subsequent 120-min study period (figure 5.1). Capillary glucose check and participant study conditions were as described earlier in section 4.3.3a

Subjective ratings of the food palatability, nausea, appetite, and other symptoms were quantified from visual analogue scale (VAS) questions (Appendix 9). The VAS scores were calculated using a ruler measuring the distance in millimetres from the far end of the 100mm horizontal line up to the vertical marking made by the participant.

4.3.4 Intravenous glucose tolerance test

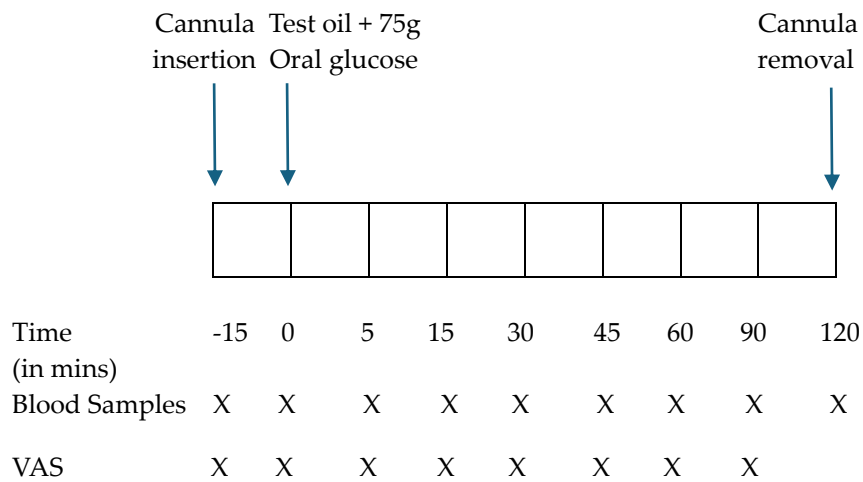
The oral glucose administration triggers substantial GLP-1 and GIP mediated incretin effect. This may mask the action of other gut peptides such as NT on glucose tolerance and insulin secretion. Insulin resistance and secretion are often measured using a variety of methods including the clamp studies and the frequently sampled intravenous glucose tolerance test (IVGTT) with minimal model analysis (270). However, these methods are often cumbersome to perform and require complex mathematical modelling.

A simplified version of the IVGTT has been tested and validated against these methods in healthy volunteers (271). The test is performed as a single rapid bolus primarily to evaluate first and second phase insulin secretion. A glucose bolus (usually 20 – 25% concentration) is injected at 300 mg / kg body weight in a contralateral vein using a large volume syringe. Blood samples are obtained at frequent intervals following glucose injection (272).

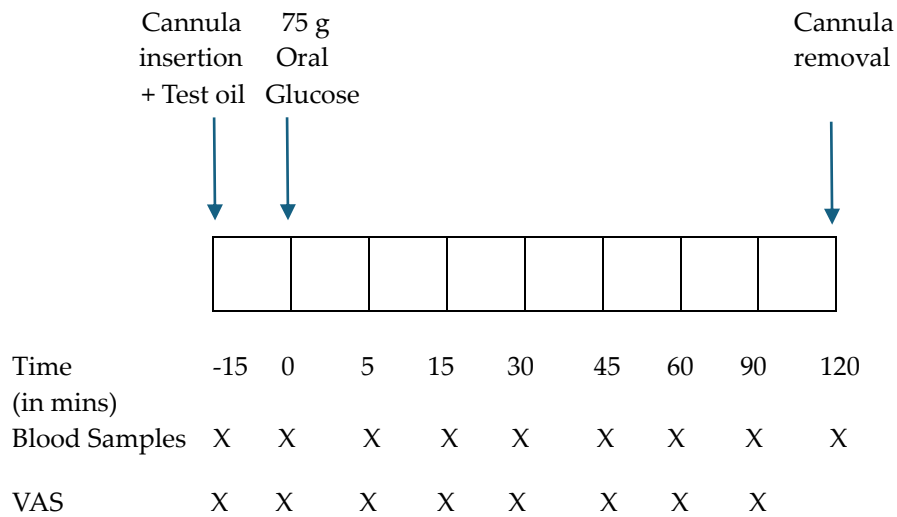
4.3.5 Experimental procedures

Three experimental protocols were examined in the participating subjects (figure 4.2). In all three protocols, baseline blood samples were obtained first after cannula insertion. In protocol 1, at time 0 mins, 75g oral glucose was administered immediately (in less than 2 minutes) after the ingestion of the test oil. In protocol 2, test oil was ingested at time – 15 mins and 75 g oral glucose was administered at time 0 mins. In protocol 3, test oil was administered at time – 15 mins and intravenous glucose bolus was administered at 300 mg / kg body weight in less than 2 minutes through a wide bore cannula in the contralateral antecubital vein.

Protocol 1



Protocol 2



Protocol 3

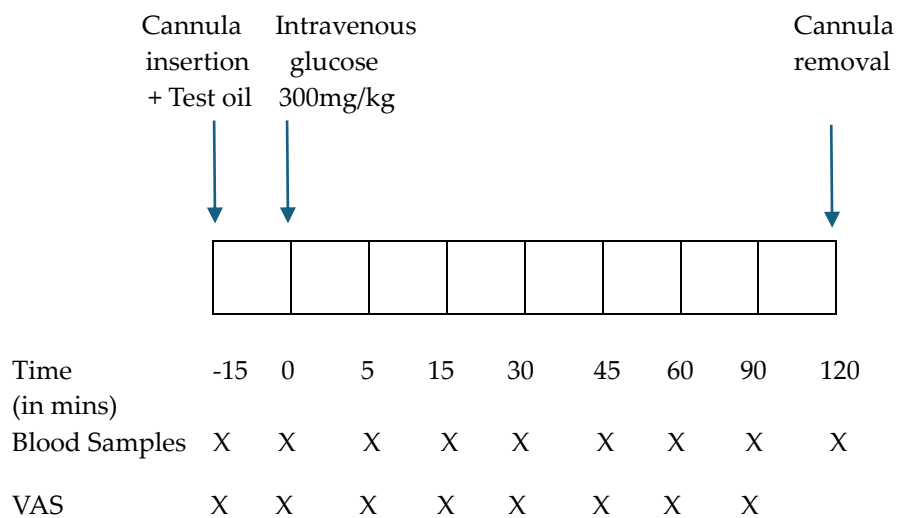


Figure 4.2: Schematic representation of the three protocols. VAS, Visual Analogue Scale

4.3.6 Sample collection and analysis

In pilot study, blood samples were collected for plasma NT. In intervention study, venous blood samples for plasma NT, GLP-1 and PYY were collected at each study time point. A 6 ml blood sample was collected in an EDTA coated tube and was centrifuged at 4000 rpm for 20 mins at 4°C within twenty minutes of collection. Plasma was separated and stored at -80°C. The gut peptides were measured using an in-house radioimmunoassay as described previously in Chapter 2 (section 2.2.4, Appendix 1). Free fatty acid samples were obtained at selected time points (-15 min, 15 min and 30 min).

Plasma Proneurotensin/Neuromedin N (Pro-NT/NMN) was measured in selected samples using a competitive enzyme immunoassay (ELISA) kit (catalogue number abx258978, Abbexa Ltd., Cambridge, UK) as per the manufacturer's instructions (251).

Serum glucose were measured using the automated Cobas® 8000 analyser (Roche, Basel, Switzerland). Serum insulin and C-peptide were measured by the Electrochemiluminescence Immunoassay (ECLIA) method using a Cobas ® C6000 platform (Roche, Basel, Switzerland). Lipid profile was measured by the enzymatic colorimetric method using a Cobas ® C8000 platform (Roche, Basel, Switzerland). In intervention study, Free Fatty acid Comprehensive Profile C8 – C26 was measured using Gas Chromatography-Mass Spectrometry Stable Isotope Dilution Analysis (Mayo Clinic, Rochester, USA).

4.3.7 Sample size calculation

The sample size was not calculated for the pilot study due to its exploratory nature.

Violi et al have investigated the effect of 10g of EVOO (compared against corn oil) on 2 hour postprandial glucose in healthy subjects; they found a mean glucose difference of 1.19 mmol/l and standard deviation of the paired difference of 0.66 mmol/l (127).

Considering the cross-over design, I computed the minimum sample size using a two-tailed paired Student's t-test. This accounted (a) a minimum detectable glucose variation between EVOO and coconut oil $|\delta|$ 0.5 mmol/l, (b) standard deviation of the paired differences SD = 0.6 mmol/l, (c) type I error probability $\alpha = 0.05$ and power $1 - \beta = 0.80$. This gave a minimum sample size of 14 participants for the two-treatment crossover study design in the intervention studies (273, 274).

4.4 Statistical Methods

Data are presented as mean (standard deviation, SD or standard error of mean, SEM) or median (interquartile range, IQR). The Shapiro–Wilk test and graphical analysis of histogram and QQ plots were used to assess data distribution. Total area under the curve (AUC) was calculated from – 15 mins to 120 mins using the trapezoidal rule (252). Incremental and decremental area under the curve, defined as the net AUC above or below fasting level (-15 mins), were similarly calculated using the trapezoidal rule. For pilot study analysis, repeated measures ANOVA was used for the estimation of differences in response to incremental doses of EVOO; pairwise comparisons were carried out using a Bonferroni multiple comparison post hoc test. For intervention study, two-way repeated measures ANOVA with Tukey's multiple comparison post hoc test was used for the estimation of differences in response to test oil; a linear mixed effects model was used when data had >1% missing values. Mean imputation for randomly missing values (<1% of total data) was performed by replacing missing values with the mean value, calculated using all relevant observations. An alternative analysis using the mixed-effects model confirmed no significant deviations in results. Paired t test was used to estimate differences in AUC. Statistical significance was assessed at the level of $P < 0.05$. All statistical analyses were performed using Prism 9.1 (GraphPad Software, San Diego, CA, USA).

4.5 Results

4.5.1 Pilot Study

4.5.1.1 Study population characteristics

7 healthy volunteers with BMI 21.03 – 28.06 kg/m² [mean($\pm$ SD) 23.50 ($\pm$ 2.33)] and age 28–40 years [mean($\pm$ SD) 34.14 ($\pm$ 3.64)] participated in the study. 5 participants received 10 – 40mls of EVOO whereas 2 participants received 30 and 40mls only. Their baseline characteristics at the time of recruitment are shown in Table 4.1.

Table 4.1: Clinical Characteristics of the population	
Age (years)	34.14 ($\pm$ 3.64)
Males (n)	3
Weight (kg)	64.75 ($\pm$ 14.5)
BMI (kg/m ²)	23.50 ($\pm$ 2.33)
Waist circumference (cm)	82.14 ($\pm$ 9.12)
Systolic blood pressure (mmHg)	121.4($\pm$ 5.13)
Diastolic blood pressure (mmHg)	70.14 ($\pm$ 6.84)
HbA1c (mmol/mol)	33.71 ($\pm$ 6.1)
Fasting serum glucose (mmol/l)	5.1 ($\pm$ 0.56)
Total Cholesterol (mmol/l)	4.88 ($\pm$ 1.11)
Triglycerides (mmol/l)	0.87 ($\pm$ 0.33)
Data are reported as mean $\pm$ SD	

4.5.1.2 Effect of incremental doses of EVOO on gut hormones

4.5.1.2a Neurotensin (NT)

Fasting plasma NT did not differ in participants on different study days [mean ($\pm$ SD) on each EVOO dose: 10 mls 26.45 pmol/l ($\pm$ 22.45), 20 mls 22.04 pmol/l ($\pm$ 3.98), 30 mls 23.97 pmol/l ($\pm$ 15.37), 40 mls 30.04 pmol/l ($\pm$ 13.08), P = 0.68]. Following EVOO ingestion, NT increased from baseline and reached its peak at 60 minutes (after 10 and 20 mls) and 90 minutes after 30 mls (figure 4.3 (a) and (c)). NT remained elevated at 120 minutes. There was minimal NT change with the 40 mls dose of EVOO in

comparison to 10 – 30 mls over the 120 minutes study period. The total and incremental AUC of NT on 10 – 30 mls of EVOO were not statistically significant (figure 4.3 (b) and (d), total AUC $P = 0.24$, incremental AUC $P = 0.33$). However, incremental AUC of NT on 20mls was significantly higher than on 40mls dose (mean difference 1698 pmol/l x min, $P = 0.02$).

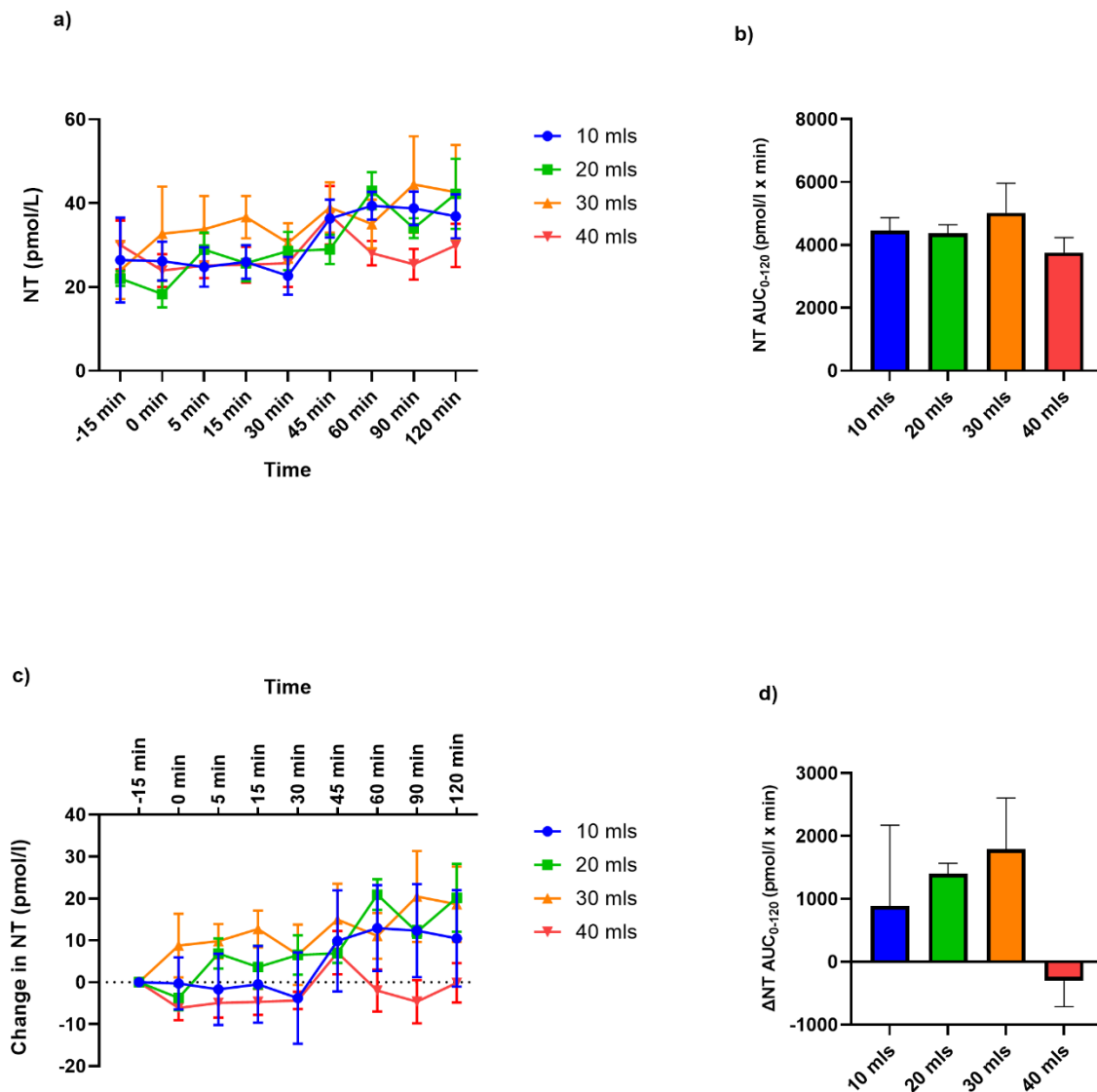


Figure 4.3. Plasma NT concentrations measured over 120 minutes following consumption of 10 – 40 mls dose of EVOO: a) absolute NT concentration, b) area under the curve of absolute NT concentration, c) change in NT concentration from baseline

and d) area under the curve of change in NT concentration from baseline. $n = 5$. Data presented as mean $\pm$ SD.

4.5.1.2b ProNeurotensin/Neuromedin N (Pro-NT/NMN)

On 10mls and 20mls doses of EVOO, Pro-NT/NMN was measured at 0, 15, 30 and 60 mins. Pro-NT at time 0 did not differ in participants on different study days [mean ($\pm$ SD) on each EVOO dose: 10 mls 167.3 pg/ml ($\pm$ 12.48), 20 mls 155.3 pg/ml ($\pm$ 11.26), $P = 0.166$]. Following EVOO ingestion, Pro-NT initially dropped at 15 mins but subsequently rose by 30 mins. On 20mls EVOO, there was a trend towards rise in Pro-NT at 30 and 60 mins (figure 4.4 (a) and (c)). The total and incremental AUC of Pro-NT on 10 and 20 mls of EVOO were not statistically significant (figure 4.4 (b) and (d), total AUC $P = 0.57$, incremental AUC $P = 0.33$).

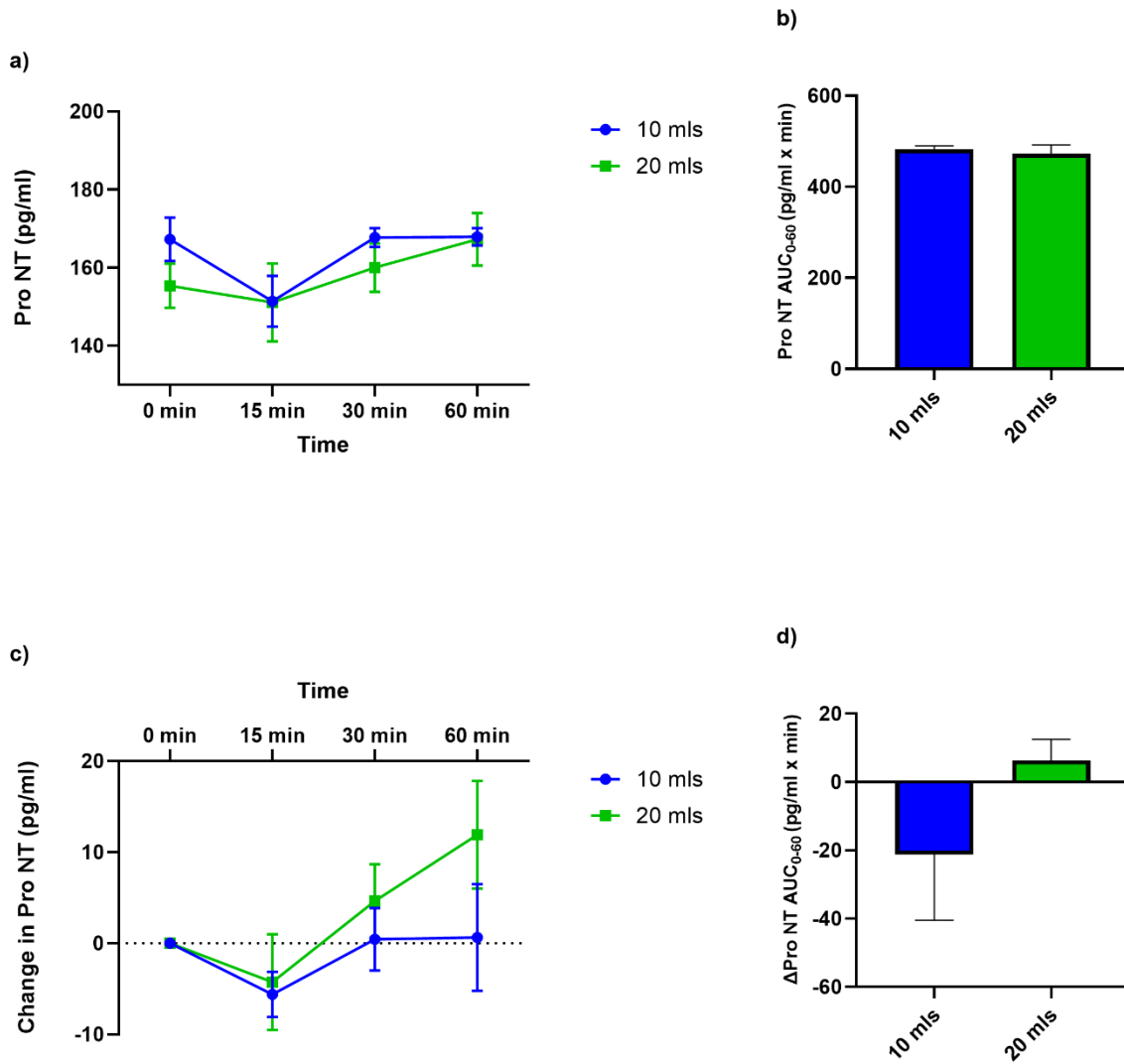


Figure 4.4. Plasma Pro-NT concentrations measured over 60 minutes following consumption of 10 and 20 mls dose of EVOO: a) absolute Pro-NT concentration, b) area under the curve of absolute Pro-NT concentration, c) change in Pro-NT concentration from baseline and d) area under the curve of change in Pro-NT concentration from baseline. $n = 4$. Data presented as mean $\pm$ SD.

4.5.1.3 Effect of incremental doses of EVOO on glucose, insulin and c-peptide

4.5.1.3a Glucose

Fasting serum glucose did not differ in participants on different study days [mean ($\pm$ SD) on each EVOO dose: 10 mls 5.12 mmol/l ($\pm$ 0.6), 20 mls 5.14 mmol/l ($\pm$ 0.4), 30 mls 4.96 mmol/l ($\pm$ 0.5), 40 mls 4.83 mmol/l ($\pm$ 0.4), $P = 0.56$]. There was significant effect of time ($P < 0.0001$) but not EVOO dose ($P = 0.4721$) on serum glucose concentrations (figure 4.5 (a) and (c)). The total and decremental AUC of serum glucose on 10 – 40 mls of EVOO were not statistically significant (figure 4.5 (b) and (d), total AUC $P = 0.35$, decremental AUC $P = 0.43$).

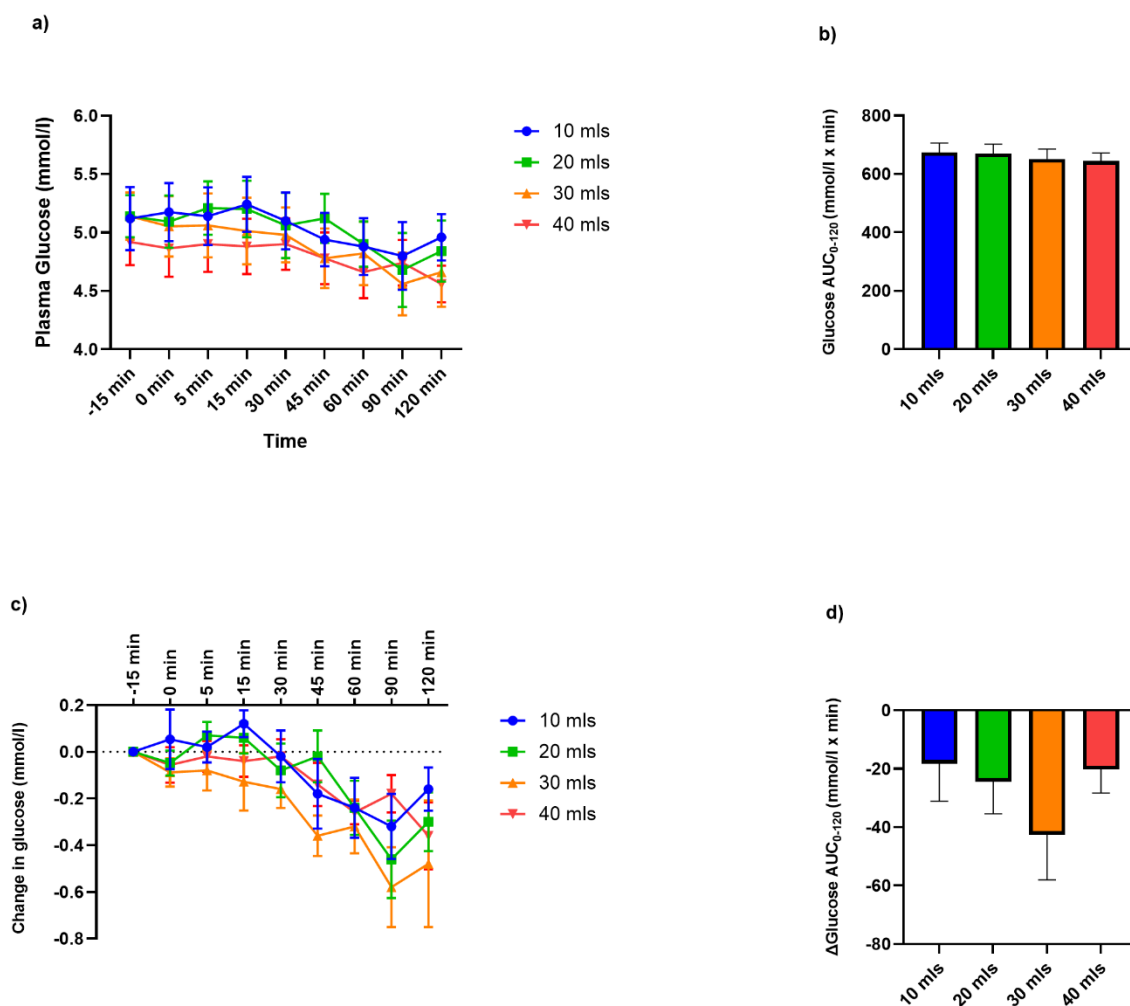


Figure 4.5. Plasma glucose concentrations measured over 120 minutes following consumption of 10 - 40 mls dose of EVOO: a) absolute glucose concentration, b) area

under the curve of absolute glucose concentration, c) change in glucose concentration from baseline and d) area under the curve of change in glucose concentration from baseline. $n = 5$. Data presented as mean $\pm$ SD.

4.5.1.3b Insulin

Fasting serum insulin did not differ in participants on different study days [mean ($\pm$ SD) on each EVOO dose: 10 mls 112.8 pmol/l ($\pm$ 67.94), 20 mls 100.1 pmol/l ($\pm$ 60.09), 30 mls 106.4 pmol/l ($\pm$ 54.81), 40 mls 87.88 mmol/l ($\pm$ 44.24), $P = 0.63$]. There was no significant effect of EVOO dose on serum insulin concentration. The total and net AUC of serum insulin on 10 – 40 mls of EVOO were not statistically significant (figure 4.6 (a) to (d), total AUC $P = 0.79$, net AUC $P = 0.61$).

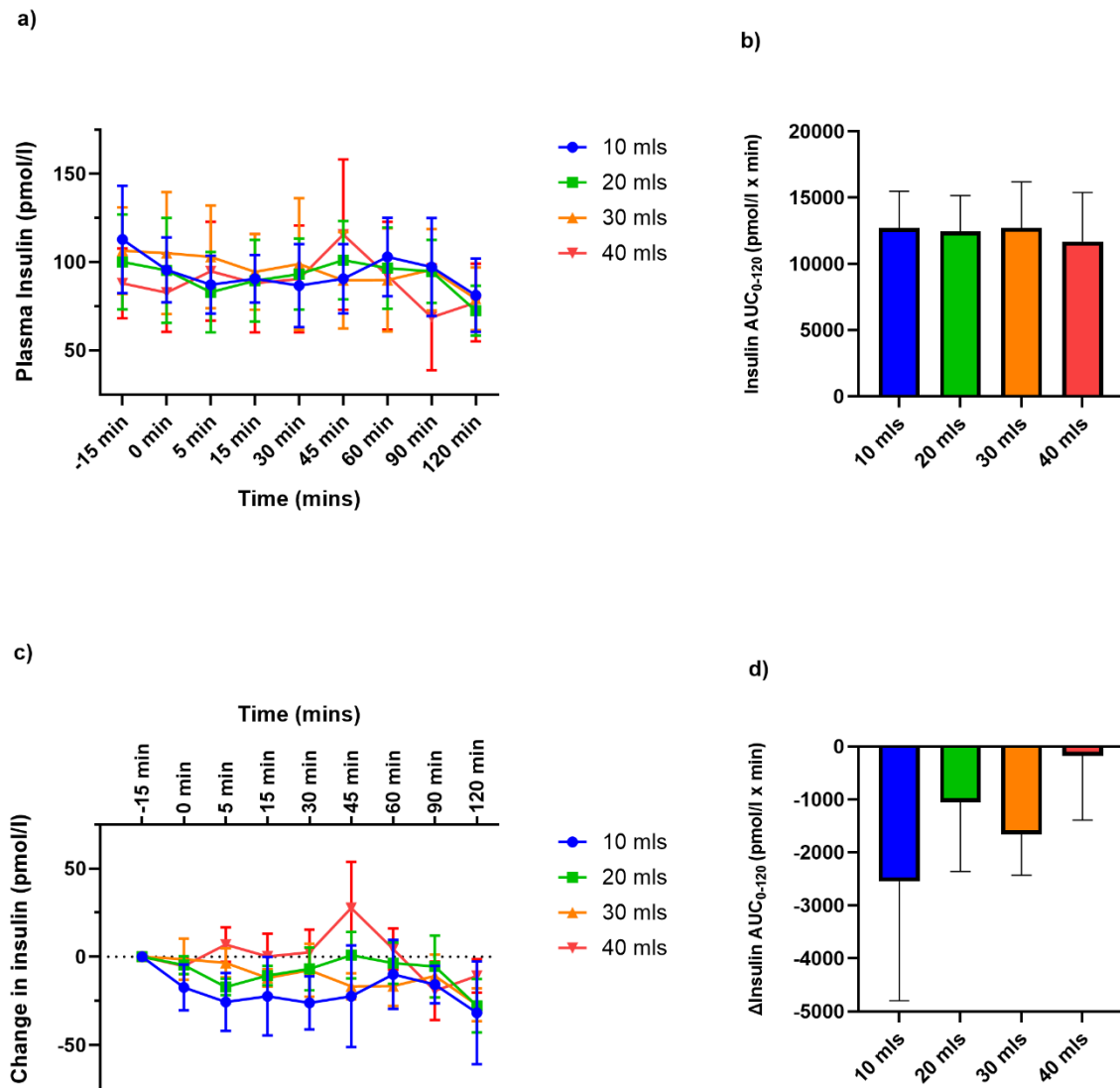


Figure 4.6. Plasma insulin concentrations measured over 120 minutes following consumption of 10 - 40 mls dose of EVOO: a) absolute insulin concentration, b) area under the curve of absolute insulin concentration, c) change in insulin concentration from baseline and d) area under the curve of change in insulin concentration from baseline. $n = 5$. Data presented as mean $\pm$ SD.

4.5.1.3c C-peptide

There was no significant effect of EVOO dose on serum C peptide concentration. The total and net AUC of serum C peptide on 10 – 40mls of EVOO were not statistically significant (figure 4.7, total AUC $P = 0.59$, net AUC $P = 0.70$).

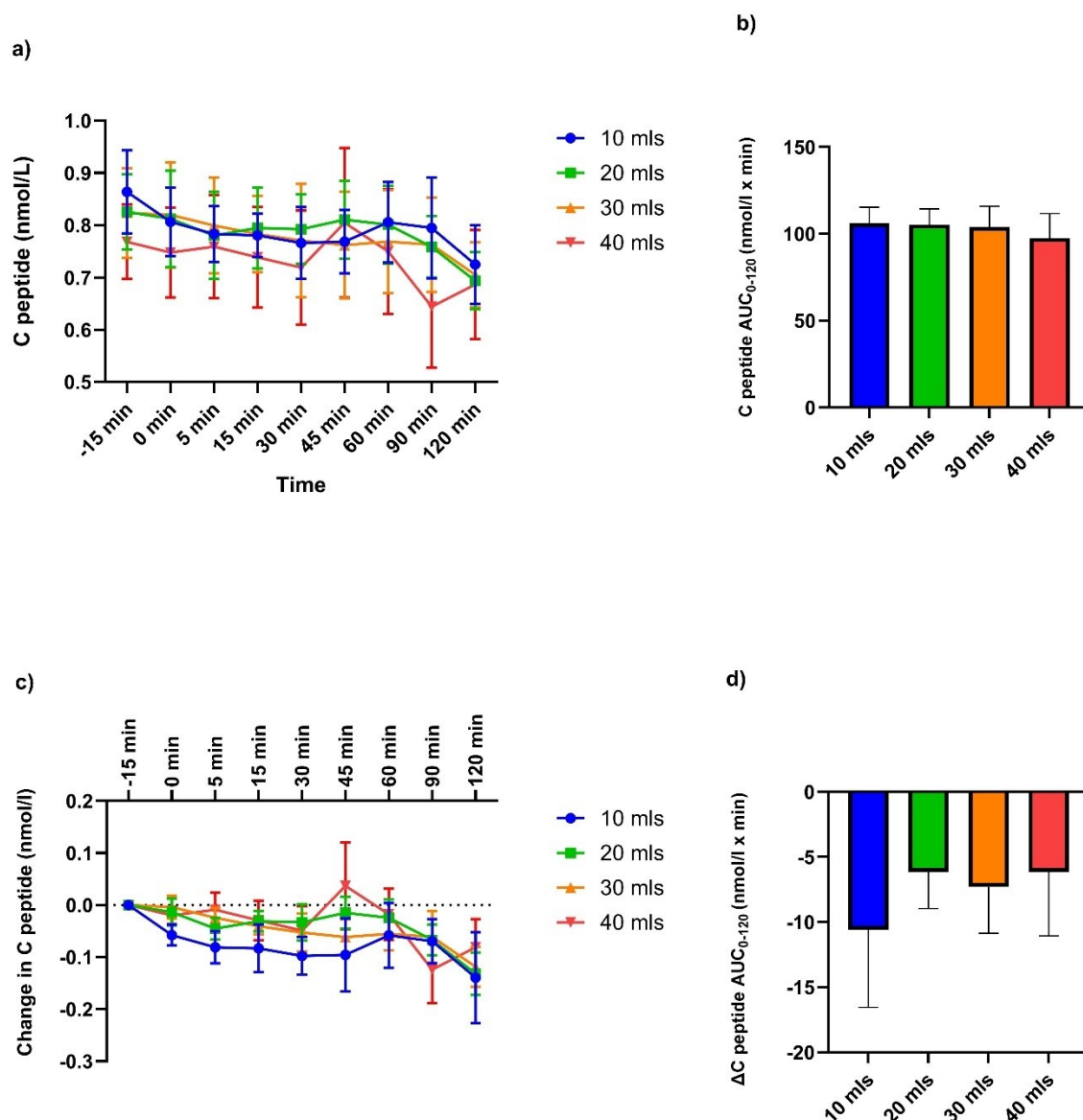


Figure 4.7. Plasma C-peptide concentrations measured over 120 minutes following consumption of 10 - 40 mls dose of EVOO: a) absolute C-peptide concentration, b) area under the curve of absolute C-peptide concentration, c) change in C-peptide

concentration from baseline and d) area under the curve of change in C-peptide concentration from baseline. $n = 5$. Data presented as mean $\pm$ SD.

4.5.1.4 Adverse events

No major adverse events were reported. However, most participants ($n = 5$) preferred the EVOO dose of up to 20 mls due to reduced palatability at higher doses.

4.5.2 Main intervention studies

4.5.2.1 Participant recruitment and baseline characteristics

61 volunteers were screened for participation in the study. Of those screened, 38 participants were recruited. A total of 24 participants completed both study visits.

The reasons for the remaining participants who were recruited but not completed the study are given below:

- 10 participants dropped out after completing study visit 1
- 1 participant felt unwell during visit 1 and withdrew consent
- 3 participants were noted to have diabetic range hyperglycaemia in the study visit 1 and were not asked to attend for visit 2

Only the data of the 24 participants who completed both study visits is included in this thesis. The baseline characteristics of the included participants are presented in Table 4.2.

Table 4.2 Demographic and Clinical Characteristics of the participants	
Age (years)	37.17 ($\pm$ 4.93)
Males – n (%)	15 (62.5)
Ethnic group – n (%)	
Asian (Filipino)	10 (41.67)
Asian (South Asian)	7 (29.16)

Asian (Arab)	6 (25)
Caucasian	1 (4)
Weight (kg)	71.25 ($\pm$ 11.58)
BMI (kg/m ²)	25.56 ($\pm$ 3.17)
Waist circumference (cm)	87.83 ($\pm$ 8.09)
Systolic blood pressure (mmHg)	122.6 ($\pm$ 8.58)
Diastolic blood pressure (mmHg)	74.17 ($\pm$ 7.20)
HbA1c (mmol/mol)	35.79 ($\pm$ 4.12)
Fasting serum glucose (mmol/l)	5.26 ($\pm$ 0.34)
Total Cholesterol (mmol/l)	5.05 ($\pm$ 0.86)
Triglycerides (mmol/l)	1.57 ($\pm$ 0.80)
Calculated LDL (mmol/l)	3.34 ($\pm$ 0.89)
HDL (mmol/l)	1.30 ($\pm$ 0.38)
Data are reported as mean $\pm$ SD or n (%). BMI, Body Mass Index; LDL, Low density lipoprotein; HDL, High density lipoprotein.	

4.5.2.2 Effect of test oil on glucose, insulin, c-peptide and lipid profile

4.5.2.2.1 Protocol 1: Effect of test oil administration immediately before oral glucose (at time 0)

15 volunteers completed the two study visits in this protocol.

(i) Glucose: Fasting serum glucose did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 5.27 mmol/l ($\pm$ 0.06) vs CO: 5.22 mmol/l ($\pm$ 0.08), $P = 0.32$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.76$) on serum glucose concentrations. The total and net AUC of serum glucose to test oils were not significantly different (figure 4.8 (a) to (d), total AUC $P = 0.49$, net AUC $P = 0.93$).

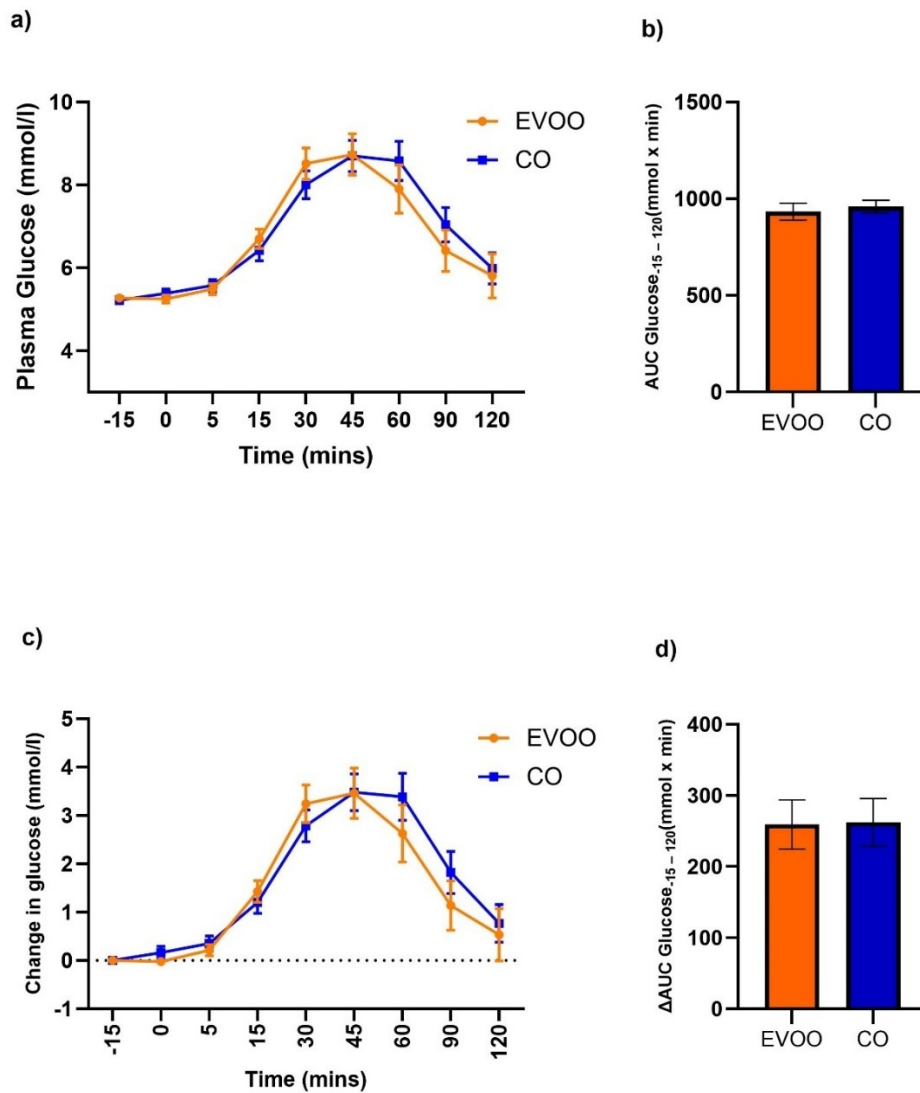


Figure 4.8. Plasma glucose measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute glucose concentration, b) area under the curve of absolute glucose concentration, c) change in glucose concentration from baseline and d) area under the curve of change in glucose concentration from baseline. $n = 15$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) Insulin: Fasting serum insulin did not differ in participants on different study days [mean ($\pm$ SEM) on EVOO: 89.94 pmol/l ($\pm$ 12.41), vs CO: 76.12 pmol/l ($\pm$ 8.31), $P = 0.15$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.97$) on serum

insulin concentrations. The total and net AUC of serum insulin to test oils were not significantly different (figure 4.9 (a) to (d), total AUC $P = 0.77$, net AUC $P = 0.57$).

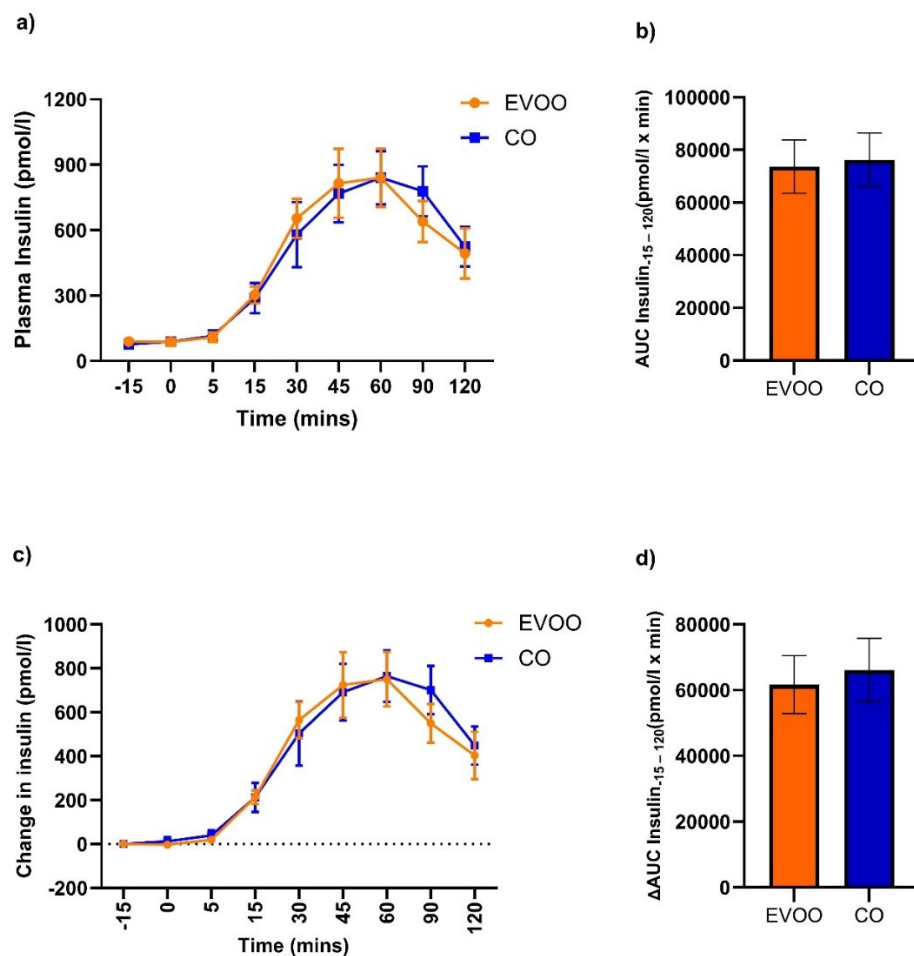


Figure 4.9. Plasma insulin measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute insulin concentration, b) area under the curve of absolute insulin concentration, c) change in insulin concentration from baseline and d) area under the curve of change in insulin concentration from baseline. $n = 15$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) C-peptide: Fasting serum c-peptide did not differ in participants on different study days [mean ($\pm$ SEM) on EVOO: 0.91 nmol/l ($\pm$ 0.08), vs CO: 0.88 nmol/l ($\pm$ 0.08), $P = 0.43$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.90$) on

serum c-peptide concentrations. The total and net AUC of serum c-peptide to test oils were not significantly different (figure 4.10 (a) to (d), total AUC $P = 0.66$, net AUC $P = 0.53$).

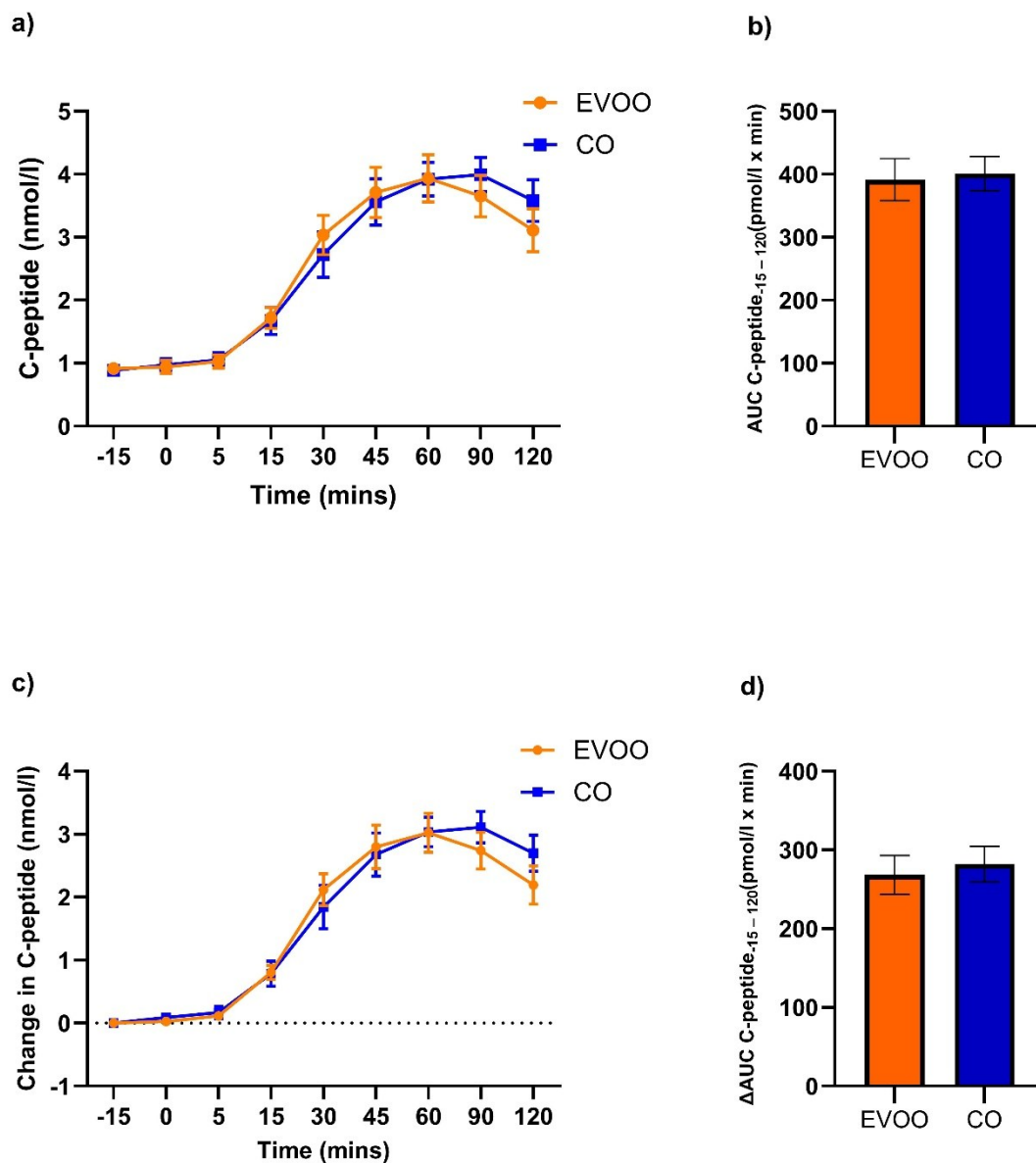


Figure 4.10. Plasma C-peptide measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute C-peptide concentration, b) area under the curve of absolute C-peptide concentration, c) change in C-peptide concentration from baseline and d) area under

the curve of change in C-peptide concentration from baseline. $n = 15$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iv) Lipid profile: There was significant effect of time ($P = 0.02$) but not test oil on change in serum triglyceride levels (figure 4.11 (a) and (b)). The changes in serum total cholesterol, HDL and calculated LDL were not significantly different following test oils (data not shown).

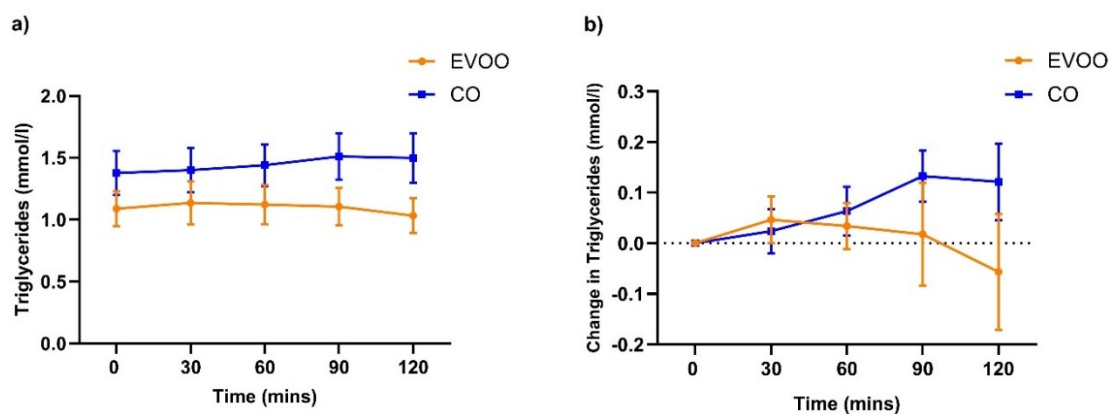


Figure 4.11. Plasma triglycerides measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute triglyceride concentration, b) change in triglycerides from baseline. $n = 15$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.2.2 Protocol 2: Effect of test oil preload 15 minutes before oral glucose

9 participants completed the two study visits in this protocol.

(i) Glucose: Fasting serum glucose did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 5.34 mmol/l ($\pm$ 0.16) vs CO: 5.57 mmol/l ($\pm$ 0.13), $P = 0.15$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.51$) on serum glucose concentrations. The total and net AUC of serum glucose to test oils were not significantly different (figure 4.12 (a) to (d), total AUC $P = 0.94$, net AUC $P = 0.58$).

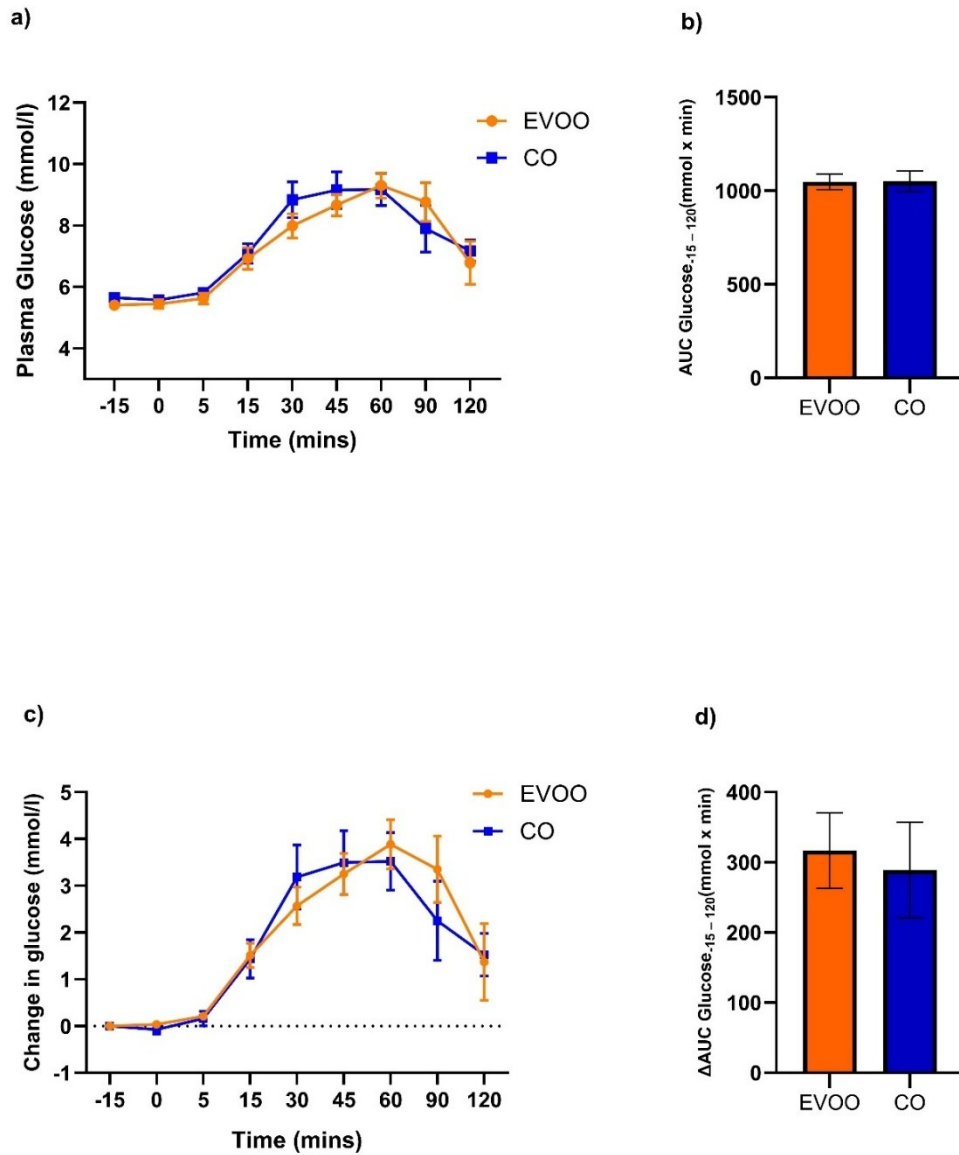


Figure 4.12. Plasma glucose measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute glucose concentration, b) area under the curve of absolute glucose concentration, c) change in glucose concentration from baseline and d) area under the curve of change in glucose concentration from baseline. $n = 9$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) Insulin: Fasting serum insulin did not differ in participants on different study days [mean ($\pm$ SEM) on EVOO: 65.01 pmol/l ($\pm$ 9.95), vs CO: 81.75 pmol/l ($\pm$ 13.45), $P = 0.16$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.97$) on serum insulin concentrations. The total and net AUC of serum insulin to test oils were not significantly different (figure 4.13 (a) to (d), total AUC $P = 0.70$, net AUC $P = 0.52$).

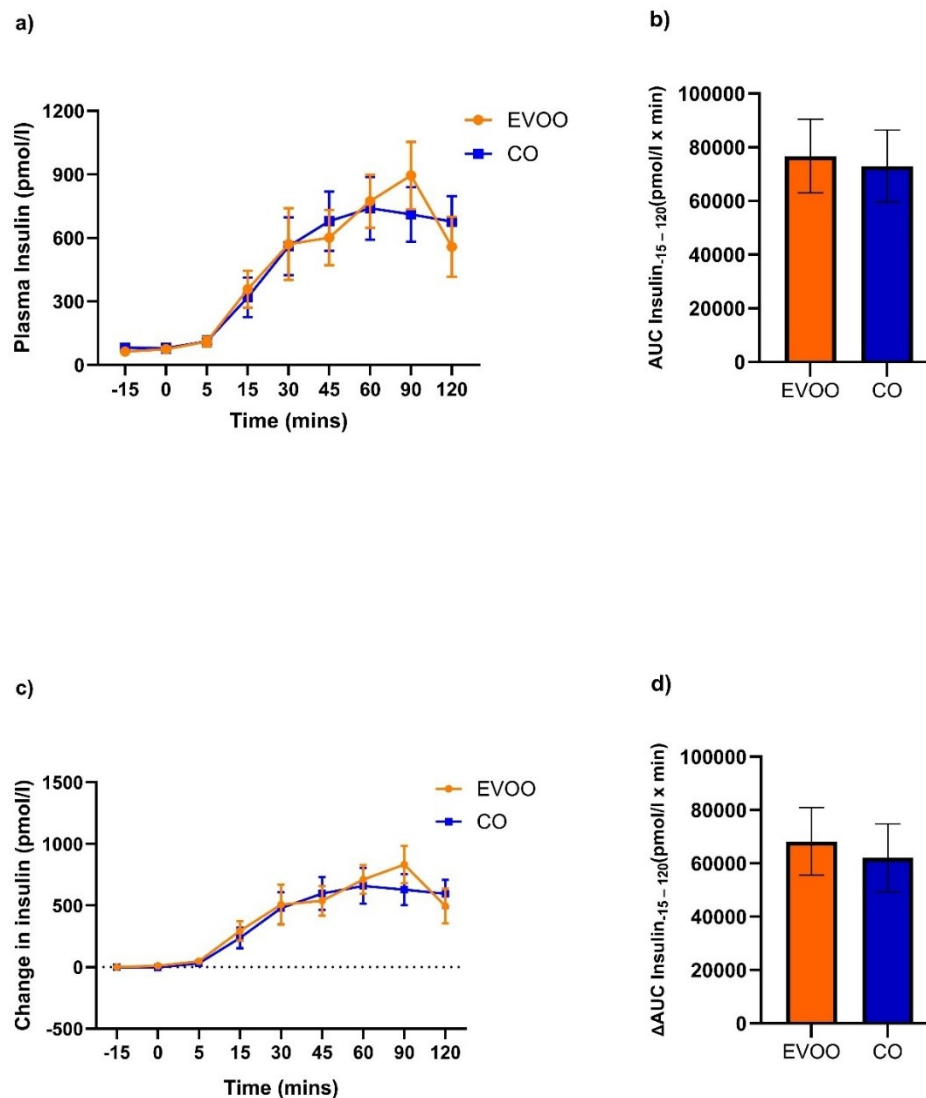


Figure 4.13. Plasma insulin measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute insulin concentration, b) area under the curve of absolute insulin concentration, c) change in insulin concentration from baseline and d) area under the

curve of change in insulin concentration from baseline. $n = 9$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) C-peptide: Fasting serum c-peptide did not differ in participants on the two study days [mean ($\pm$ SEM) on EVOO: 0.73 nmol/l ($\pm$ 0.07), vs CO: 0.82 nmol/l ($\pm$ 0.09), $P = 0.28$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.72$) on serum c-peptide concentrations. The total and net AUC of serum c-peptide to test oils were not significantly different (figure 4.14 (a) to (d), total AUC $P = 0.62$, net AUC $P = 0.33$).

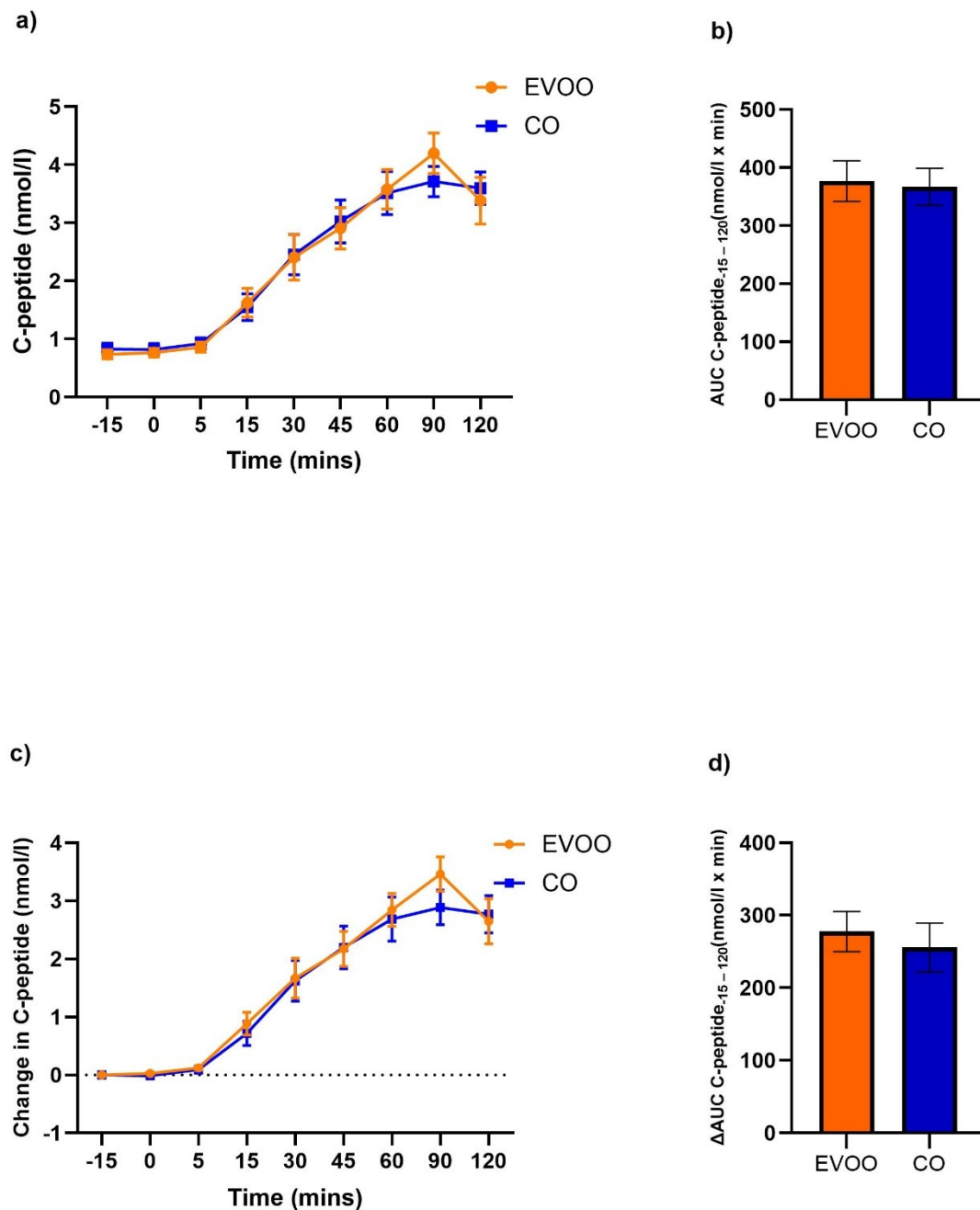


Figure 4.14. Plasma C-peptide measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute C-peptide concentration, b) area under the curve of absolute C-peptide concentration, c) change in C-peptide concentration from baseline and d) area under the curve of change in C-peptide concentration from baseline. $n = 9$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iv) Lipid profile: Although the triglyceride elevations were observed to be higher following EVOO ingestion, this did not reach statistical significance (95% CI: -0.07 to 0.39, $P = 0.15$). There was a significant effect of time ($P = 0.01$) but not test oil on change in serum triglyceride levels (figure 4.15 (a) and (b)). The changes in serum total cholesterol, HDL and calculated LDL were not significantly different on the two study days (data not shown).

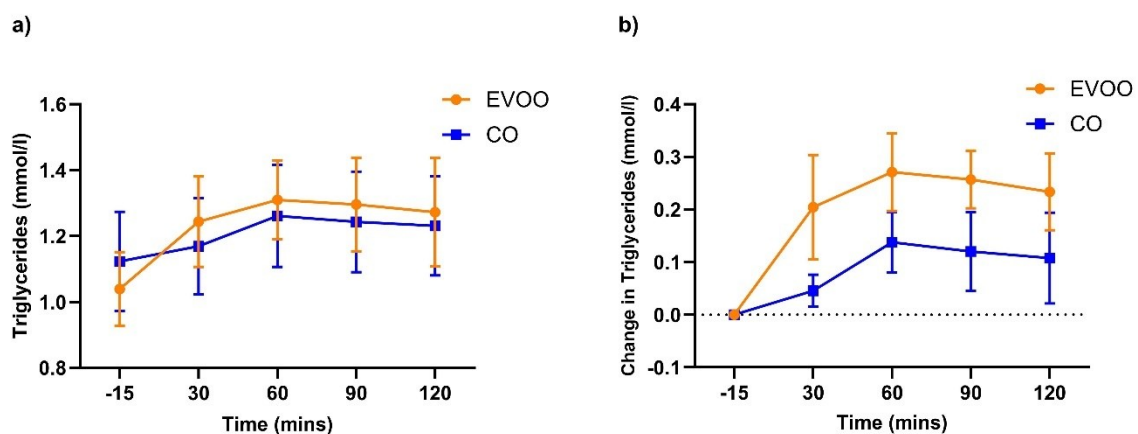


Figure 4.15. Plasma triglycerides measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute triglyceride concentration, b) change in triglycerides from baseline. $n = 9$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.2.3 Protocol 3: Effect of test oil preload 15 minutes before intravenous glucose

6 participants completed the two study visits in this protocol.

(i) Glucose: Fasting serum glucose did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 5.63 mmol/l ($\pm$ 0.13) vs CO: 5.62 mmol/l ($\pm$ 0.14), $P = 0.94$]. Plasma glucose dropped below baseline by 60 minutes [mean ($\pm$ SEM) EVOO: 5.30 mmol/l ($\pm$ 0.65) vs CO: 5.56 mmol/l ($\pm$ 0.64)] and remained lower at 120 minutes [mean ($\pm$ SEM) EVOO: 4.40 mmol/l ($\pm$ 0.10) vs CO: 4.42 mmol/l ($\pm$ 0.23)]. Despite a lower peak glucose concentration observed following EVOO administration at 5 mins [mean ($\pm$ SEM) EVOO: 11.78 mmol/l ($\pm$ 0.76) vs CO 13.18 mmol/l ($\pm$ 0.52)], the difference was not statistically significant ($P = 0.06$]. The total and net AUC of serum glucose were not significantly different (figure 4.16 (a) to (d), total AUC $P = 0.35$, net AUC $P = 0.38$).

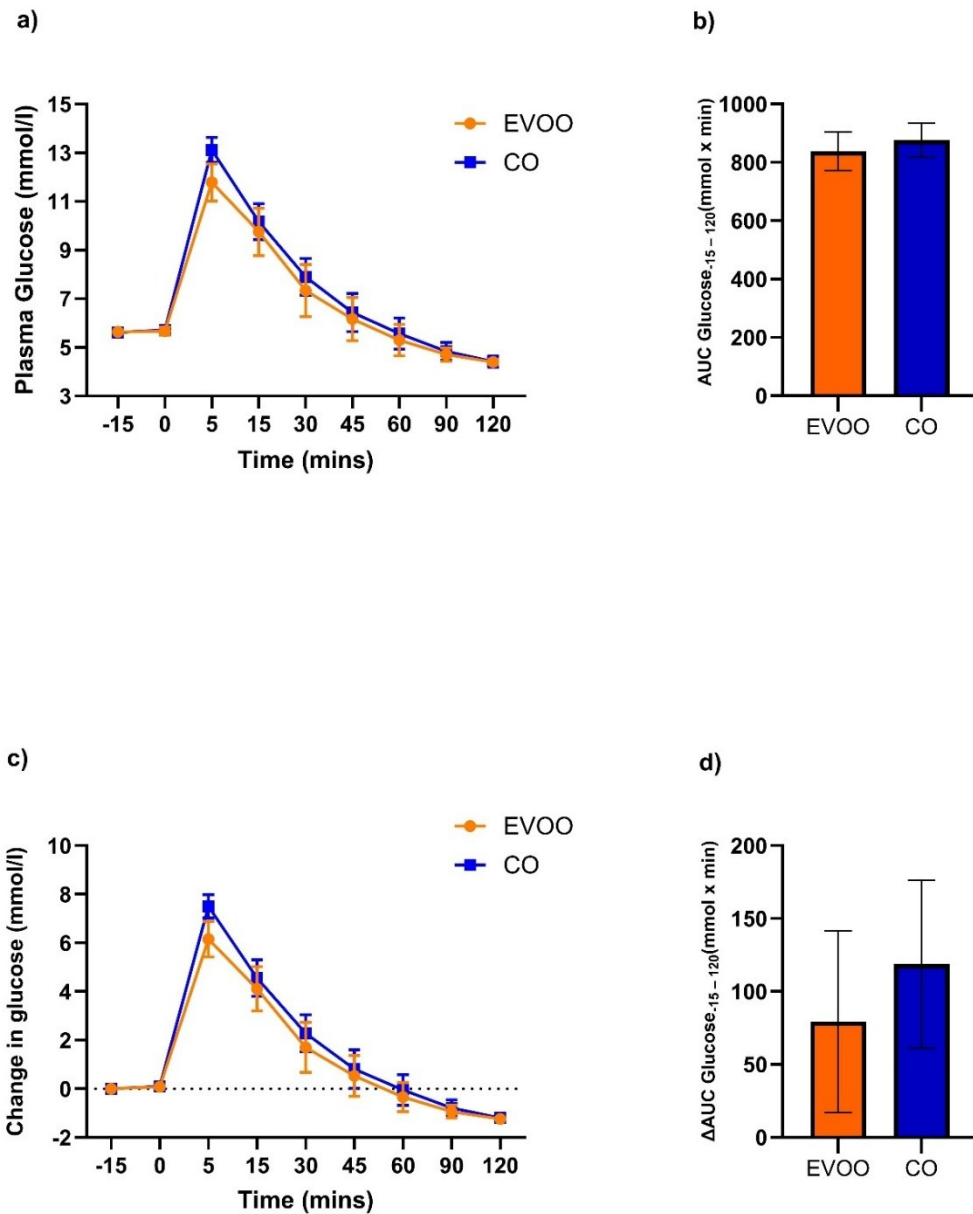


Figure 4.16. Plasma glucose measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute glucose concentration, b) area under the curve of absolute glucose concentration, c) change in glucose concentration from baseline and d) area under the curve of change in glucose concentration from baseline. $n = 6$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) Insulin: Fasting serum insulin did not differ in participants on different study days [mean ($\pm$ SEM) on EVOO: 71.92 pmol/l ($\pm$ 10.29), vs CO: 74.55 pmol/l ($\pm$ 10.93), $P = 0.83$]. Although the peak insulin concentration was greater following EVOO [mean ($\pm$ SEM) EVOO: 587.90 pmol/l ($\pm$ 202.41) vs CO: 447.40 pmol/l ($\pm$ 84.90)], it was not statistically significant ($p = 0.54$). There was significant effect of time ($P = 0.002$) but not test oil ($P = 0.56$) on serum insulin concentrations. The total and net AUC of serum insulin to test oils were not significantly different (figure 4.17 (a) to (d), total AUC $P = 0.23$, net AUC $P = 0.16$).

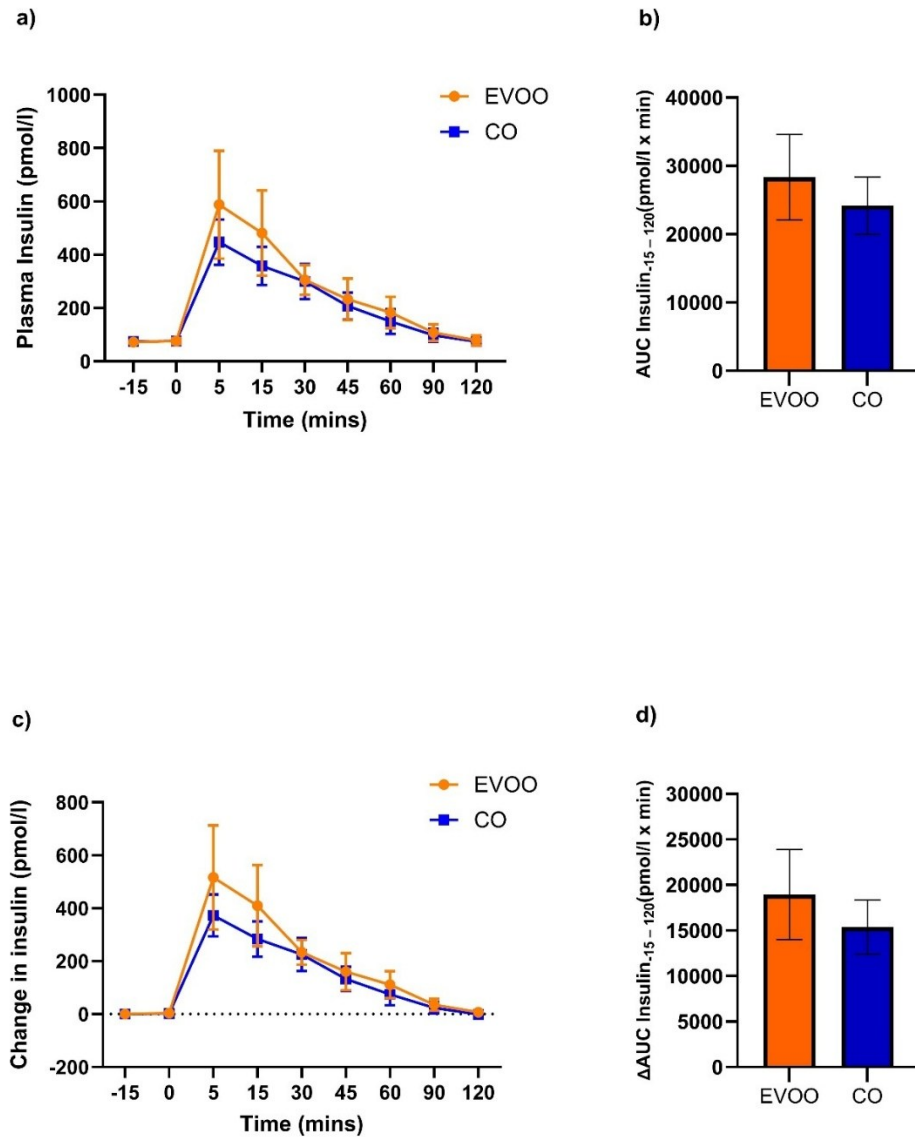
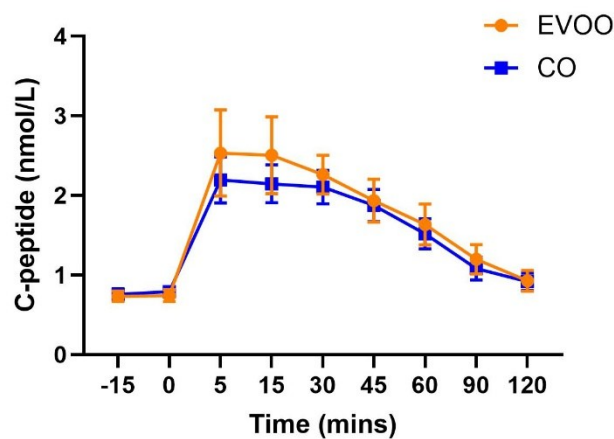


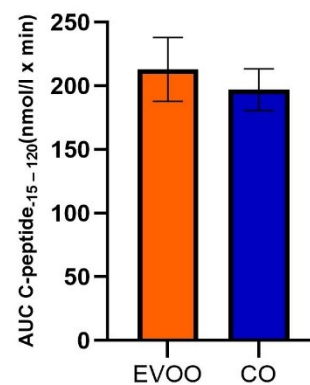
Figure 4.17. Plasma insulin measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute insulin concentration, b) area under the curve of absolute insulin concentration, c) change in insulin concentration from baseline and d) area under the curve of change in insulin concentration from baseline. $n = 6$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) C peptide: Fasting serum c-peptide did not differ in participants on the two study days [mean ($\pm$ SEM) on EVOO: 0.73 nmol/l ($\pm$ 0.06), vs CO: 0.76 nmol/l ($\pm$ 0.07), $P = 0.75$]. There was significant effect of time ($P < 0.0001$) but not test oil ($P = 0.60$) on serum c-peptide concentrations. The total and net AUC of serum c-peptide to test oils were not significantly different (figure 4.18 (a) to (d), total AUC $P = 0.32$, net AUC $P = 0.23$).

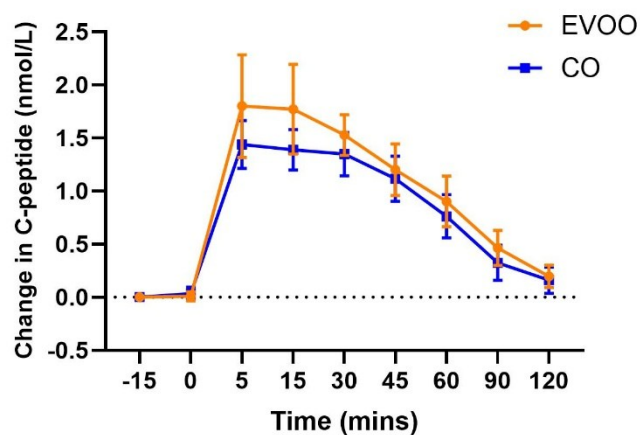
a)



b)



c)



d)

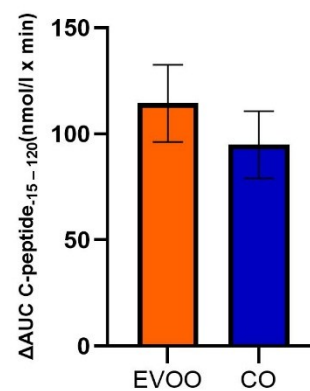


Figure 4.18. Plasma C-peptide measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute C-peptide concentration, b) area under the curve of absolute C-peptide concentration, c) change in C-peptide concentration from baseline and d) area under the curve of change in C-peptide concentration from baseline. $n = 6$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iv) Lipid profile: There was significant effect of time ($P = 0.02$) but not test oil ($P = 0.57$) on change in serum triglyceride levels (figure 4.19 (a) and (b)). The changes in serum total cholesterol, HDL and calculated LDL were not significantly different on the two study days (data not shown).

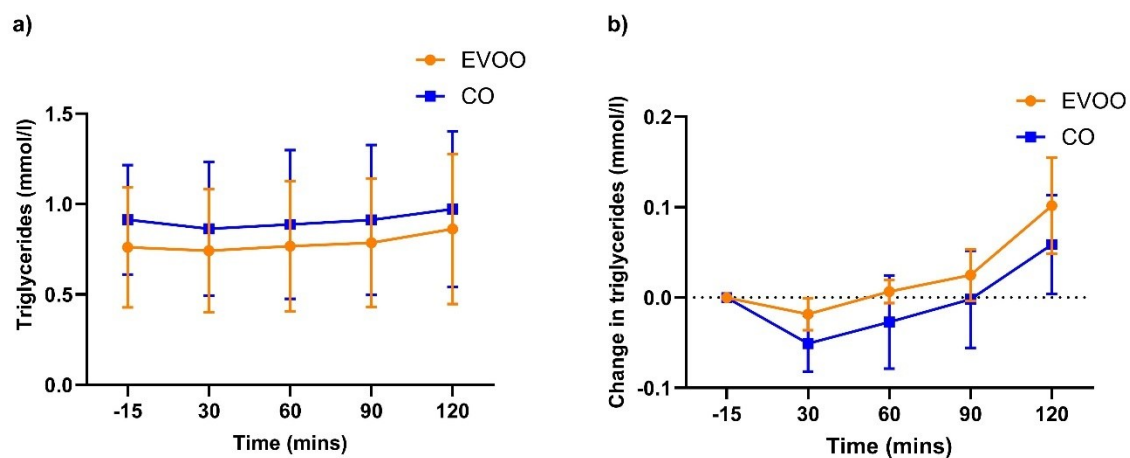


Figure 4.19. Plasma triglycerides measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute triglyceride concentration, b) change in triglycerides from baseline. $n = 6$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.3 Effect of test oil on gut hormones

4.5.2.3.1 Protocol 1: Effect of test oil administration immediately before oral glucose (at time 0)

(i) NT: Fasting plasma NT did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 43.90 pmol/l ($\pm$ 4.18) vs CO: 48.05 pmol/l ($\pm$ 2.73), $P = 0.19$]. There was significant effect of time ($P = 0.008$) but not test oil ($P = 0.41$) on NT concentration. A treatment $\times$ time interaction was observed for the test oils, approaching statistical significance ($F(8, 192) = 1.859$, $P = 0.07$). Although the net AUC of serum NT was much higher post EVOO, this did not reach statistical significance (figure 4.20 (b) and (d), total AUC $P = 0.58$, net AUC $P = 0.20$).

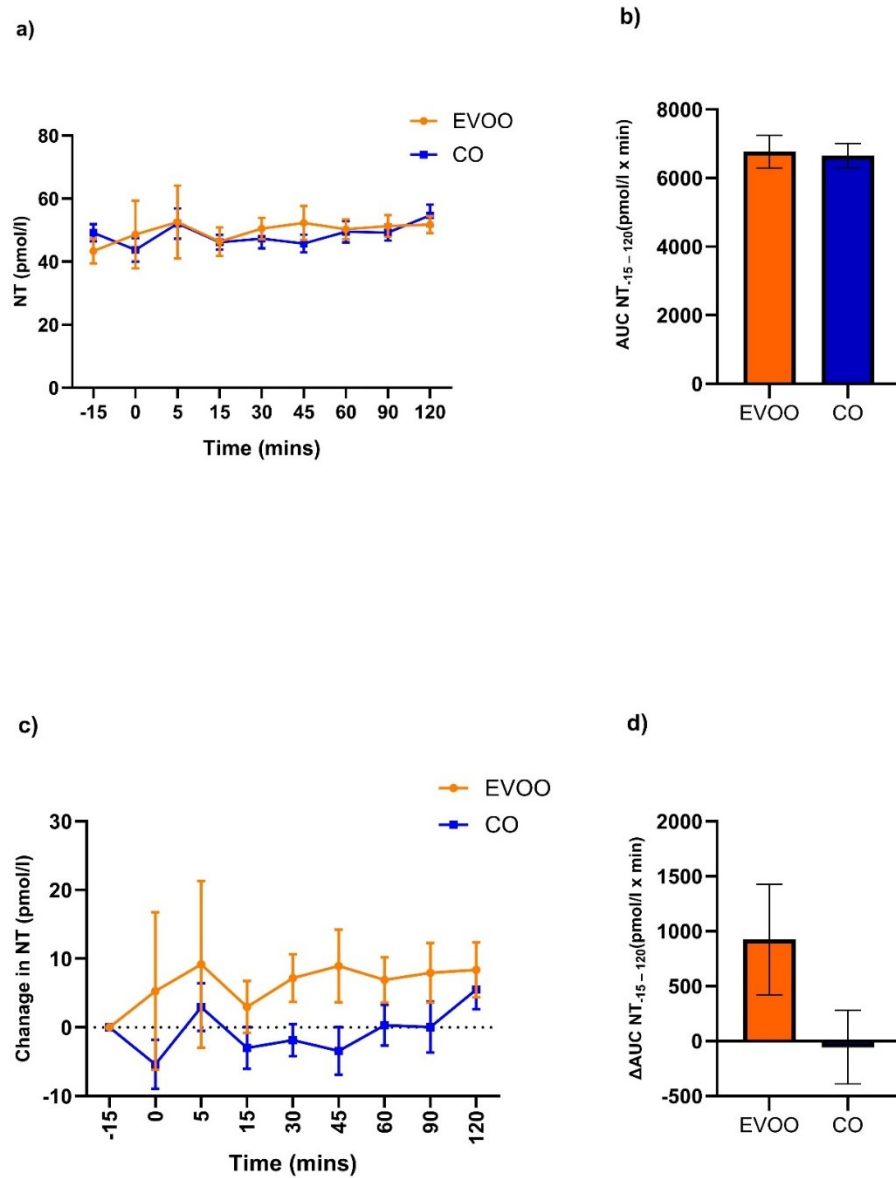


Figure 4.20. Plasma NT measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute NT concentration, b) area under the curve of absolute NT concentration, c) change in NT from baseline and d) area under the curve of change in NT concentration from baseline. $n = 14$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) GLP-1: Fasting plasma GLP-1 was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 51.99 pmol/l ($\pm$ 2.94) vs CO: 54.58 pmol/l ($\pm$ 5.55), $P = 0.57$]. There was significant effect of time ($p = 0.0009$) but not test oil ($P = 0.49$) on GLP-1 concentrations. The total and net AUC of GLP-1 response to test oils were not significantly different (figure 4.21 (a) to (d), total AUC $P = 0.61$, net AUC $P = 0.65$).

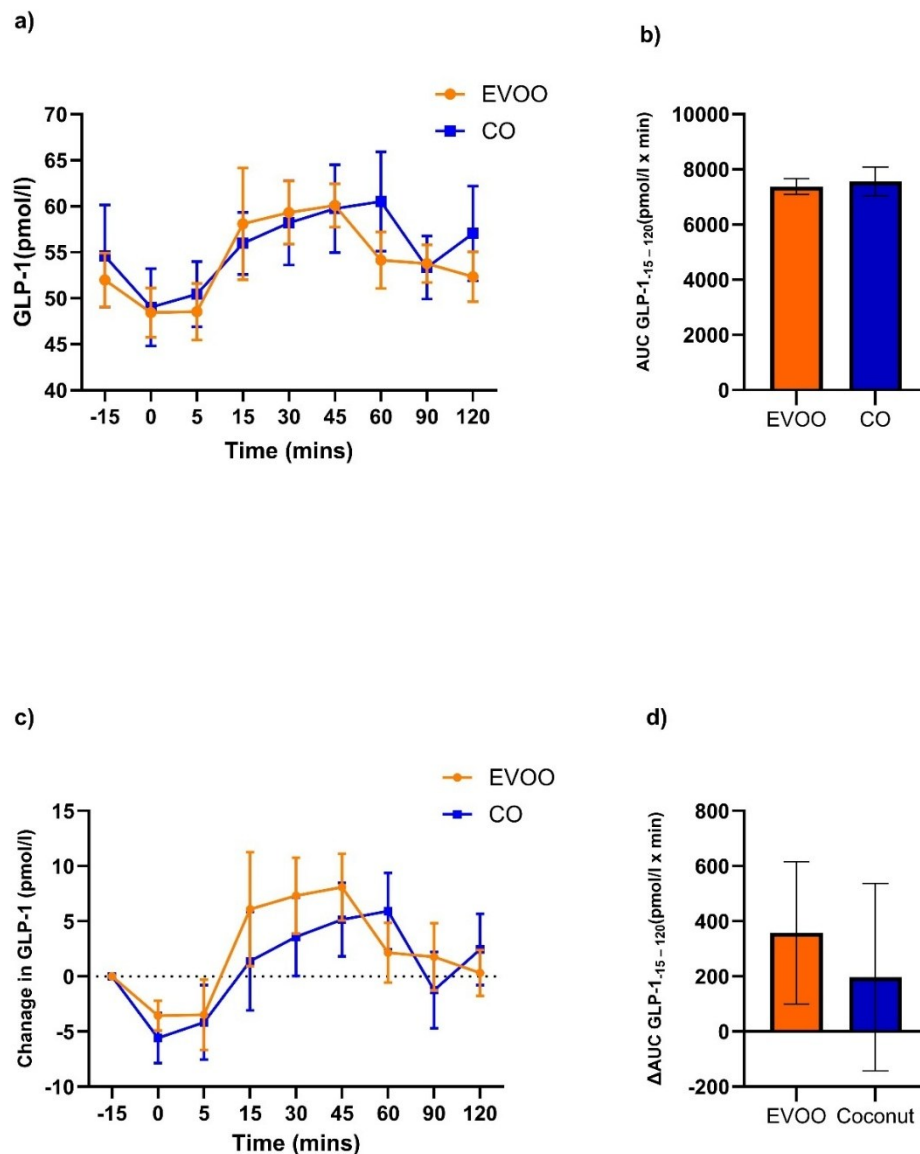


Figure 4.21. Plasma GLP-1 measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute GLP-1 concentration, b) area under the curve of absolute GLP-1 concentration, c) change in GLP-1 from baseline and d) area under the curve of change

in GLP-1 concentration from baseline. $n = 13$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) PYY: Fasting plasma PYY was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 93.13 pmol/l ($\pm$ 7.87) vs CO: 101.99 pmol/l ($\pm$ 11.76), $P = 0.24$]. EVOO was noted to exert a significant effect on PYY concentrations ($P = 0.03$); PYY levels were significantly higher at 30, 45 and 90 minutes post oil ingestion (figure 5.16 (c), $P < 0.05$). The total and net AUC of PYY concentration was not significantly different (figure 4.22 (b) and (d), total AUC $P = 0.08$, net AUC $P = 0.11$).

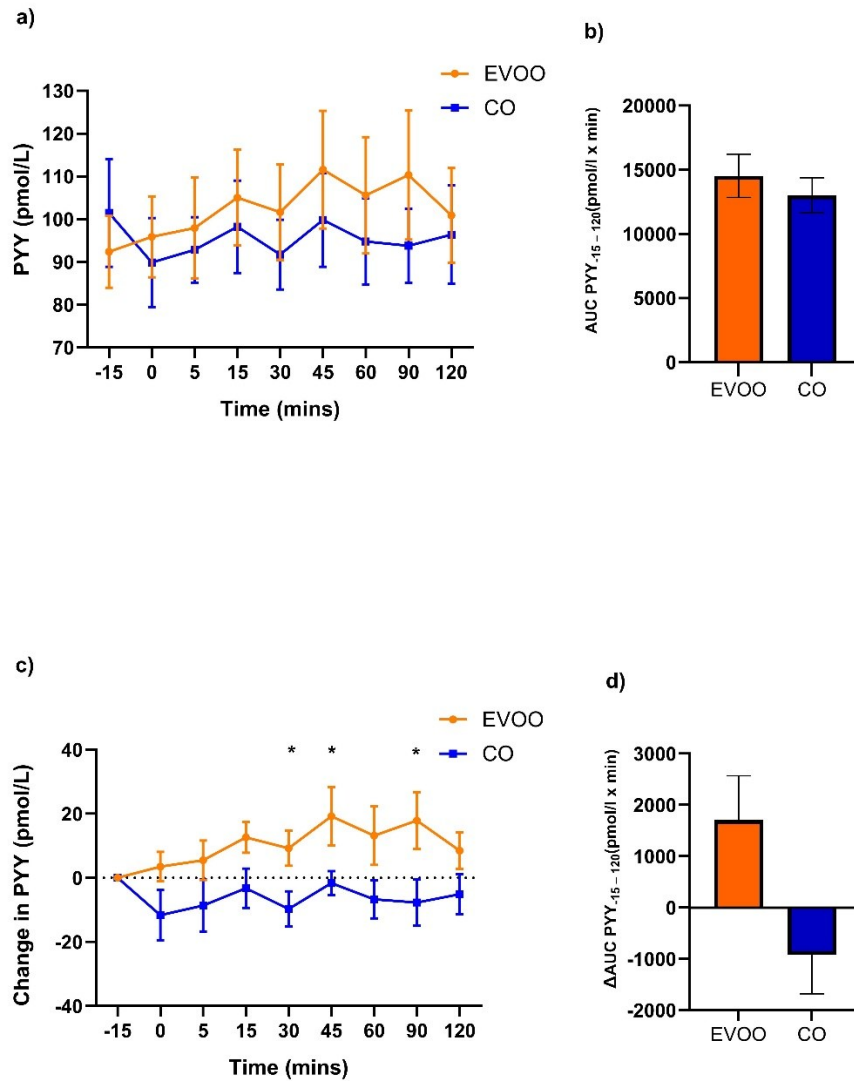


Figure 4.22. Plasma PYY measured over 120 minutes following test oils administration, with oils administered immediately before oral glucose (Protocol 1): a) absolute PYY concentration, b) area under the curve of absolute PYY concentration, c) change in PYY from baseline and d) area under the curve of change in PYY concentration from baseline. $n = 13$. Data presented as mean $\pm$ SEM. * represents $P < 0.05$, EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.3.2 Protocol 2: Effect of test oil preload 15 minutes before oral glucose

(i) NT: Fasting plasma NT did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 48.52 pmol/l ($\pm$ 7.68) vs CO: 45.95 pmol/l ($\pm$ 5.56), $P = 0.53$]. The total and net AUC of NT response to test oils were not significantly different (figure 4.23 , total AUC $p = 0.94$, net AUC $P = 0.34$).

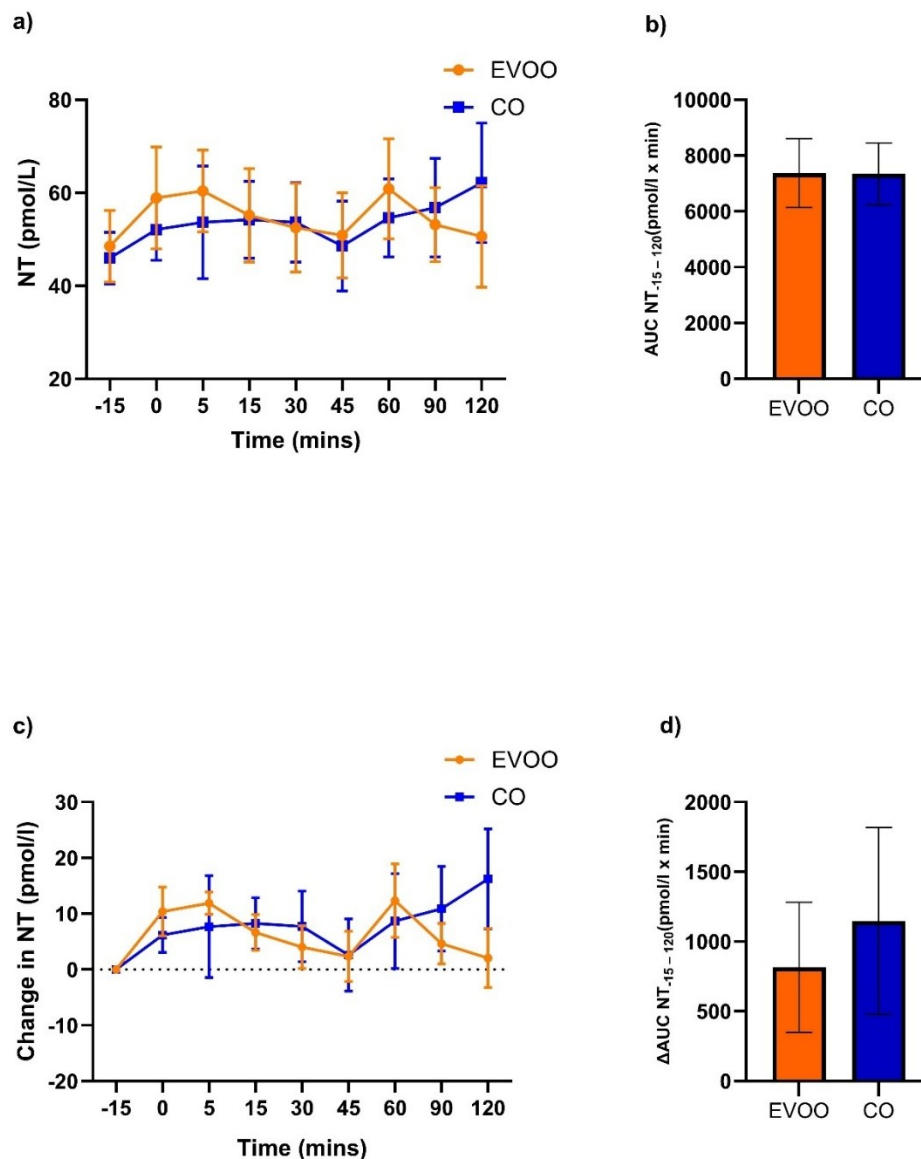


Figure 4.23. Plasma NT measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute neurotensin concentration, b) area under the curve of absolute NT concentration, c) change in NT from baseline and d) area under the curve of change in NT concentration

from baseline. $n = 7$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) Pro-NT: Pro-NT was also measured at selected time points in this protocol. Although the net AUC following EVOO ingestion was greater than CO, ProNT concentration was not significantly different (net AUC, $P = 0.61$, figure 4.24 (a) and (b)).

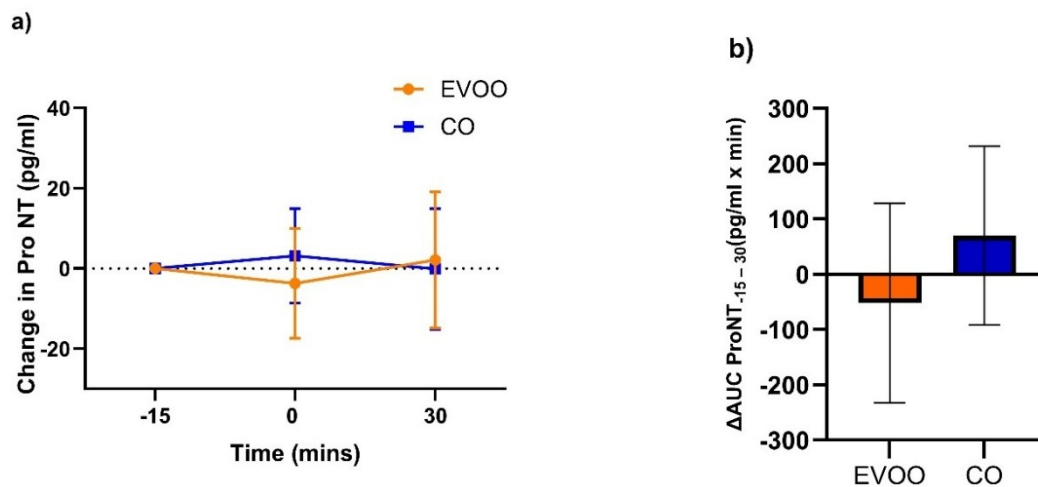


Figure 4.24. Plasma Pro-NT measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) change in Pro-NT concentration, b) area under the curve of change in Pro-NT concentration from baseline. $n = 9$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) GLP-1: Fasting plasma GLP-1 was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 59.23 pmol/l ($\pm$ 4.44) vs CO: 65.41 pmol/l ($\pm$ 4.50), $P = 0.21$]. There was significant effect of time ($P = 0.0003$) but not test oil ($P = 0.92$) on GLP-1 concentrations. The total and net AUC of GLP-1 response to test oils were not significantly different (figure 4.25 (a) to (d), total AUC $P = 0.76$, net AUC $P = 0.51$).

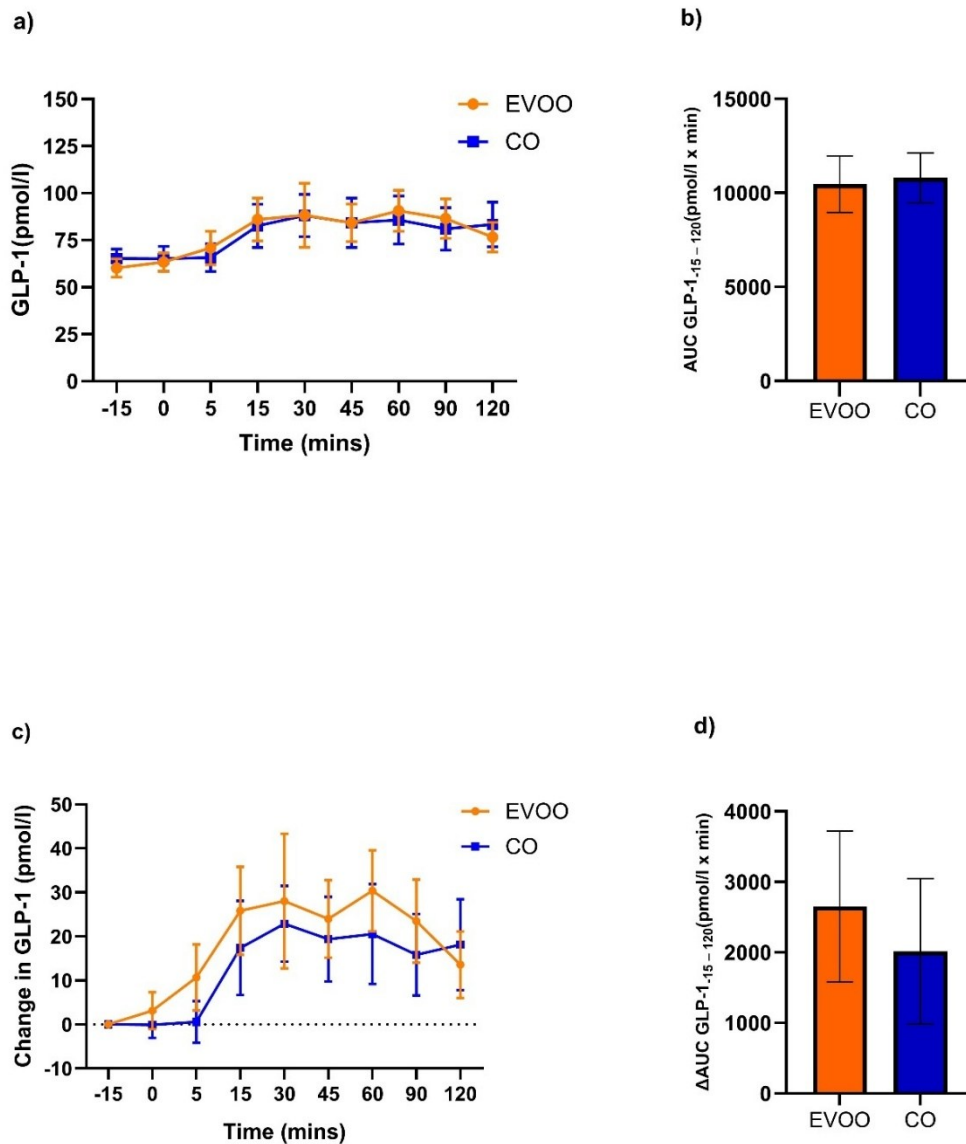


Figure 4.25. Plasma GLP-1 measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute GLP-1 concentration, b) area under the curve of absolute GLP-1 concentration, c) change in GLP-1 from baseline and d) area under the curve of change in GLP-1 concentration from baseline. $n = 7$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iv) PYY: Fasting plasma PYY was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 49.81 pmol/l ($\pm$ 7.16) vs CO: 60.29 pmol/l ($\pm$ 7.72), $P = 0.07$].

PYY levels were significantly higher at 5 minutes post EVOO (figure 4.26 (c)). The total and net AUC of PYY concentration was not significantly different (figure 4.26, total AUC $P = 0.97$, net AUC $P = 0.08$).

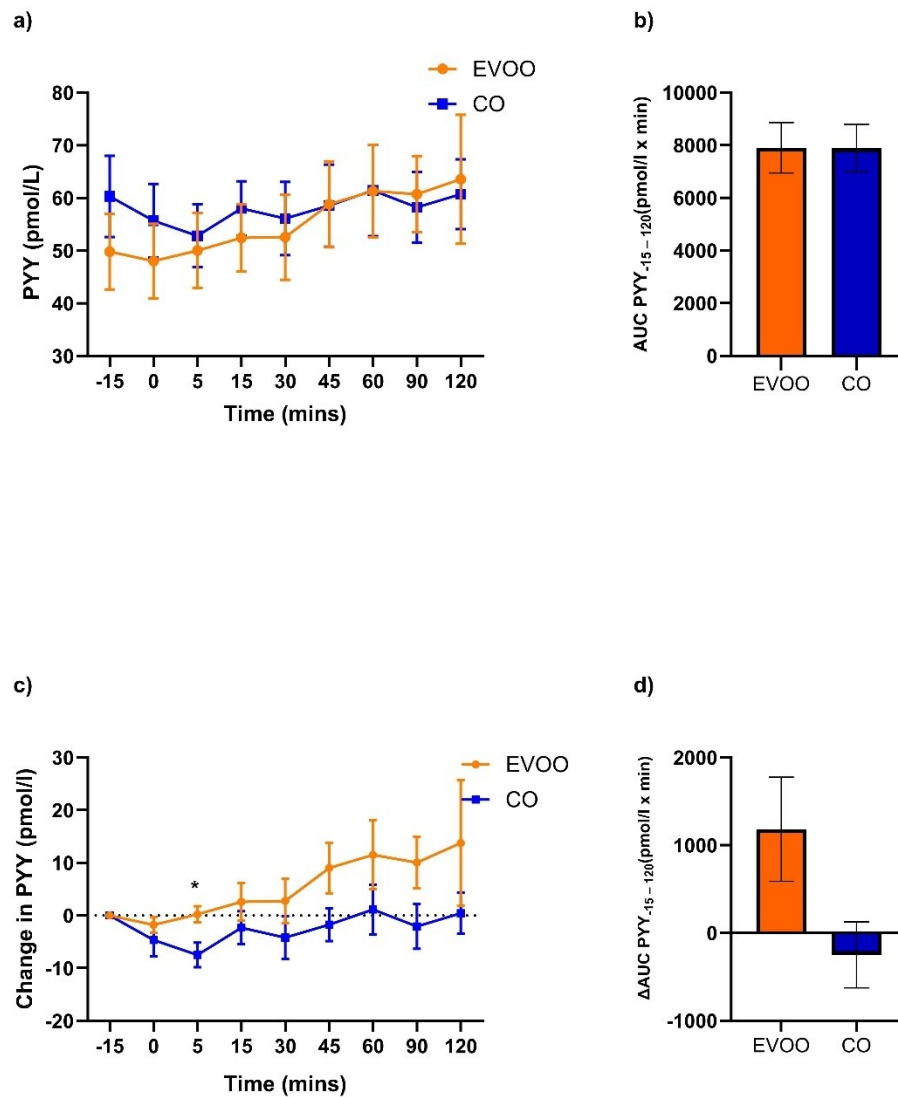


Figure 4.26. Plasma PYY measured over 120 minutes following test oils administration, with oils administered 15 minutes before oral glucose (Protocol 2): a) absolute PYY concentration, b) area under the curve of absolute PYY concentration, c) change in PYY from baseline and d) area under the curve of change in PYY concentration from baseline. $n = 9$. Data presented as mean $\pm$ SEM. * represents $P < 0.05$, EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.3.3 Protocol 3: Effect of test oil preload 15 minutes before intravenous glucose

(i) NT: Fasting plasma NT did not differ in participants on the two study days [mean ($\pm$ SEM) EVOO: 39.63 pmol/l ($\pm$ 7.05) vs CO: 44.40 pmol/l ($\pm$ 9.82), $P = 0.63$]. NT concentration was not significantly different after test oils (figure 4.27 (a) and (c)). The total and net AUC of NT response to test oils was not statistically significant (figure 4.27 (b) and (d), total AUC $P = 0.31$, net AUC $P = 0.57$).

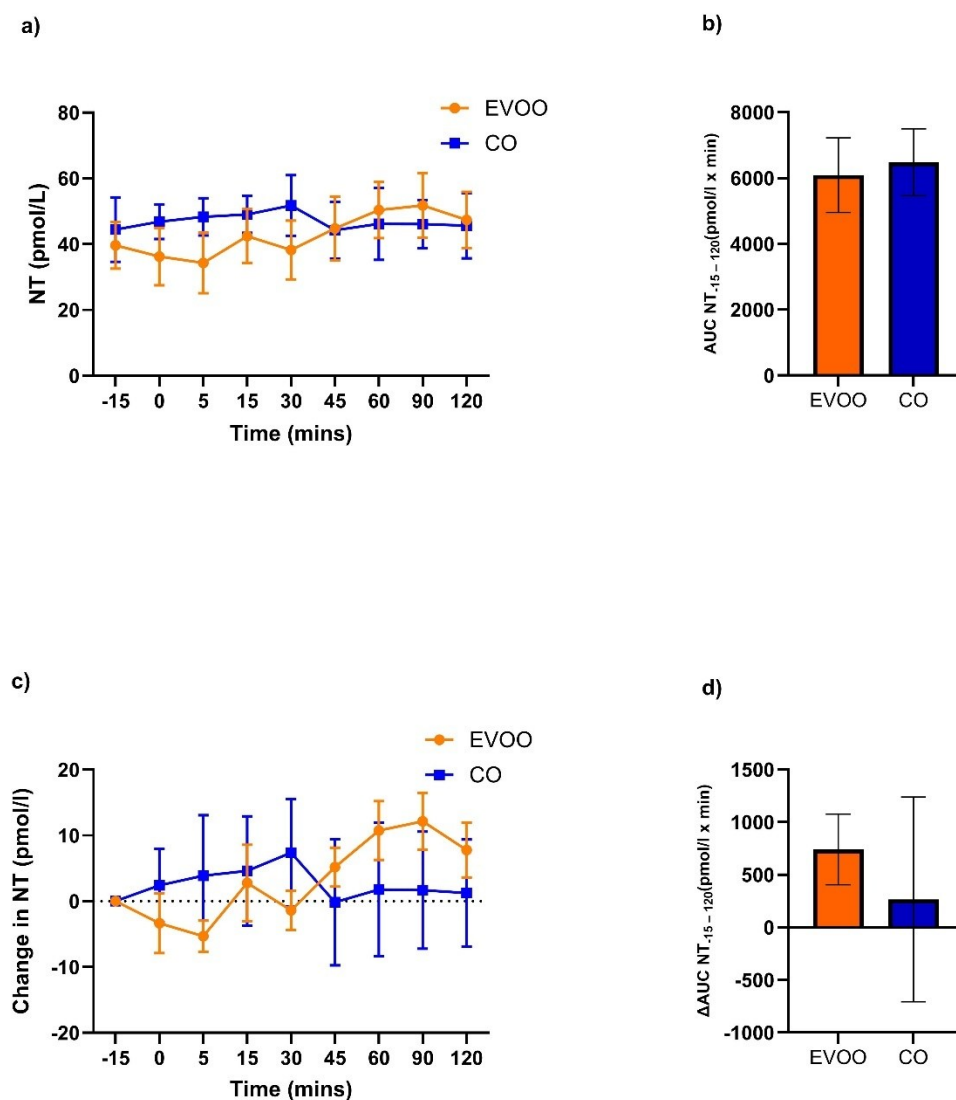


Figure 4.27. Plasma NT measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute NT concentration, b) area under the curve of absolute NT concentration, c) change in

NT from baseline and d) area under the curve of change in NT concentration from baseline. $n = 5$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(ii) Pro-NT: Pro-NT was also measured at selected time points in this protocol. Pro-NT was not significantly different following ingestion of the test oils (figure 4.28 (a) and (b), net AUC, $P = 0.24$).

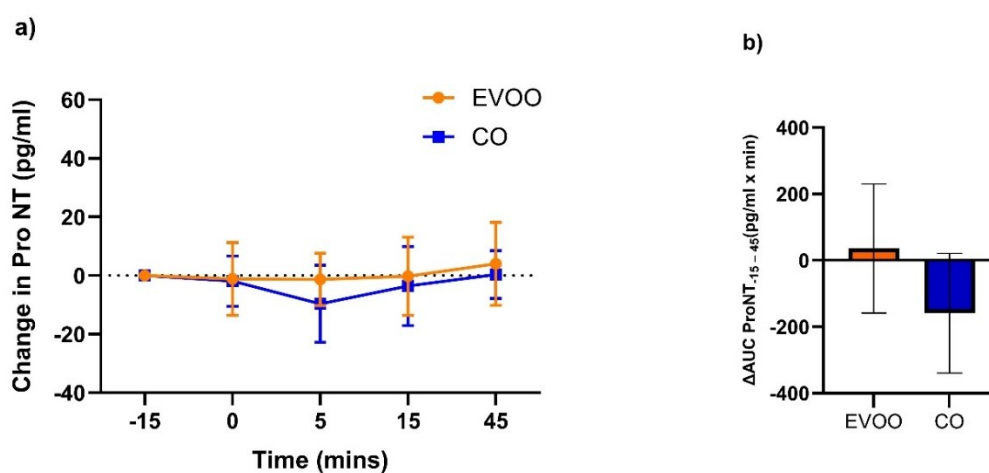


Figure 4.28. Plasma Pro-NT measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) change in Pro-NT concentration, b) area under the curve of change in ProNT concentration from baseline. $n = 7$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iii) GLP-1: Fasting plasma GLP-1 was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 50.67 pmol/l ($\pm$ 6.56) vs CO: 48.10 pmol/l ($\pm$ 2.61), $P = 0.66$]. GLP-1 concentration was not significantly different after test oils. The total and net AUC of GLP-1 response to test oils were not statistically significant (figure 4.29 (a) to (d), total AUC $P = 0.82$, net AUC $P = 0.94$).

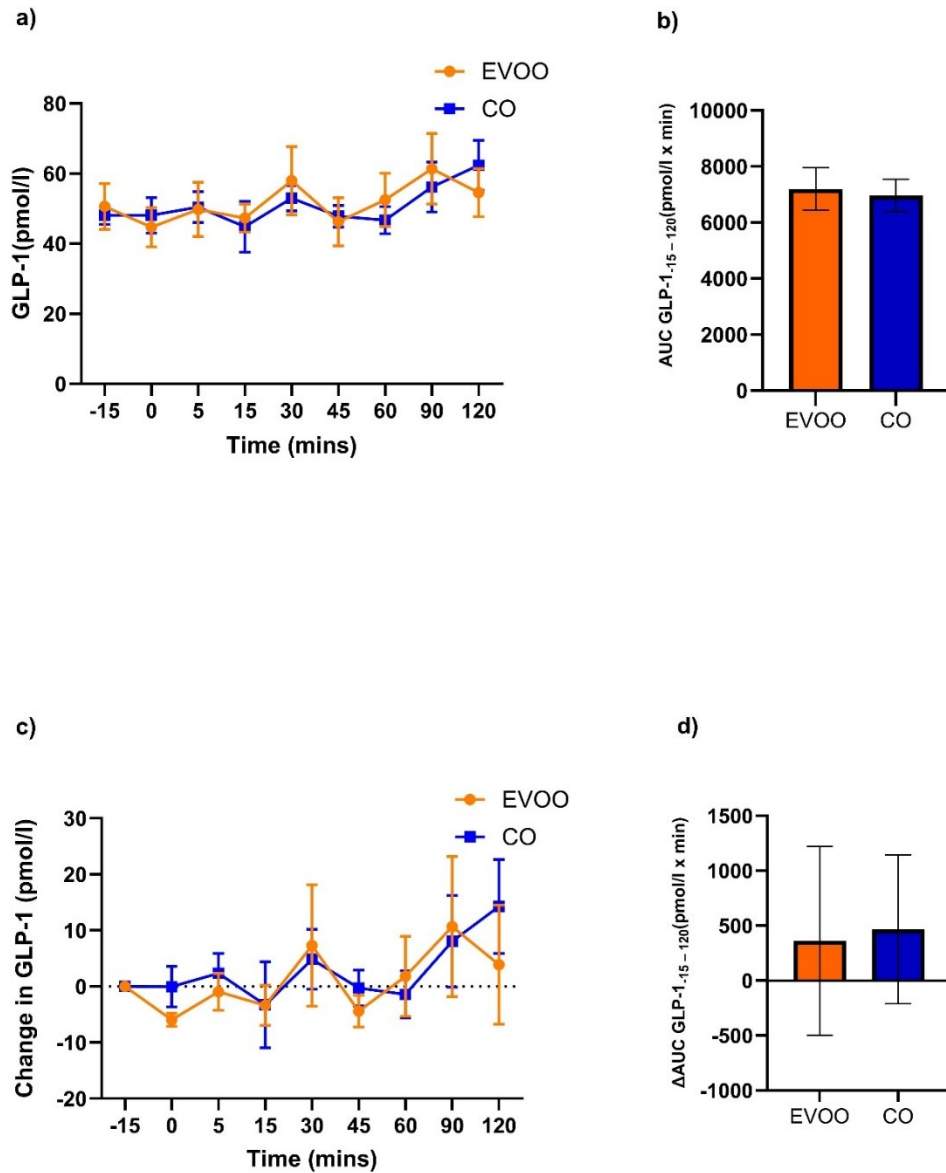


Figure 4.29. Plasma GLP-1 measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute GLP-1 concentration, b) area under the curve of absolute GLP-1 concentration, c) change in GLP-1 from baseline and d) area under the curve of change in GLP-1 concentration from baseline. $n = 4$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

(iv) PYY: Fasting plasma PYY was not different amongst participants on the two study days [mean ($\pm$ SEM) EVOO: 55.82 pmol/l ($\pm$ 3.88) vs CO: 63.23 pmol/l ($\pm$ 4.07), $P = 0.33$]. PYY concentration was not significantly different after test oils. The total and net AUC of PYY response to test oils was not significantly different (figure 4.30 (a) to (d), total AUC $P = 0.93$, net AUC $P = 0.43$).

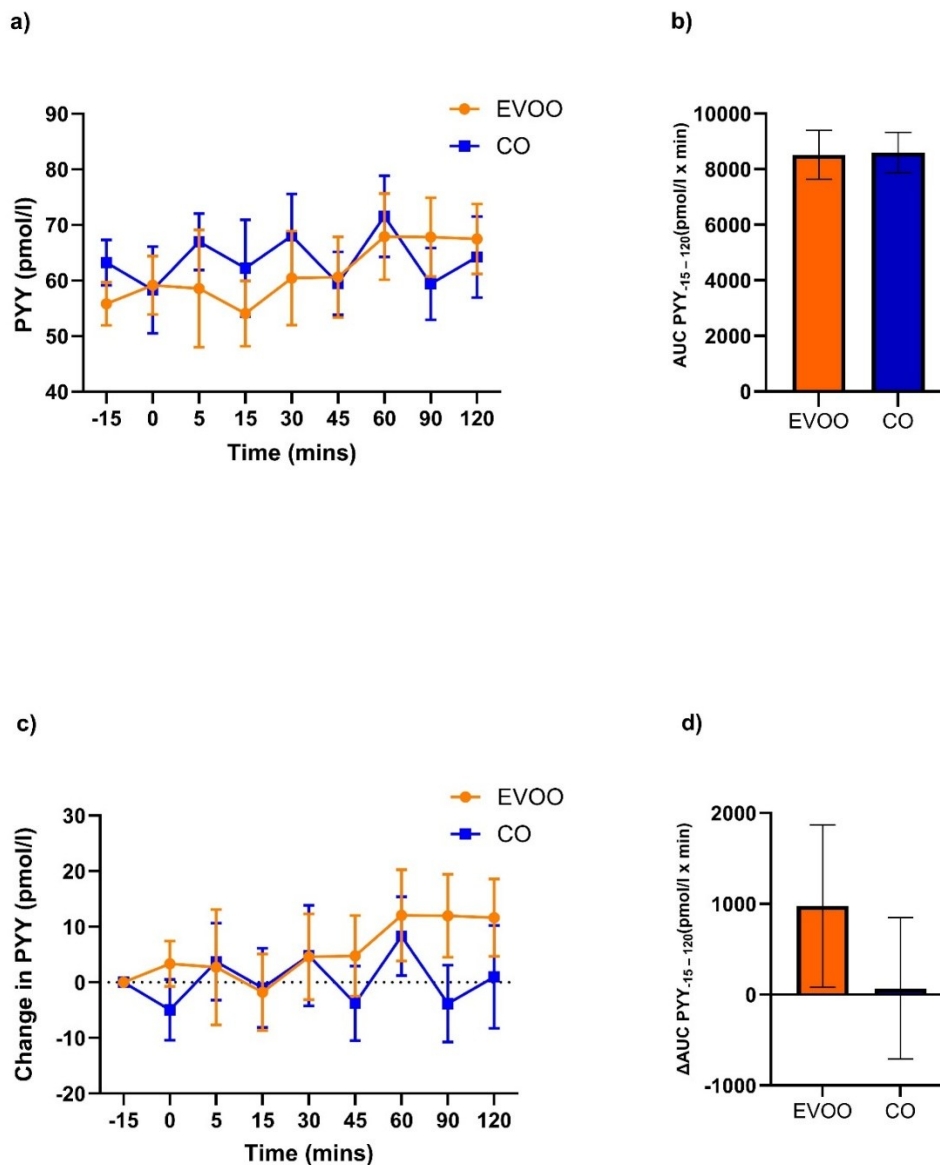


Figure 4.30. Plasma PYY measured over 120 minutes following test oils administration, with oils administered 15 minutes before intravenous glucose (Protocol 3): a) absolute PYY concentration, b) area under the curve of absolute PYY concentration, c) change in PYY from baseline and d) area under the curve of change

in PYY concentration from baseline. $n = 5$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil.

4.5.2.4 Free fatty acids (FFA) analysis

4.5.2.4.1 Protocol 1: Effect of test oil administration immediately before oral glucose (at time 0)

Paired data was available for 10 participants at the three time points. There were no significant differences in the postprandial concentration of plasma FFAs between the test oils (table 4.3, figure 4.31).

Table 4.3: Free fatty acid profile before and after oil ingestion (Protocol 1)						
Fatty acid	EVOO			CO		
	-15 mins	15 mins	30 mins	-15 mins	15 mins	30 mins
Oleic acid (nmol/ml)	1956.5 $\pm$ 165.2	2038.4 $\pm$ 156.1	2060.5 $\pm$ 197.3	2122.5 $\pm$ 190.6	2233.2 $\pm$ 221.9	2195.6 $\pm$ 208.6
Linoleic acid (nmol/ml)	3693.6 $\pm$ 222.6	3803.4 $\pm$ 263.2	3906.7 $\pm$ 251.9	3956.5 $\pm$ 267.3	4127.2 $\pm$ 267.9	4125.2 $\pm$ 304.3
Palmitic acid (nmol/ml)	2608.4 $\pm$ 205.9	2667.6 $\pm$ 204.5	2726.9 $\pm$ 236.2	2760.1 $\pm$ 240.7	2876.5 $\pm$ 270.3	2872.2 $\pm$ 294.0
Palmitoleic acid (nmol/ml)	231.4 $\pm$ 39.6	235.6 $\pm$ 46.7	252.9 $\pm$ 44.0	271.8 $\pm$ 49.7	281.9 $\pm$ 51.7	278.3 $\pm$ 48.2
Total Fatty acids (mmol/l)	12.16 $\pm$ 0.8	12.52 $\pm$ 0.8	12.84 $\pm$ 0.9	13.10 $\pm$ 0.9	13.68 $\pm$ 1.0	13.70 $\pm$ 1.2
Total MUFA (mmol/l)	2.54 $\pm$ 0.2	2.59 $\pm$ 0.2	2.67 $\pm$ 0.3	2.75 $\pm$ 0.3	2.88 $\pm$ 0.3	2.83 $\pm$ 0.3
Total PUFA (mmol/l)	5.58 $\pm$ 0.3	5.76 $\pm$ 0.4	5.91 $\pm$ 0.4	6.06 $\pm$ 0.4	6.33 $\pm$ 0.4	6.39 $\pm$ 0.5
Total Saturated FA (mmol/l)	4.06 $\pm$ 0.3	4.19 $\pm$ 0.3	4.26 $\pm$ 0.3	4.30 $\pm$ 0.3	4.47 $\pm$ 0.4	4.46 $\pm$ 0.4
Data are reported as mean $\pm$ SEM. EVOO, Extra Virgin Olive Oil, CO, Coconut Oil, MUFA, Monounsaturated fatty acids, PUFA, Polyunsaturated fatty acids.						

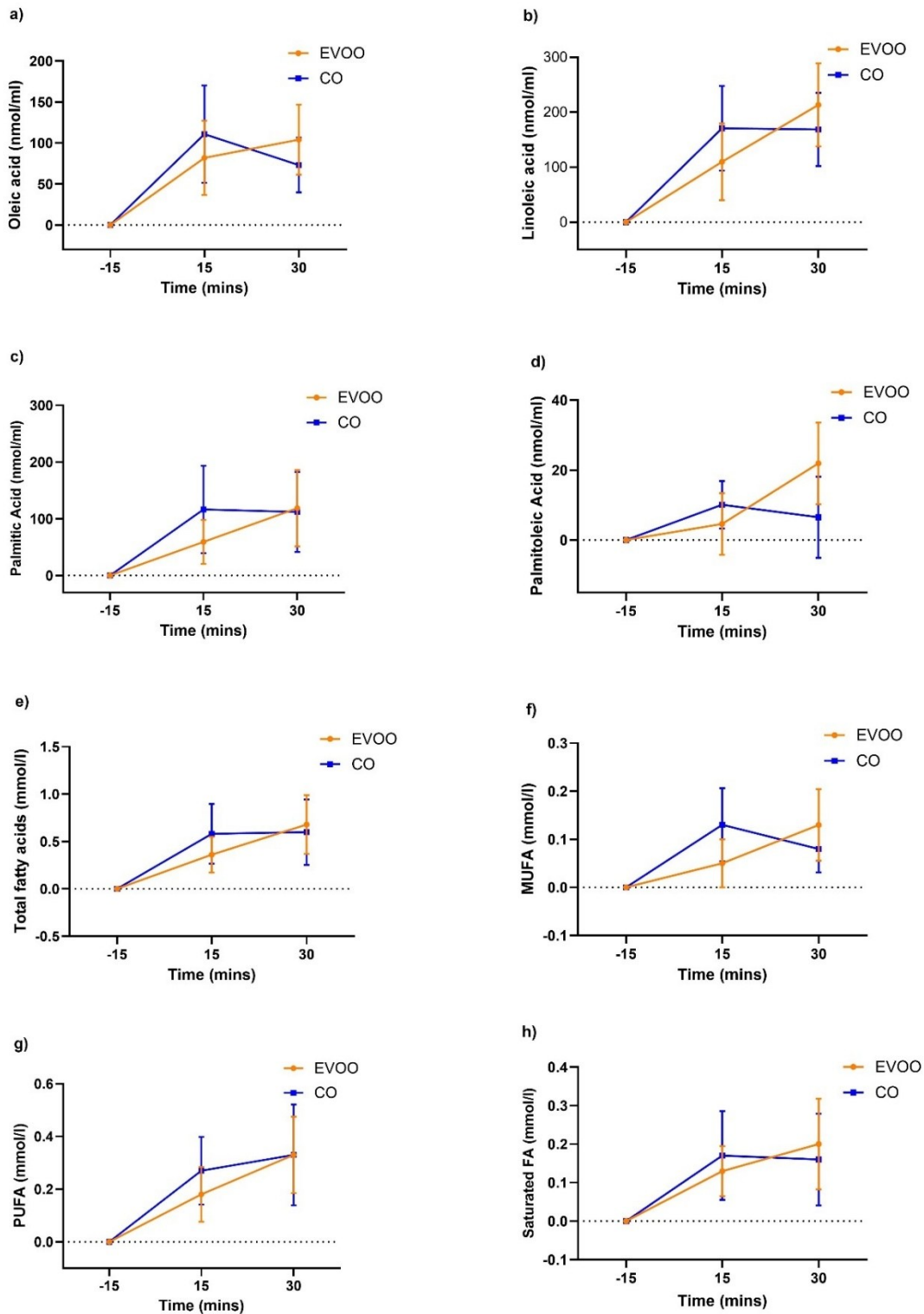


Figure 4.31. Changes in free fatty acid concentrations over 30 minutes following test oils (Protocol 1): a) Oleic acid, b) Linoleic acid, c) Palmitic acid, d) Palmitoleic acid, e) Total fatty acids, f) MUFA g) PUFA, h) Saturated fatty acids. $n = 10$. Data presented as

mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

4.5.2.4.2 Protocol 2: Effect of test oil preload 15 minutes before oral glucose

Paired data was available for 8 participants at the three times points. There were no significant differences in the postprandial concentration of plasma FFAs between the two test oils (table 4.4, figure 4.32).

Table 4.4: Free fatty acid profile before and after oil ingestion (Protocol 2)						
Fatty acid	EVOO			CO		
	-15 mins	15 mins	30 mins	-15 mins	15 mins	30 mins
Oleic acid (nmol/ml)	1906.5 $\pm$ 152.8	1976.6 $\pm$ 165.6	1975.8 $\pm$ 143.5	1954.3 $\pm$ 194.3	1844.7 $\pm$ 178.6	1950.1 $\pm$ 195.3
Linoleic acid (nmol/ml)	2956.0 $\pm$ 152.9	3022.9 $\pm$ 185.0	2978.7 $\pm$ 197.6	3308.6 $\pm$ 209.7	3333.0 $\pm$ 245.1	3283.4 $\pm$ 206.8
Palmitic acid (nmol/ml)	2316.7 $\pm$ 202.4	2399.3 $\pm$ 219.8	2313.0 $\pm$ 181.3	2407.4 $\pm$ 223.4	2403.7 $\pm$ 249.6	2423.8 $\pm$ 249.4
Palmitoleic acid (nmol/ml)	271.2 $\pm$ 42.2	245.1 $\pm$ 33.1	265.4 $\pm$ 37.4	223.6 $\pm$ 39.6	203.6 $\pm$ 38.7	220.1 $\pm$ 39.7
Total Fatty acids (mmol/l)	10.59 $\pm$ 0.6	11.59 $\pm$ 1.0	10.80 $\pm$ 0.5	11.28 $\pm$ 0.7	11.16 $\pm$ 0.8	11.17 $\pm$ 0.7
Total MUFA (mmol/l)	2.45 $\pm$ 0.2	2.74 $\pm$ 0.3	2.55 $\pm$ 0.2	2.47 $\pm$ 0.3	2.35 $\pm$ 0.2	2.48 $\pm$ 0.2
Total PUFA (mmol/l)	4.64 $\pm$ 0.2	5.26 $\pm$ 0.5	4.81 $\pm$ 0.1	5.15 $\pm$ 0.3	5.21 $\pm$ 0.3	5.11 $\pm$ 0.3
Total Saturated FA (mmol/l)	3.44 $\pm$ 0.3	3.58 $\pm$ 0.3	3.42 $\pm$ 0.2	3.60 $\pm$ 0.3	3.59 $\pm$ 0.3	3.56 $\pm$ 0.3
Data are reported as mean $\pm$ SEM. EVOO, Extra Virgin Olive Oil, CO, Coconut Oil, MUFA, Monounsaturated fatty acids, PUFA, Polyunsaturated fatty acids.						

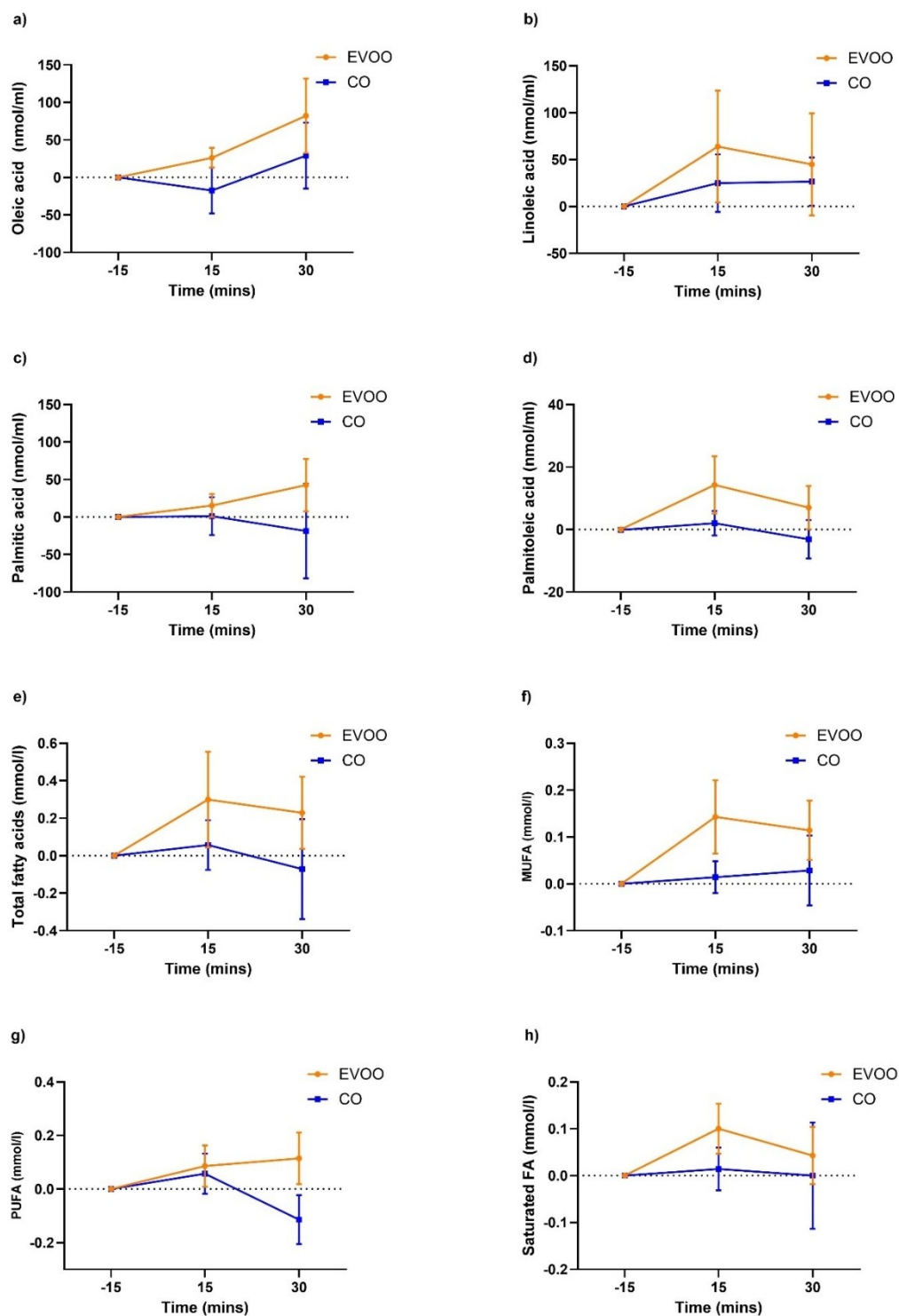


Figure 4.32. Changes in free fatty acid concentrations over 30 minutes following test oils (Protocol 2): a) Oleic acid, b) Linoleic acid, c) Palmitic acid, d) Palmitoleic acid, e) Total fatty acids, f) MUFA g) PUFA, h) Saturated fatty acids. $n = 8$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

4.5.2.4.3 Protocol 3: Effect of test oil preload 15 minutes before intravenous glucose

Paired data was available for 7 participants at the three times points. There were significant differences in the postprandial concentration of plasma FFAs following the test oils (table 4.5, figure 4.33).

Table 4.5: Free fatty acid profile before and after oil ingestion (Protocol 3)						
Fatty acid	EVOO			CO		
	-15 mins	15 mins	30 mins	-15 mins	15 mins	30 mins
Oleic acid (nmol/ml)	1494.5 ± 200.3	1525.2 ± 198.4	1728 ± 175.5 ^b	1629.9 ± 184.6	1650 ± 184.8	1889.3 ± 192.2
Linoleic acid (nmol/ml)	2697.5 ± 161.7	2788.5 ± 233.8	3007.9 ± 224.7 ^b	2902.1 ± 219.7	2888.6 ± 218.6	3157.4 ± 185.4 ^{a,b}
Palmitic acid (nmol/ml)	2034.0 ± 174.7	2035.2 ± 164.2	2200.9 ± 137.3 ^b	2132.3 ± 156.0	2123.0 ± 150.4	2382.6 ± 179.8
Palmitoleic acid (nmol/ml)	153.3 ± 15.8	146.7 ± 12.1	168.7 ± 9.7	162.0 ± 13.7	164.0 ± 14.4	191.0 ± 15.3 ^{a,b}
Total Fatty acids (mmol/l)	9.75 ± 0.7	9.97 ± 0.8	10.84 ± 0.7 ^b	10.26 ± 0.8	10.28 ± 0.7	11.41 ± 0.7 ^{a,b}
Total MUFA (mmol/l)	1.93 ± 0.2	1.97 ± 0.2	2.19 ± 0.2 ^b	2.06 ± 0.2	2.10 ± 0.2	2.39 ± 0.2
Total PUFA (mmol/l)	4.7 ± 0.3	5.0 ± 0.4	5.4 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.4 ± 0.4
Total Saturated FA (mmol/l)	3.1 ± 0.2	3.0 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.6 ± 0.3
Data are reported as mean ± SEM. EVOO, Extra Virgin Olive Oil, CO, Coconut Oil, MUFA, Monounsaturated fatty acids, PUFA, Polyunsaturated fatty acids. The superscript letters indicate significant differences (a: -15 vs 30 mins, b: 15 vs 30 mins, $P < 0.05$).						

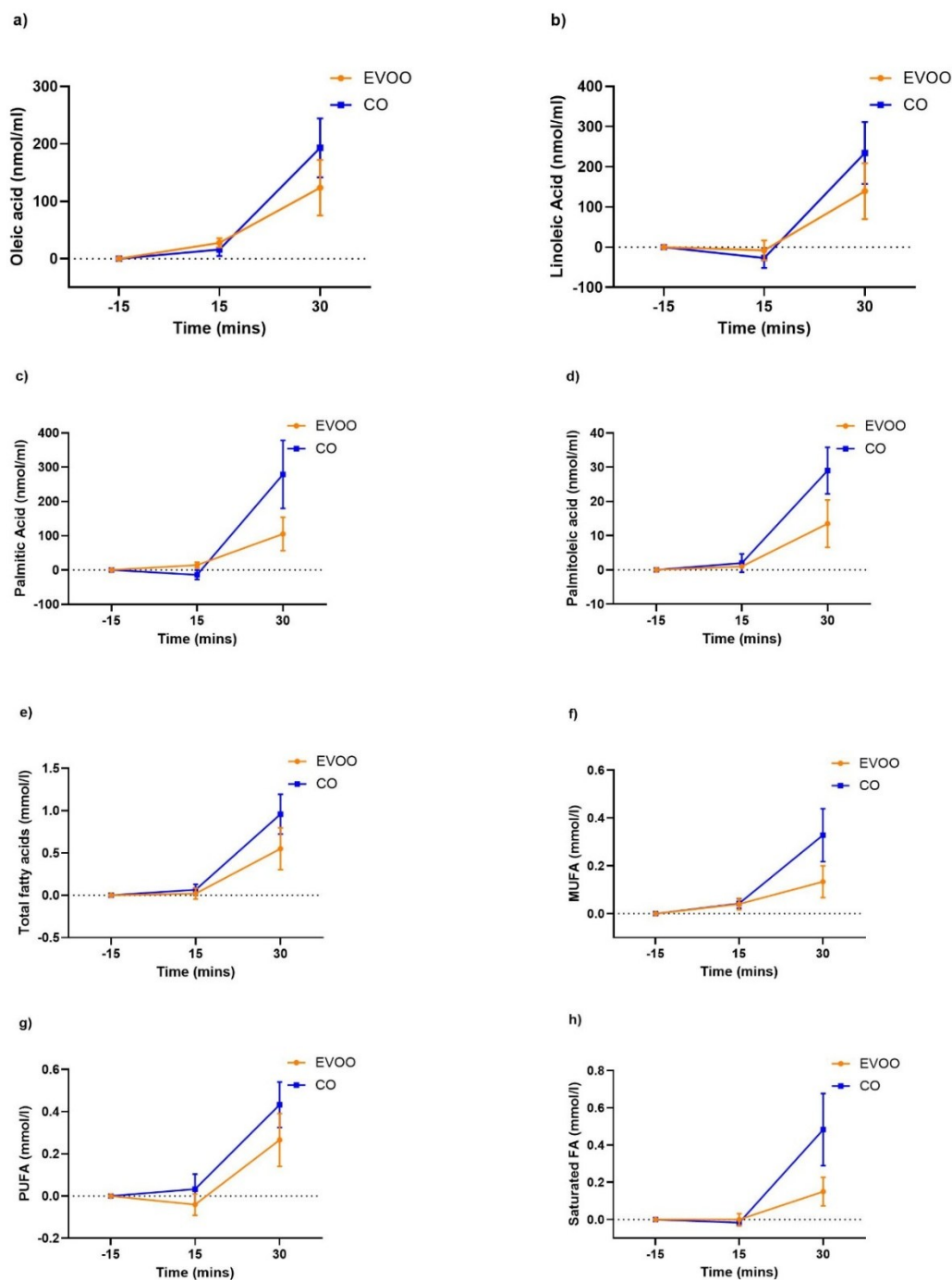


Figure 4.33. Changes in free fatty acid concentrations over 30 minutes following test oils (Protocol 3): a) Oleic acid, b) Linoleic acid, c) Palmitic acid, d) Palmitoleic acid, e) Total fatty acids, f) MUFA g) PUFA, h) Saturated fatty acids. $n = 7$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

4.5.2.5 Visual Analogue Scales

Time course and baseline data for hunger, fullness, nausea and palatability are presented below. Data for irritability, anxiety, sleepiness are included in Appendix 10.

4.5.2.5.1 Protocol 1: Effect of test oil administration immediately before oral glucose (at time 0)

(i) Hunger: At baseline, mean scores were similar [mean ($\pm$ SEM) EVOO: 40.91 mm ($\pm$ 7.35) vs CO: 30.18 mm ($\pm$ 7.58), $P = 0.25$], reflecting moderate hunger levels. Hunger levels decreased immediately after oil consumption at time 0 and 5 minutes ($P < 0.05$, figure 4.34a). The satiating effect of oil appeared to diminish afterwards with mean hunger levels close to baseline by 90 minutes. There were no significant differences in hunger levels across time points between the two test oils.

(ii) Fullness: Fullness scores showed the opposite trend, starting low at baseline [mean ($\pm$ SEM) EVOO: 18.50 mm ($\pm$ 7.63) vs CO: 21.8 mm ($\pm$ 7.39), $P = 0.76$] and rising immediately after ingesting oil. Fullness decreased gradually over time but did not reach baseline by 90 minutes (figure 4.34b). There were no significant differences in fullness levels across time points between the two test oils.

(iii) Nausea: At baseline, mean nausea scores were low across all participants [mean ($\pm$ SEM) EVOO: 3.09 mm ($\pm$ 1.39) vs CO: 1.54 mm ($\pm$ 0.45), $P = 0.24$]. Although nausea score increased after oil ingestion, this was not significant (figure 4.34c).

(iv) Palatability: Palatability was assessed immediately after ingestion of test oils. Score reduced gradually over time, indicating persistent but improved aftertaste (figure 4.34d).

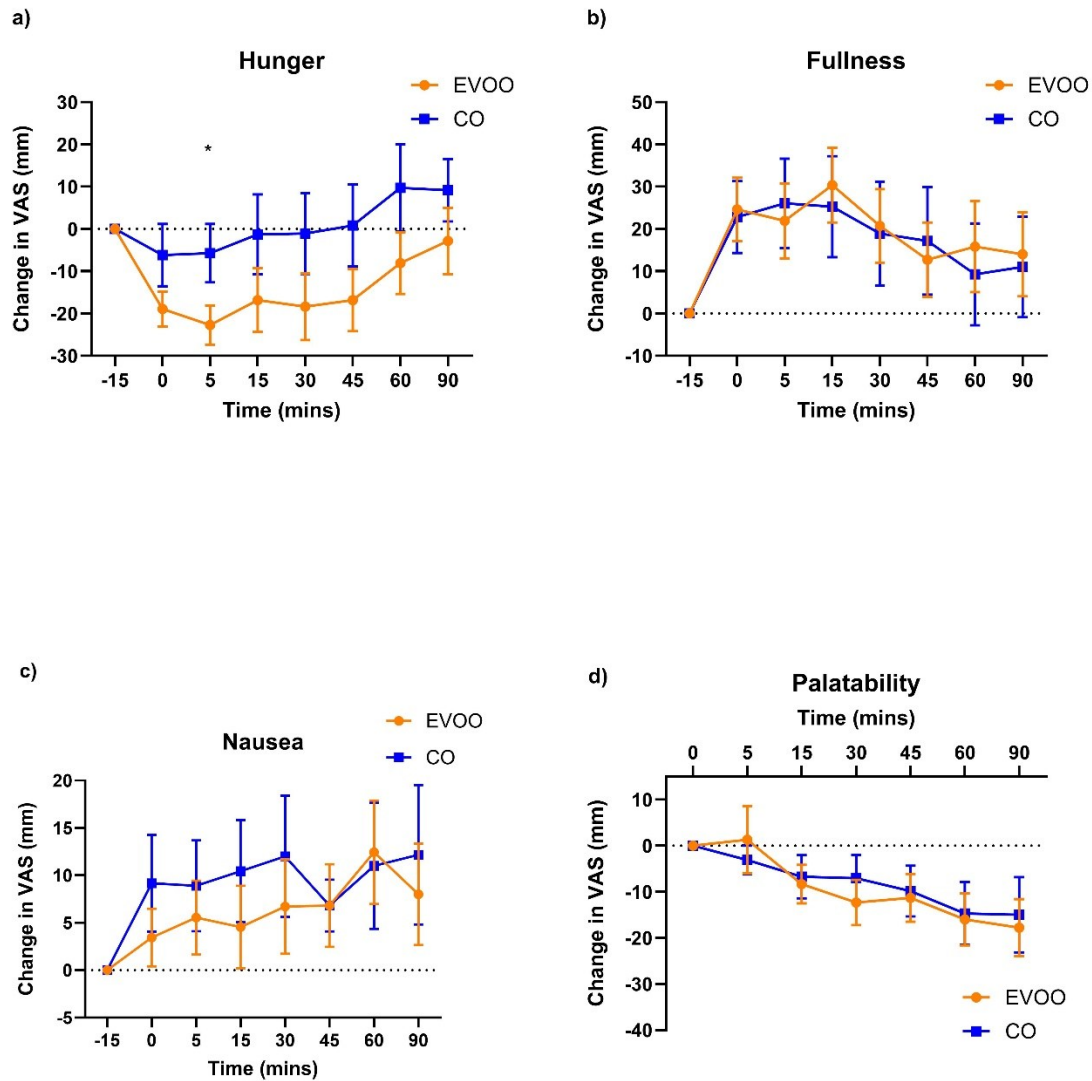


Figure 4.34. Change in VAS measured over 90 minutes following test oils (Protocol 1): a) Hunger scores b) Fullness c) Nausea d) Palatability. $n = 11$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.

4.5.2.5.2 Protocol 2: Effect of test oil preload 15 minutes before oral glucose

(i) Hunger: At baseline, mean scores were similar [mean ($\pm$ SEM) EVOO: 31.28 mm ($\pm$ 12.68) vs CO: 34.71 mm ($\pm$ 11.76), $P = 0.53$], reflecting moderate hunger levels. Hunger scores dropped after oil consumption but returned to baseline levels after EVOO ingestion by 30 minutes. The effects of CO on hunger were more prolonged (figure

4.35a). However, there were no significant differences in hunger levels across time points between the two test oils.

(ii) Fullness: Participants were relatively full at baseline [mean ($\pm$ SEM) EVOO: 49.44 mm ($\pm$ 12.53) vs CO: 37.89 mm ($\pm$ 10.84), $P = 0.17$]. After EVOO ingestion, fullness scores decreased rapidly over time and remained below baseline at 90 minutes (figure 4.35b). There were no significant differences in fullness levels across time points between the two test oils.

(iii) Nausea: At baseline, mean nausea scores were low across all participants [mean ($\pm$ SEM) EVOO: 3.0 mm ($\pm$ 1.80) vs CO: 6.87 mm ($\pm$ 3.92), $P = 0.15$]. Although nausea score increased after oil ingestion, this was not significant (figure 4.35c).

(iv) Palatability: Palatability was assessed immediately after ingestion of test oils. Score reduced gradually over time, indicating persistent but improved aftertaste (figure 4.35d).

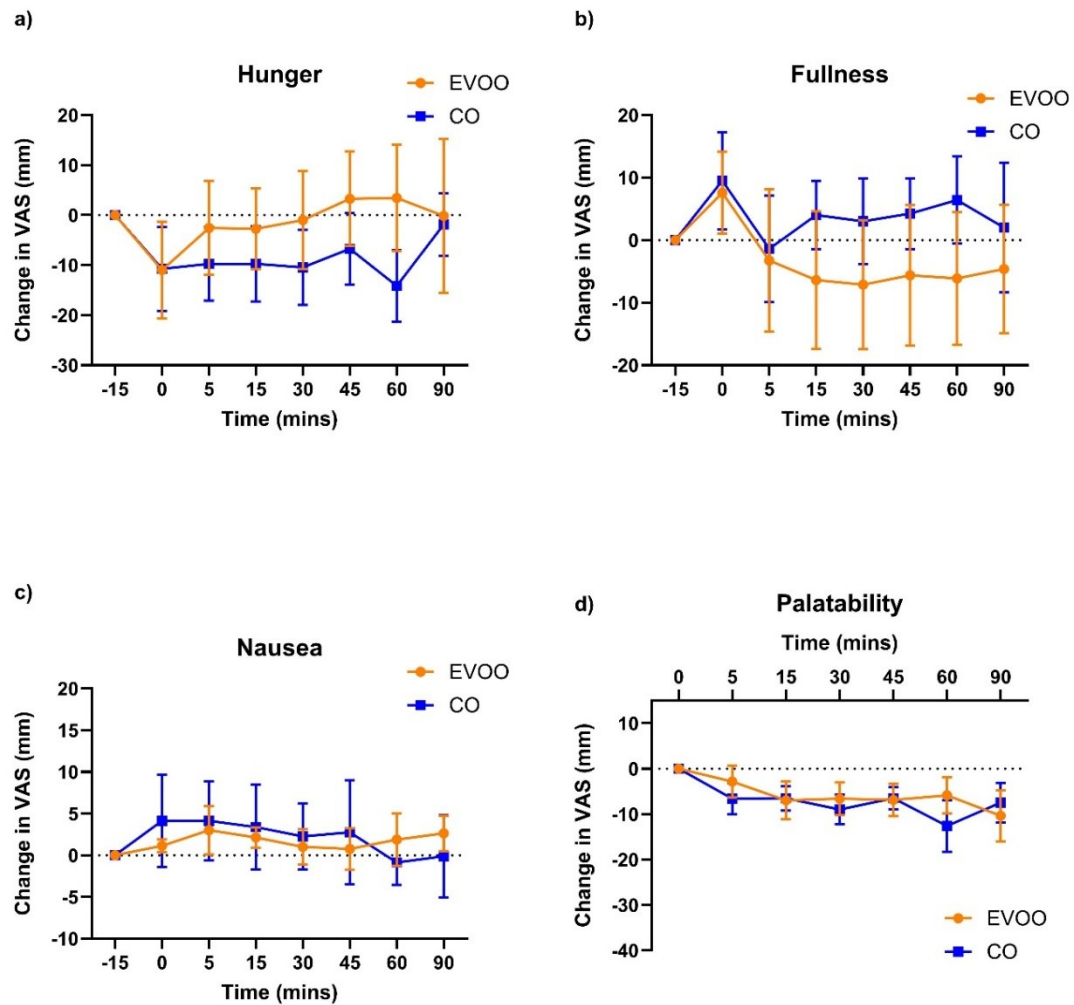


Figure 4.35. Change in VAS measured over 90 minutes following test oils (Protocol 2): a) Hunger scores b) Fullness c) Nausea d) Palatability. $n = 8$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.

4.5.2.5.3 Protocol 3: Effect of test oil preload 15 minutes before intravenous glucose

(i) Hunger: At baseline, mean scores were similar [mean ($\pm$ SEM) EVOO: 24.50 mm ($\pm$ 13.24) vs CO: 33.16 mm ($\pm$ 15.03), $P = 0.16$], reflecting moderate hunger levels. Hunger scores dropped after oil consumption but returned to baseline levels by 45 minutes (figure 4.36a). However, there were no significant differences in hunger levels across time points between the two test oils.

(ii) Fullness: Fullness increased rapidly after oil ingestion but returned near baseline by 5 minutes (figure 4.36b). There were no significant differences in fullness levels across time points between the two test oils.

(iii) Nausea: At baseline, mean nausea scores were low across all participants [mean ($\pm$ SEM) EVOO: 3.17 mm ($\pm$ 1.14) vs CO: 4.00 mm ($\pm$ 1.61), $P = 0.22$]. There was minor elevation in nausea score after oil intake but this was not significant (figure 4.36c).

(iv) Palatability: Palatability score reduced gradually over time after EVOO ingestion, indicating persistent but improved aftertaste (figure 4.36d).

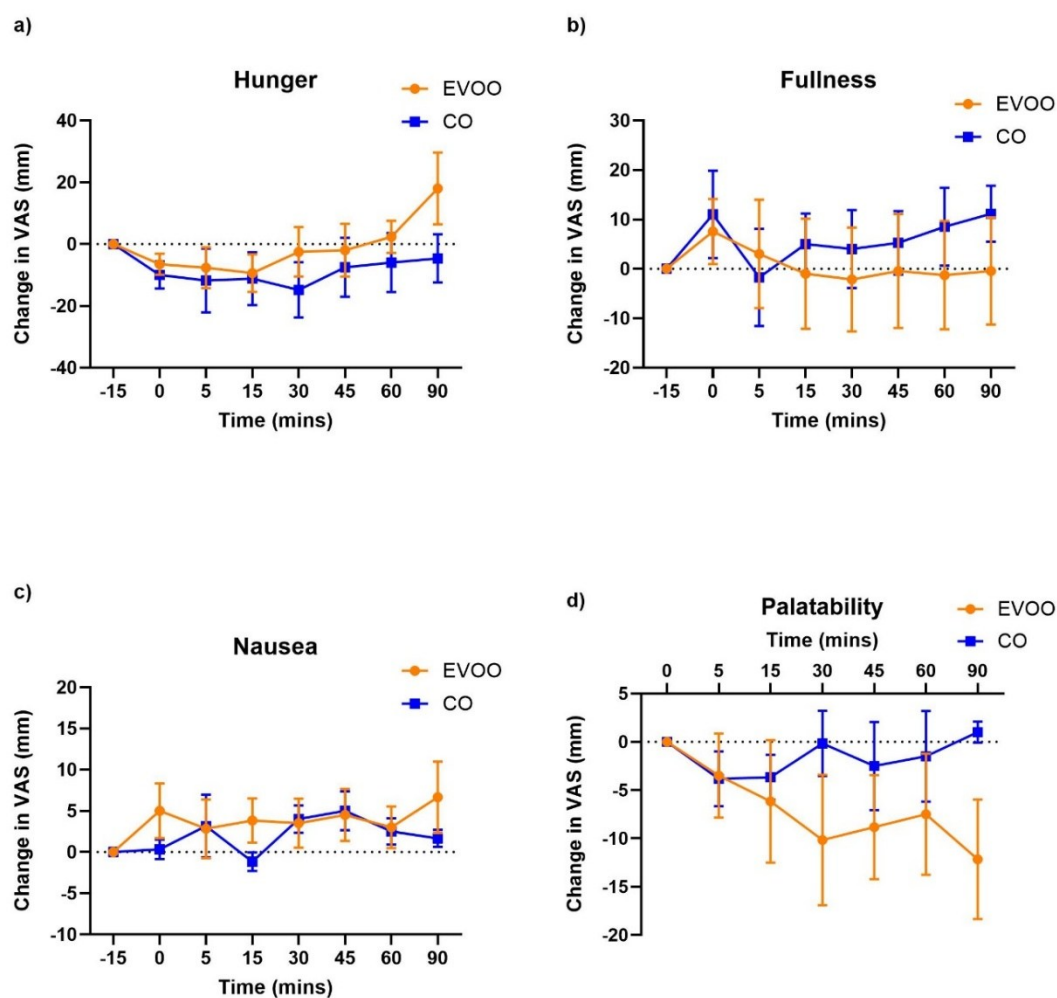


Figure 4.36. Change in VAS measured over 90 minutes following test oils (Protocol 3): a) Hunger scores b) Fullness c) Nausea d) Palatability. $n = 6$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.

The glycaemic outcomes and the gut hormone results are summarised in the table 4.6:

Table 4.6: Summary of glycaemic and gut hormone results			
Outcome	Protocol 1 (n = 15)	Protocol 2 (n = 9)	Protocol 3 (n = 6)
Glucose	↑ over time; no difference in total or net AUC between EVOO vs CO	↑ over time; no difference between EVOO and CO	Peak glucose lower with EVOO at 5 min (Δ -1.33 mmol/L, P = 0.06); total & net AUC not significant
Insulin	↑ over time; no difference in total or net AUC	↑ over time; no difference in total or net AUC	Peak higher and AUC numerically higher with EVOO, but not statistically significant
C peptide	↑ over time; no difference in total or net AUC	↑ over time; no difference in total or net AUC	Peak higher with EVOO at 5 min; no difference in total or net AUC
NT	No difference between oils (total or net AUC)	No difference between oils (total or net AUC)	No difference between oils (total or net AUC)
GLP-1	No difference between oils (total or net AUC)	No difference between oils (total or net AUC)	No significant change; no difference between oils
PYY	EVOO associated with higher PYY at several time points (30, 45, 90 min); AUC not different	EVOO associated with higher PYY at several time points (30, 45, 90 min); AUC not different	No difference between EVOO and CO
Pro-NT	Not measured	Net AUC numerically higher with EVOO, but not statistically significant	No difference between EVOO and CO (net AUC not significant)
EVOO, Extra Virgin Olive Oil, CO, Coconut Oil, AUC, area under the curve, ↑, increase, NT, Neurotensin, GLP-1, Glucagon-Like peptide 1, PYY, Peptide YY, Pro-NT, Proneurotensin			

4.6 Post hoc Power calculations:

In protocol 3, using IV glucose tolerance test, the mean difference in net AUC of glucose over 120 minutes, between EVOO and CO, was 39.37 mmol/l x min and standard deviation of the paired difference was 100.3. Using this data in a two-tailed paired Student's t test, setting a significance level of 0.05 and study power at 0.8, the minimum sample size of 53 participants was calculated for a two-treatment cross over study design. Since the mean difference in glucose at 30 minutes between the two oils was 0.567 mmol/l with a standard deviation of 1.93, the minimum sample size was calculated to be 92 (using the significance of 0.05 and study power of 0.8).

In protocol 2, using oil 15 minutes before oral glucose, the mean difference in net AUC of glucose measured over 120 minutes was 27.85 and standard deviation of the paired difference was 135.1. Using this data in a two-tailed paired Student's t test, setting a significance level of 0.05 and study power at 0.8, the minimum sample size of 187 participants was calculated for a two-treatment cross over study design.

4.7 Discussion

4.7a. Pilot studies:

The pilot studies in the first part of the chapter were designed to assess the effect of incremental doses of EVOO on plasma NT concentrations and on glucose, insulin and c peptide levels.

Plasma NT concentrations increased in the dose-dependent manner in response to the 10 – 30 mls dose of EVOO. However, 40 mls of EVOO did not trigger a notable rise in plasma NT. The rise in plasma NT is thought to be mediated through MUFA stimulation of EECs in proximal small intestine via neural or endocrine pathways. Comparing lower doses of EVOO, the minimal effect of 40 mls dose could be explained by stronger inhibition of gastric emptying. This may minimize the exposure of NT secreting EECs to MUFAs. This amount of fat has been shown to slow down the stomach emptying in both healthy volunteers and those with diabetes (255, 256). Some reports have shown more profound effects of MUFA than saturated fat (257-260). These effects are thought to be mediated primarily by the actions of CCK, which relaxes the gastric fundus, contracts the pyloric sphincter and reduces peristalsis in the small intestine (261). Unsaturated fat has been shown to trigger CCK release more potently than the saturated fat (262).

A similar response was observed in Pro-NT/NMN to 20 mls EVOO dose at 30 and 60 minutes after EVOO ingestion. The mechanism behind the initial, albeit, insignificant

drop in Pro-NT/NMN at time 15 minutes remains unclear. There was dose dependent drop in glucose concentration with 10 – 30 mls of EVOO. However, the corresponding insulin and C-peptide levels dropped below fasting levels post EVOO and remained low during the course of the study. It is probable that NT may exert its effects through insulin independent mechanism. Alternatively, the drop in glucose during the study could be due to the time effect of fasting. This may be further explored using a non-calorific nutritional control such as water.

Since 20 mls of EVOO dose was better tolerated by the participants and was associated with a notable NT and Pro-NT rise, I went on to carry out further clinical studies using this dose.

4.7b Main intervention studies

The findings of the main intervention studies using 20 mls of EVOO suggest that EVOO may improve acute glucose tolerance, although the studies did not demonstrate a notable rise in plasma NT concentrations, suggesting that NT may not mediate this putative effect.

Glucose concentrations were significantly influenced by time across all three protocols reflecting the expected post-glucose load increase. In Protocol 3, the glucose levels dropped below baseline at 90 minutes following administration of both oils. Although not statistically significant, the peak glucose concentration after EVOO was lower at 5 minutes (mean difference -1.33 mmol/l, $P = 0.06$) than CO, suggesting a potentially greater glucose lowering effect of EVOO. Moreover, bypassing the gut-mediated action of major incretin hormones, such as GLP-1 and GIP, may have unmasked the modest glucose-lowering effect of EVOO through other mechanisms. The relatively shorter duration between oil intake and oral glucose load in protocol 1 may not have allowed sufficient time for EVOO to exert its effects on glucose metabolism. Nevertheless, the results of protocol 3 must be interpreted with caution due to fewer

participant numbers ($n = 6$) which may have inflated observed differences due to random variation.

Plasma insulin levels concomitantly increased significantly over time in all three protocols, reflecting the physiological response to glucose administration. In Protocol 3, although not significantly different, the total and net AUCs of insulin concentration were higher following EVOO compared to CO. C-peptide, a marker of endogenous insulin secretion, showed a similar pattern. These observations, though not significant, may suggest a subtle influence of EVOO in augmenting circulating insulin levels in comparison to the CO control.

The MedDiet has been implicated in weight loss and improved glycaemia (275, 276). EVOO, a key component of this diet, is rich in MUFAs. Long term use of EVOO has been linked to both better glycaemic control and prevention of T2DM (277). While these effects are generally attributed to its antioxidant properties, the role of gut nutrient sensing and peptide-mediated glucose regulation, particularly acute glucose tolerance, remains underexplored. To date, only one study has investigated the effect of EVOO on postprandial glucose handling in healthy adults, reporting improved glucose levels and higher insulin and incretin (GLP-1 and GIP) concentrations (127). However, the EVOO in that study was consumed as part of a Mediterranean meal, making it difficult to isolate the effect of EVOO on glucose tolerance from other macronutrient components. Additionally, insulin and gut hormone measurements were limited to a single 120-minute postprandial timepoint, thus restricting insight into the temporal changes in these markers of glucose homeostasis.

Animal studies in our lab indicate that OO administration may improve acute glucose tolerance through NT-modulated insulin release through NTSR1 receptors (unpublished data). The very short half-life of NT in circulation (≈ 30 seconds in rodents) has led to challenges in studying its endocrine effects (278). Although Protocol 1 showed a modest increase in the NT levels following EVOO at 30 and 45 minutes compared to CO (mean difference 30 mins: 4.06 pmol/l, $P = 0.18$, mean

difference 45 mins: 6.1 pmol/l, $P = 0.35$), these findings were not replicated in other protocols. Pro-NT, a relatively stable precursor of NT released in equimolar amounts to NT, was thus, measured in Protocols 2 and 3 (279). However, Pro-NT levels did not differ significantly between groups. Multiple freeze-thaw cycles and fewer participant numbers in Protocol 3 may explain these results.

Oral glucose administration triggered a significant, though expected, rise in GLP-1 levels in both protocols 1 and 2 with no substantial difference between the two test oils. In contrast, when the gut was bypassed in protocol 3, GLP-1 response was markedly attenuated. Similarly PYY concentrations were higher following EVOO ingestion, particularly in protocols 1 and 2. The early postprandial rise in both GLP-1 and PYY, before nutrients reach the dominant L cell population in the distal gut, may be mediated through a direct nutrient interaction with the proximal population of L cells in the duodenum and upper jejunum (140, 280). Additionally, an indirect enteroendocrine neural pathway has been proposed for this early postprandial GLP-1 and PYY release (281, 282). It is worth noting that NT is co-expressed with these gut peptides and has been proposed to act synergistically in mediating these neuroendocrine functions (141).

Mild elevations in triglycerides were observed at 120 minutes post oil ingestion in protocol 1, aligning with previous work by Fawad et al who observed a peak triglyceride rise change of 0.32 mmol/l (95% CI 0.18–0.45 mmol/l, $P < 0.001$) at 3 hours post OO ingestion (237). The triglyceride elevations were not pronounced in Protocol 1 and 3. In Protocol 1, there may not have been sufficient time for triglycerides to appear in circulation. The lack of notable triglyceride rise in Protocol 3 is not fully explained but a smaller sample size may have influenced the detection of subtle changes post oil ingestion. The acute effects of OO on postprandial serum lipid concentrations have not been extensively investigated. The limited reports have shown mixed results. Violi et al noted a small rise in triglycerides (0.2 mmol/l) at 2 hours after EVOO administration (127). Longer term consumption of olive oil has been

linked to favourable effects on serum total cholesterol, LDL, HDL and triglycerides, when compared with other plant oils (283). In contrast, a more recent systematic review including 34 small randomised trials, investigating a dose-response effect of OO concluded that the changes in serum lipids were trivial and larger studies were needed (284). Despite the higher concentration of saturated fat in coconut oil, a randomised trial of a month's consumption of coconut or olive oil reported no difference in the serum lipid profiles when comparing the two oils (285).

Subjective appetite sensations and palatability were evaluated using VAS after ingestion of test oils. VAS are frequently employed tools in nutrition research that often complement the objective appetite measures such as energy intake and gut hormone responses (286, 287). Despite their ease of use, within-individual reproducibility and sensitivity to the experimental conditions, VAS have certain limitations. Substantial variation in the interpretation of VAS questions and understanding of scale may be observed between subjects (288, 289). Hunger decreased markedly after oil and glucose ingestion in Protocol 1, with a more pronounced and sustained effect following EVOO. In protocol 2, hunger suppression was modest and returned to baseline earlier with EVOO than with CO. Protocol 3 showed a similar but transient reduction in hunger for both oils. These findings suggest that the 75g oral glucose load in protocols 1 and 2 contributed noticeably to appetite suppression. Fullness ratings followed reciprocal patterns. Previous investigation has shown that the saturation level of dietary fats in an isoenergetic mixed meal does not differentially affect satiety or subsequent energy intake (290).

Mild transient nausea was commonly reported following both oils across all protocols. Palatability declined slightly over time suggesting mild aftertaste persistence, but no notable differences were observed between oils. Overall, VAS results showed minimal difference between the two oils across all three protocols. This may be due to comparable caloric content, oil consistency and effects on gastric emptying. Moreover,

the low number of participants, particularly in protocols 2 and 3, may have limited detection of subtle differences.

EVOO ingestion led to modest but relatively sustained increases in plasma free fatty acids, particularly MUFAs, likely reflecting its richer composition. However, in protocol 3, elevation of free fatty acids was more pronounced following CO. Although this could be due to a smaller sample size, Lyte et al observed a much higher postprandial levels of non-esterified fatty acids (at 60 and 120 minutes) following CO ingestion in a mixed meal, in comparison to olive, grapeseed and fish oils. Nevertheless, treatment meal x time interaction was not significantly different in this study (289).

These clinical studies aimed to explore the role of NT in postprandial glucose handling in healthy adults. The cross-over design minimised inter-individual variability and the inclusion of three distinct protocols with varying preload intervals enabled multiple mechanistic assessments. Nevertheless, several limitations must be acknowledged. Participant heterogeneity, while enhancing generalisability, may have introduced biological variability in the glycaemic responses observed. The relatively short-term evaluation of postprandial glucose homeostasis may not have captured the delayed physiological effects of dietary fat on glucose tolerance. The short plasma half-life of NT posed challenges in analysing its putative role and while Pro-NT was used as a proxy, sample degradation may have affected accuracy. Most key metabolic outcomes such as glucose, insulin and c-peptide showed no significant differences between oils, limiting conclusive interpretation. The observed differences in protocol 3 require cautious interpretation given the limited statistical power. The observed effects of EVOO were small. The substantial inter-individual variability in postprandial glucose handling may have further limited statistical power despite the crossover design. Additionally, the absence of a control arm without oil preload restricts the evaluation of the glucose-lowering action of EVOO.

Future work should consider larger, more homogenous cohorts and adopt intravenous glucose tolerance tests to better isolate the role of NT and its precursors. Use of the Pro-NT assay across extended time points along with control arms would allow a more detailed evaluation of EVOO's effects on postprandial glucose handling. Participant characteristics such as BMI, glucose tolerance, ethnic background and levels of physical activity should be factored into the study design to minimise confounding biological variation.

Chapter 5: General discussion

T2DM is a complex metabolic disorder responsible for considerable morbidity, mortality and high socioeconomic burden. It is one of the leading health issues of the past century (2). Diabetes management has witnessed remarkable progress in the last 20 years. T2DM remission, previously considered unattainable, is now achievable in those who are able to achieve and maintain marked weight loss, through energy restriction or following metabolic surgery.

Understanding gut peptide physiology, notably of the incretins (GLP-1 and GIP), has led to a paradigm shift in the pharmacological management of T2DM and obesity. Continued progress in incretin-based pharmacotherapies has led to the development of increasingly potent glucose and body weight lowering single peptide agents such as Liraglutide and Semaglutide and more recently the unimolecular peptide based dual agonist, Tirzepatide. Despite this incredible success, these agents are largely inaccessible to the majority of global T2DM population who live in low and middle-income countries (291). Moreover, current therapeutic strategies are often hindered by adverse effects and limited long-term compliance. At least, 4 in 5 adults with diabetes are estimated to be living in middle or low income countries (2). It is thus desirable and important to explore other affordable therapeutic avenues such as dietary modification that exploit intestinal nutrient sensing, thereby utilising ‘functional’ benefits of foods beyond their nutritional value. The delivery of good quality diabetes care especially in poorer countries is an even greater challenge. In such economies, effective diabetes management through employing dietary strategies that are affordable, culturally appropriate and sustainable, is more crucial.

The MedDiet has gained popularity in recent years due to its various health benefits attributed primarily to its high content of fruits, vegetables, legumes, nuts, and notably, olive oil. Extra virgin olive oil (EVOO) is produced by manually crushing and pressing olives without using heat or chemical solvents (292). Considered

nutritionally rich, many cardiometabolic benefits of EVOO are attributed to its bioactive compounds (such as polyphenols) with antioxidant properties and MUFAs. The high MUFA concentration has been shown to reduce many cardiovascular risk factors when it is substituted into the diet in place of saturated fats (293).

Neurotensin (NT), a 13 amino acid peptide, is secreted in response to fat ingestion from enteroendocrine N cells in distal small bowel. It is also co-secreted with GLP-1 and PYY from enteroendocrine cells located more proximally in the small intestine. Gastrointestinal NT is reported to regulate gut motility, promote fat absorption and coordinate energy balance (294). Oleic acid, a major MUFA in EVOO (55 – 83%), stimulates NT release (145, 295, 296). The suggested glucoregulatory roles of peripherally secreted NT are its effects on insulin secretion, glucose homeostasis and pancreatic beta cell preservation. NT's role in mediating glucose homeostasis remains unclear. Earlier research proposed its dual actions on glucose homeostasis; NT can stimulate insulin and glucagon secretion at low glucose concentrations but inhibits their release when glucose or arginine levels are high (161, 162). These effects are attributed to differential receptor expression and interactions in pancreatic islets. However, this observed dual role of NT in glucose regulation was not replicated in other studies (163, 164).

The aim of this thesis was to investigate the glucoregulatory role of an EVOO preload and if this is mediated by NT.

5.1 Neurotensin and Metabolic Health

NT exhibits dual action on energy balance depending on its site of action, exerting anorexigenic effects centrally while promoting lipid absorption and storage peripherally. Its stable precursor, pro-neurotensin (Pro-NT), has been linked in observational studies to obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), and cardiovascular events (172).

Previous studies have not investigated the association of the circulating, metabolically active NT with glycaemic markers or microvascular complications of diabetes. Through a cross-sectional analysis in a cohort of 802 individuals, I was able to demonstrate positive, albeit weak, correlations between fasting plasma NT levels and key metabolic markers, such as HbA1c, fasting blood glucose, total cholesterol, LDL, and triglycerides. NT levels were higher in individuals with T2DM compared to those with normoglycaemia. Moreover, NT levels were also higher in females. However, no direct association was found between NT and the presence of microvascular complications of diabetes. These results align with previous reports of positive associations between elevated fasting Pro-NT concentrations and presence or development of diabetes. Barchetta et al found circulating Pro-NT levels to be significantly higher in people with T2DM (194.8 ± 115.6 vs 154.2 ± 74.2 pmol/L, $p = 0.038$) (297). Pro-NT has also been linked to a higher risk of incident diabetes in multiple longitudinal cohorts; Melander et al. noted a 28% increased risk of developing diabetes in those with higher Pro-NT over 15 years (184). Fawad et al. also observed this increased risk, though only among women, suggesting potential sex-specific differences in NT signalling (185). Collectively, these findings suggest that NT could be a biomarker of metabolic impairment, likely reflecting its role in dietary fat absorption and obesity. However, the causal role of NT in the development of metabolic dysfunction remains to be established. One possible explanation of the weak or absence of associations observed in our study may be due to the unstable nature of NT in circulation. In rodents, the half-life of NT has been estimated to be only 30 seconds; this complicates investigating its pathophysiological relevance in the development of metabolic disorders (278, 298).

An unexpected finding was the negative correlation of NT with FIB-4 scores. FIB-4 scores are used in predicting the presence and severity of NAFLD (299). Higher FIB-4 scores are indicative of more advanced liver fibrosis. The inverse correlation between NT and FIB-4 is not fully explained. A possible reason could be a more rapid

degradation of NT in those with worsening liver disease; however, this requires further investigation in larger cohorts with biopsy proven NAFLD to draw definitive conclusions.

5.2 Fat intake and glucose tolerance

Following this analysis, I explored the existing literature for the acute effects of addition of dietary fat into a carbohydrate meal and MUFA rich diets (compared against SFA) on postprandial glucose excursion through a systematic review and meta-analyses. A review of existing literature suggests different types of dietary fat have highly variable effects on glycaemic response. Many reports have not seen a difference in glucose lowering with addition of fat (300-304). Conversely, a number of studies noted varied effects of fat addition on glucose lowering (234, 268, 305-307). Our analysis showed that the addition of fat leads to a nonsignificant attenuation of postprandial glucose rise without a visible effect on insulin levels. The delayed gastric emptying caused by fat addition may explain this response. Moreover, dietary fat has been shown to trigger an immediately blunted but more sustained incretin secretion than carbohydrate alone; this may play a role in glucose lowering (211).

Contrary to the previous evidence showing that the chronic administration of fat, and particularly MUFA, may lower glucose and HbA1c; a single meal of MUFA rich diet did not appear to exert an immediate effect on postprandial glucose lowering, in our pooled analysis. However, the acute SFA intake led to a greater insulin response suggesting a possible transient effect on insulin resistance. Further analyses should include longer postprandial monitoring (beyond 2 hours) especially as dietary fat slows gastric emptying. Isolating fat effects by comparing standardized meals and macronutrient composition may reveal acute effects on postprandial glycaemic response. In addition, investigating the acute glucoregulatory effects of plant vs animal sources of dietary fat (especially MUFA) may yield more insights (266).

5.3 Olive Oil and Neurotensin Secretion: Dose–Response Insights

These data analyses were further expanded by investigating the dose-dependent effects of EVOO on NT secretion in healthy volunteers. I carried out the pilot crossover studies using incremental doses of EVOO. The NT concentrations increased significantly with 10–30 mL doses of EVOO, peaking at 60–90 minutes post-ingestion. Interestingly, the 40 mL dose of EVOO did not elicit a further increase in NT, likely due to delayed gastric emptying limiting MUFA exposure in the proximal intestine (253).

These findings confirmed that the EVOO, rich in oleic acid, can effectively stimulate NT secretion, with 20–30 mL appearing to be the optimal dose range in these studies. A decline in glucose levels was observed following EVOO ingestion, suggesting that NT may have a glucose-lowering effect. Alternatively, this glucose dip could reflect a longer period in fasting.

5.4 Olive Oil Preload and Glucose Tolerance

Following the pilot studies, I evaluated if a 20ml preload of EVOO could acutely improve glucose tolerance compared to coconut oil (CO) control in healthy volunteers. Through a series of crossover protocols (including oral and intravenous glucose tolerance tests), the study tested the hypothesis that EVOO improves postprandial glucose responses by stimulating NT.

Although no statistically significant changes were observed in glucose, insulin, or C-peptide AUCs, a trend toward lower postprandial glucose levels was noted following EVOO, especially in protocol 3 employing the intravenous glucose. This suggests a potential glucoregulatory effect that may not have been captured within this protocol's power limitations or timing window.

Interestingly, although NT increased after EVOO administration, these changes were not significantly different from the saturated fatty acid rich CO, suggesting that the glucose changes may not be mediated by NT. The short half-life and rapid metabolism of NT may have influenced the accurate measurement of its response. It reinforces the importance of measuring Pro-NT or investigating local tissue concentrations for a more detailed mechanistic evaluation.

To date, NT has not been considered to be a suitable target for pharmacotherapeutic translation. This is primarily due to its central effects on temperature regulation, and on dopamine signalling, which have potential unfavourable implications in addiction, schizophrenia and Parkinson's disease (308). However, selective exploitation of its peripheral actions may have a therapeutic potential. In mice, a combination of a long acting pegylated form of NT with Liraglutide demonstrated synergism in lowering food intake and weight; however, the combination peptides did not affect acute glucose tolerance (174).

This thesis outlines evidence for the potential role of dietary lipids in glycaemic management. Manipulation of the diet especially altering its macronutrient components may improve glucose tolerance modestly and thus may have a role to play in diabetes management. Future work should use measurement of stable NT assays and its precursors over a longer observation period to delineate its role in glucose homeostasis. This unexplored area of gut peptide physiology may have significant therapeutic potential.

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Appendices

Appendix 1. Radio-immunoassay (RIA) for gut peptides

2.1 RIA Procedure

The assays were performed in duplicate tubes. Each tube contained RIA buffer (0.06M phosphate buffer, 0.3% bovine serum albumin BSA, 0.2% Tween-20) and 100 µl of plasma was added into each tube. A total volume of 700 µl of reaction mixture per tube was left for incubation for three days at 4°C. Following tubes were also added at the beginning of each assay:

1. Non-specific binding (NSB) tubes: radiolabelled antigen without any antibody to check non-specific binding of the unknown antigen to the radiolabel.
2. Half and twice the required volume of labelled antigen added to check the assay sensitivity.
3. Zero tubes: tubes without sample distributed throughout the assay to detect baseline drift within the assay.
4. Excess antibody tubes: Antibody added at six times the amount required for other tubes to assess the immunological integrity and binding of radiolabelled antigen.

The free and antibody bound peptide label were separated using charcoal adsorption method (2.4% charcoal, 0.24% dextran in 0.1M PBS supplemented with gelatine). A 250 µl of dextran coated charcoal was added to each tube, followed by centrifugation at 4°C, 1500xg for 20 mins. The supernatant containing bound peptide is aspirated and transferred to a separate collection tube. Both charcoal pellet containing free peptide and supernatant fractions were counted for 2 minutes using gamma scintillation counter (LB2111 Multi Crystal Gamma Counter, Berthold Technologies, Bad Wildbad, Germany). The peptide concentration in each sample was interpolated from the

standard curve using a non-linear plot in Graphpad Prism 9.5.1 (GraphPad Software, San Diego, CA, USA).

2.2 GLP-1 assay

The GLP-1 immunoreactivity was measured using an established in-house RIA (309). The GLP-1 antibody was produced in rabbits against GLP-17-36 amide coupled to BSA. The antibody cross-reacts fully with all amidated forms of GLP-1 but does not cross-react with glycine extended forms (GLP-1₁₋₃₇ and GLP-1₇₋₃₇) or any other known pancreatic or GI peptides. The GLP-1 labels were prepared by iodogen method and purified by reverse HPLC method (193). The assay was incubated for three days at 4°C before separation using charcoal absorption. Further details on the assay procedures are described as above (section 2.1).

2.3 PYY assay

PYY-like immunoreactivity was measured with a previously established in-house radioimmunoassay (310). The antiserum to PYY (Y21) was produced against synthetic porcine PYY₃₋₃₆ coupled to BSA using glutaraldehyde. This antibody cross-reacts fully with both PYY₃₋₃₆ and PYY₁₋₃₆, but not with pancreatic polypeptide, neuropeptide Y, or other known gut peptides. The PYY radiolabels were prepared by iodogen method and purified by reverse HPLC method (193). The assay was incubated for three days at 4°C before separation of the free and antibody-bound label by charcoal adsorption. Further details on the assay procedures are described as above (section 2.1).

Appendix 2. Logistic Regression Analysis of Predictors Associated with Microvascular Complications in Individuals with Type 2 Diabetes

	B	SE	Odds Ratio	(95% CI)	P value
Age (years)	0.046	0.009	1.047	(1.029 – 1.065)	< .001
Sex	-0.134	0.168	0.874	(0.630 – 1.214)	0.423
Weight (kg)	-0.002	0.005	0.998	(0.989 – 1.008)	0.736
BMI (kg/m ²)	-0.001	0.014	0.999	(0.972 – 1.027)	0.960
Waist circumference (cm)	0.014	0.007	1.014	(0.999 – 1.029)	0.061
Systolic BP (mmHg)	0.014	0.005	1.014	(1.004 – 1.024)	0.061
Diastolic BP (mmHg)	-0.012	0.008	0.989	(0.973 – 1.004)	0.156
Smoking	0.202	0.238	1.224	(0.767 – 1.952)	0.397
Diabetes duration (years)	-0.014	0.012	0.986	(0.963 – 1.009)	0.238
Hypertension	0.665	0.186	1.945	(1.350 – 2.802)	<0.001
Dyslipidaemia	0.537	0.234	1.711	(1.081 – 2.708)	0.022
HbA1c (mmol/mol)	0.025	0.006	1.026	(1.014 – 1.038)	<0.001
Fasting Glucose (mmol/l)	0.067	0.027	1.069	(1.014 – 1.127)	0.013
HOMA-IR	-0.170	0.132	0.843	(0.898 – 1.060)	0.197
Creatinine (umol/l)	0.014	0.004	1.014	(1.006 – 1.022)	<0.001
Total cholesterol (mmol/l)	-0.039	0.065	0.962	(0.846 – 1.093)	0.553
LDL (mmol/l)	-0.130	0.089	0.878	(0.738 – 1.045)	0.143
HDL (mmol/l)	-0.049	0.255	0.952	(0.578 – 1.568)	0.847
Triglycerides (mmol/l)	0.038	0.086	0.038	(0.877 – 1.229)	0.661
Neurotensin (pmol/l)	-0.001	0.003	0.999	(0.993 – 1.006)	0.812
Log Neurotensin (pmol/l)	-0.078	0.395	0.925	(0.427 – 2.005)	0.844

Table 2.4: Univariate logistic regression analysis. The presence of any microvascular complication is the dependent variable.

	B	SE	Odds Ratio	(95% CI)	P value
Intercept	-4.914	0.923	0.007		< .001
Age (years)	0.039	0.01	1.04	(1.021 – 1.06)	< .001
Systolic BP (mmHg)	0.005	0.006	1.005	(0.994 – 1.016)	0.387
Hypertension	0.236	0.208	1.266	(0.843 – 1.901)	0.256
Dyslipidaemia	0.239	0.261	1.27	(0.762 – 2.117)	0.359
Creatinine (umol/l)	0.012	0.004	1.012	(1.004 – 1.021)	0.003
HbA1c (mmol/mol)	0.025	0.008	1.025	(1.01 – 1.041)	0.001
Glucose (mmol/l)	-0.004	0.036	0.996	(0.928 – 1.069)	0.908

Table 2.5: Multivariable binary logistic regression analysis. The presence of any microvascular complication is the dependent variable.

Appendix 3. Database search strategies

Ovid MEDLINE(R)

Searched: 22/09/2022

- 1 diet* fat*.mp.
- 2 exp Dietary Fats/
- 3 exp Fats, Unsaturated/
- 4 exp Diet, High-Fat/
- 5 exp Diet, Fat-Restricted/
- 6 (fat* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 7 (oil* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 8 (lipid* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 9 (fatty acid* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

- 10 ((monounsaturated or mono-unsaturated or polyunsaturated or poly-unsaturated or unsaturated) adj5 fat*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 11 exp Fish Oils/ or exp Plant Oils/
- 12 exp Fatty Acids/ad [Administration & Dosage]
- 13 (fat* tolera* test* or FTT).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 14 ((Vegetable* or Olive or Rape* or sunflow* or Linseed*) adj5 oil*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 15 ((monounsaturated or mono-unsaturated or polyunsaturated or poly-unsaturated or unsaturated) adj5 oil*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 16 exp Fat Emulsions, Intravenous/
- 17 (healthy adj5 (adult* or participant* or volunteer* or subject* or individual* or control*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 18 exp Healthy Volunteers/
- 19 (nondiabet* or non-diabet* or nonobes* or non-obes* or normoglyc* or normoglyc*).mp. [mp=title, abstract, original title, name of substance word, subject heading

word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

20 (Normal glucose tolerance or NGT).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

21 glyc?emi*.mp.

22 exp Blood Glucose/

23 ((postprandial* or post prandial or post-prandial*) adj5 glucose).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

24 ((postprandial* or post prandial or post-prandial*) adj5 insulin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

25 (glucose adj5 (toleran* or level* or reading* or measure* or chang* or index* or output* or concentr* or profile* or response* or fall* or drop*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

26 (insulin adj5 (secret* or level* or measure* or chang* or output* or concentr* or profile* or rise* or incr* or respons* or releas*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

- 27 (OGTT or IVGTT).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 28 exp Glucose Tolerance Test/
- 29 exp Postprandial Period/
- 30 (area under the curve or area under curve or AUC).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 31 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16
- 32 17 or 18 or 19 or 20
- 33 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30
- 34 31 and 32 and 33
- 35 limit 34 to (english language and humans)

Pubmed

Searched: 22/09/2022

("dietary fat*" [tw] OR "fat intake" [tw] OR "fat ingestion" [tw] OR "fat load*" [tw] OR "fat emulsi*" [tw] OR "fat administr*" [tw] OR "fat additi*" [tw] OR "fat supplement*" [tw] OR "fat rich*" [tw] OR "fat toleran*" [tw] OR "fat challen*" [tw] OR "fat consum*" [tw] OR "oil intake" [tw] OR "oil ingestion" [tw] OR "oil load*" [tw] OR "oil emulsi*" [tw] OR "oil administr*" [tw] OR "oil additi*" [tw] OR "oil supplement*" [tw] OR "oil rich*" [tw] OR "oil toleran*" [tw] OR "oil challen*" [tw] OR "oil consum*" [tw] OR "lipid intake" [tw] OR "lipid ingestion" [tw] OR "lipid load*" [tw] OR "lipid emulsi*" [tw] OR "lipid administr*" [tw] OR "lipid additi*" [tw] OR "lipid supplement*" [tw] OR "lipid rich*" [tw] OR "lipid toleran*" [tw] OR "lipid challen*" [tw] OR "lipid consum*" [tw] OR "fatty acid intake" [tw] OR "fatty acid ingestion" [tw] OR "fatty acid ingestion" [tw] OR "fatty acid load*" [tw] OR "fatty acid emulsi*" [tw] OR "fatty acid administr*" [tw] OR "fatty acid additi*" [tw] OR "fatty acid supplement*" [tw] OR "fatty acid rich*" [tw] OR "fatty acid toleran*" [tw] OR "fatty acid challen*" [tw])

OR "fatty acid consum*" [tw] OR "monounsaturated fat*" [tw] OR "mono-unsaturated fat*" [tw] OR "mono-unsaturated fat*" [tw] OR "polyunsaturated fat*" [tw] OR "poly-unsaturated fat*" [tw] OR "fat tolerance test" [tw] OR "Plant Oils"[MeSH] OR "Dietary Fats"[MeSH] OR "Diet, High-Fat"[MeSH] OR "Diet, Fat-Restricted"[MeSH] OR "Fat Emulsions, Intravenous"[MeSH] OR "Fatty Acids"[MeSH]) AND ("healthy subject*" [tw] OR "healthy adult*" [tw] OR "healthy volunteer*" [tw] OR "healthy participant*" [tw] OR "healthy control*" [tw] OR "healthy individual*" [tw] OR "nondiabet*" [tw] OR "non-diabet*" [tw] OR "nonobes*" [tw] OR "normogly*" [tw] OR "normo-gly*" [tw] OR "normal glucose toleran*" [tw] OR "Healthy Volunteers"[MeSH]) AND ("glycemi*" [tw] OR "glycaemi*" [tw] OR "postprandial glucose" [tw] OR "post-prandial glucose" [tw] OR "postprandial insulin" [tw] OR "post-prandial insulin" [tw] OR "glucose readin*" [tw] OR "glucose respon*" [tw] OR "glucose level*" [tw] OR "glucose measur*" [tw] OR "glucose chang*" [tw] OR "glucose index*" [tw] OR "glucose output*" [tw] OR "glucose conc*" [tw] OR "glucose profi*" [tw] OR "glucose fall*" [tw] OR "glucose drop*" [tw] OR "insulin respon*" [tw] OR "insulin level*" [tw] OR "insulin measur*" [tw] OR "insulin chang*" [tw] OR "insulin index*" [tw] OR "insulin output*" [tw] OR "insulin conc*" [tw] OR "insulin profi*" [tw] OR "insulin rise*" [tw] OR "insulin releas*" [tw] OR "insulin incr*" [tw] OR "Oral glucose tolerance test" [tw] OR "intravenous glucose tolerance test" [tw] OR "OGTT" [tw] OR "IVGTT" [tw] OR "area under the curve" [tw] OR "area under curve" [tw] OR "AUC" [tw] OR "Blood Glucose"[MeSH] OR "Postprandial Period"[MeSH] OR "Glucose Tolerance Test"[MeSH] OR "Area Under Curve"[MeSH])

Embase

Searched: 22/09/2022

- 1 diet* fat*.mp.
- 2 exp fat intake/ or exp lipid diet/
- 3 Oil*.mp.
- 4 exp vegetable oil/ or exp fish oil/
- 5 Lipid*.mp.
- 6 Fatt* acid*.mp.

- 7 exp fatty acid/
- 8 fat* in*.mp.
- 9 monounsaturated fat*.mp.
- 10 mono-unsaturated fat*.mp.
- 11 exp monounsaturated fatty acid/
- 12 polyunsaturated fat*.mp.
- 13 poly-unsaturated fat*.mp.
- 14 exp saturated fatty acid/ or exp polyunsaturated fatty acid/
- 15 fat* load*.mp.
- 16 fat* administr*.mp.
- 17 oil* administr*.mp.
- 18 ((VEGETABLE* or OLIVE* or RAPE* or SUNFLOW* or LINSEED* or MONOUNSAT* or POLY*) and oil).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 19 ((LOW* or MODIFI* or ANIMAL* or VEGETABLE* or ACID* or MONOSAT* or POLYUNSAT* or SATURAT* or UNSATUR*) and FAT).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 20 oral* fat*.mp.
- 21 exp lipid emulsion/
- 22 lipi* emul*.mp. or exp intralipid/
- 23 lipi* administr*.mp.
- 24 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
- 25 health* adul*.mp.
- 26 exp volunteer/ or exp normal human/

- 27 health* volunt*.mp.
- 28 health* subje*.mp.
- 29 health* partici*.mp.
- 30 norm* subj*.mp.
- 31 (nondiabetic or nonobese or normoglycaemic* or normoglycemic*).mp.
[mp=title, abstract, heading word, drug trade name, original title, device
manufacturer, drug manufacturer, device trade name, keyword heading word,
floating subheading word, candidate term word]
- 32 (normoglyc* or NGT or NG or normal glucose toleran*).mp. [mp=title,
abstract, heading word, drug trade name, original title, device manufacturer, drug
manufacturer, device trade name, keyword heading word, floating subheading
word, candidate term word]
- 33 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32
- 34 glucos*.mp.
- 35 gluco* toler*.mp.
- 36 exp insulin/ or exp glucose blood level/ or exp glucose tolerance test/ or exp
glucose tolerance/
- 37 glycem*.mp.
- 38 glycaem*.mp.
- 39 exp glycemic control/
- 40 exp postprandial state/ or postprandi*.mp.
- 41 post-prandi*.mp.
- 42 insuli*.mp.
- 43 insuli* profi*.mp.
- 44 (OGTT or oral glucose tolerance test).mp. [mp=title, abstract, heading word,
drug trade name, original title, device manufacturer, drug manufacturer, device
trade name, keyword heading word, floating subheading word, candidate term
word]

- 45 (insulin and (level or measurement or index or load or secretion or test)).mp.
[mp=title, abstract, heading word, drug trade name, original title, device
manufacturer, drug manufacturer, device trade name, keyword heading word,
floating subheading word, candidate term word]
- 46 (IVGTT or intravenous glucose tolerance test).mp. [mp=title, abstract, heading
word, drug trade name, original title, device manufacturer, drug manufacturer,
device trade name, keyword heading word, floating subheading word, candidate
term word]
- 47 exp area under the curve/ or area under the curve*.mp.
- 48 exp "AUC (0-8 h)"/ or exp "AUC (0-4 h)"/ or exp "AUC (0-24 h)"/ or exp "AUC
(0-48 h)"/ or exp "AUC (0-2 h)"/ or AUC*.mp. or exp "AUC (0-12 h)"/ or exp "AUC (0-
6 h)"/
- 49 area under curve*.mp.
- 50 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or
48 or 49 1885328
- 51 24 and 33 and 50
- 52 limit 51 to (human and english language)
- 53 diet* fat*.mp.
- 54 exp fat intake/ or exp lipid diet/
- 55 (fat* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or
tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, heading word,
drug trade name, original title, device manufacturer, drug manufacturer, device
trade name, keyword heading word, floating subheading word, candidate term
word]
- 56 (oil* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement* or
tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, heading word,
drug trade name, original title, device manufacturer, drug manufacturer, device
trade name, keyword heading word, floating subheading word, candidate term
word]
- 57 (lipid* adj5 (in* or load* or emulsion* or adminstr* or additi* or supplement*
or tolera* or challeng* or consum* or rich*)).mp. [mp=title, abstract, heading word,
drug trade name, original title, device manufacturer, drug manufacturer, device

trade name, keyword heading word, floating subheading word, candidate term word]

58 ((monounsaturated or mono-unsaturated or polyunsaturated or poly-unsaturated or unsaturated) adj5 fat*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

59 (fat* tolera* test* or FTT).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

60 exp vegetable oil/

61 exp fish oil/

62 ((Vegetable* or Olive or Rape* or sunflow* or Linseed*) adj5 oil*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

63 ((monounsaturated or mono-unsaturated or polyunsaturated or poly-unsaturated or unsaturated) adj5 oil*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

64 exp lipid emulsion/

65 exp intralipid/

66 (healthy adj5 (adult* or participant* or volunteer* or subject* or individual* or control*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

67 exp volunteer/

68 exp normal human/

69 (nondiabet* or non-diabet* or nonobes* or non-obes* or normoglyc* or normoglyc*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

- 70 (Normal glucose tolerance or NGT).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 71 glyc?emi*.mp.
- 72 exp glycemic control/ or exp glucose blood level/
- 73 ((postprandial* or post prandial or post-prandial*) adj5 glucose).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 74 ((postprandial* or post prandial or post-prandial*) adj5 insulin).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 75 (glucose adj5 (toleran* or level* or reading* or measure* or chang* or index* or output* or concentr* or profile* or response* or fall* or drop*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 76 (insulin adj5 (secret* or level* or measure* or chang* or output* or concentr* or profile* or rise* or incr* or respons* or releas*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 77 (OGTT or IVGTT).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
- 78 exp glucose tolerance test/
- 79 exp postprandial state/
- 80 (area under curve or area under the curve or AUC).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

- 81 exp area under the curve/
- 82 exp fatty acid/bd, ad, du, ig, ip, iv, po, pa [Buccal Drug Administration, Drug Administration, Intraduodenal Drug Administration, Intragastric Drug Administration, Intraperitoneal Drug Administration, Intravenous Drug Administration, Oral Drug Administration, Parenteral Drug Administration]
- 83 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 82
- 84 66 or 67 or 68 or 69 or 70
- 85 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81
- 86 83 and 84 and 85
- 87 limit 86 to (human and english language)

Scopus

Searched: 21/09/2022

((TITLE-ABS-KEY (glyc?emi*)) OR (TITLE-ABS-KEY ((postprandial* OR (post AND prandial) OR post-prandial*) PRE/5 glucose)) OR (TITLE-ABS-KEY ((postprandial* OR (post AND prandial) OR post-prandial*) PRE/5 insulin)) OR (TITLE-ABS-KEY (glucose PRE/5 (toleran* OR level* OR reading* OR measure* OR chang* OR index OR output OR concentr* OR profile* OR respon* OR fall* OR drop*))) OR (TITLE-ABS-KEY (insulin PRE/5 (secret* OR level* OR reading* OR measure* OR chang* OR output OR concentr* OR profile* OR respon* OR ris* OR incr* OR releas*))) OR (TITLE-ABS-KEY (ogtt OR ivgtt OR "glucose tolerance test" OR (post AND prandial AND period))) OR (TITLE-ABS-KEY ("area under the curve" OR "area under curve" OR auc))) AND ((TITLE-ABS-KEY (healthy PRE/5 (adult* OR participant* OR volunteer* OR subject* OR individual* OR control* OR patient*))) OR (TITLE-ABS-KEY (nondiabet* OR non-diabet* OR nonobes* OR non-obes* OR normoglyc* OR normo-glyc*)) OR (TITLE-ABS-KEY ("normal glucose tolerance" OR ngt))) AND ((TITLE-ABS-KEY (diet* AND fat*)) OR (TITLE-ABS-KEY (high-fat AND diet*)) OR (TITLE-ABS-KEY (unsaturated AND fat*)) OR (TITLE-ABS-KEY (fat* PRE/5 (in* OR load OR emulsion OR adminstr* OR addit* OR supplement* OR toleran* OR challeng* OR consum* OR rich*))) OR (TITLE-ABS-KEY (oil* PRE/5 (in* OR load OR emulsion OR adminstr* OR

addit* OR supplement* OR toleran* OR challeng* OR consum* OR rich*)))
 OR (TITLE-ABS-KEY (lipid* PRE/5 (in* OR load OR emulsion OR adminstr*
 OR addit* OR supplement* OR toleran* OR challeng* OR consum* OR rich*))
) OR (TITLE-ABS-KEY ("fatty acid*" PRE/5 (in* OR load OR emulsion OR
 adminstr* OR addit* OR supplement* OR toleran* OR challeng* OR consum*
 OR rich*))) OR (TITLE-ABS-KEY ((monounsaturated OR mono-unsaturated
 OR polyunsaturated OR poly-unsaturated OR unsaturated) PRE/5 fat*)) OR (TITLE-ABS-KEY ((monounsaturated OR mono-unsaturated OR polyunsaturated
 OR poly-unsaturated OR unsaturated) PRE/5 oil*)) OR (TITLE-ABS-KEY ((vegetable* OR olive OR rape* OR sunflow* OR linseed* OR fish* OR plant*)
 PRE/5 oil*)) OR (TITLE-ABS-KEY ((fat* AND toleran* AND test*) OR ftt)))
 AND (LIMIT-TO (LANGUAGE , "English"))

CINAHL

Searched: 27/09/2022

(MH "Diet, Fat-Restricted") OR (MH "Dietary Fats+") OR (MH "Lipids+") OR (MH "Fatty Acids+") OR (MH "Fatty Acids, Unsaturated+") OR (MH "Fatty Acids, Saturated") OR "diet* fat*" OR (MH "Fats+") OR (MH "Fats, Unsaturated+") OR (MH "Oils+") OR (MH "Fish Oils") OR (MH "Plant Oils")

oil* N5 (intak* OR ingest* OR load* OR emulsion* OR administ* OR add* OR supplement* OR toleran* OR challeng* OR consum* OR rich*)

fat* N5 (intak* OR ingest* OR load* OR emulsion* OR administ* OR add* OR supplement* OR toleran* OR challeng* OR consum* OR rich*)

lipid* N5 (intak* OR ingest* OR load* OR emulsion* OR administ* OR add* OR supplement* OR toleran* OR challeng* OR consum* OR rich*)

fatty acid* N5 (intak* OR ingest* OR load* OR emulsion* OR administ* OR add* OR supplement* OR toleran* OR challeng* OR consum* OR rich*)

(monounsaturated OR mono-unsaturated OR polyunsaturated OR poly-unsaturated OR unsaturated) N5 fat*

"fat* toleran* test* OR FTT"

(monounsaturated OR mono-unsaturated OR polyunsaturated OR poly-unsaturated OR unsaturated) N5 oil*

(MH "Linseed Oil") OR (MH "Rapeseed Oil") OR (MH "Fish Oils+") OR (MH "Corn Oil") OR (MH "Cottonseed Oil") OR (MH "Olive Oil") OR (MH "Plant Oils+") OR (MH "Essential Oils+") OR (MH "Tea Tree Oil") OR (MH "Sesame Oil") OR (MH "Safflower Oil") OR (MH "Peanut Oil") OR (MH "Soybean Oil")

(MH "Fat Emulsions, Intravenous") OR "Fat Emulsions, Intravenous"

AND

healthy N5 (adult* OR participant* OR volunteer* OR subject* OR individual* OR control* OR patient* OR population*)

nondiabet* OR non-diabet* OR nonobes* OR non-obes* OR normoglyc* OR normoglyc*

normal glucose tolerance OR NGT

AND

(MH "Glycemic Control") OR (MH "Glycemic Index") OR (MH "Glycemic Load") OR "glycem*"

glycaemia OR glycemia OR glyceemic OR glycaemic

(MM "Blood Glucose") OR "blood glucose"

(postprandial* OR post prandial OR post-prandial*) N5 glucose

(postprandial* OR post prandial OR post-prandial*) N5 insulin

glucose N5 (toleran* OR level* OR reading* OR measure* OR chang* OR index* OR output* OR concentra* OR profile* OR respon* OR fall* OR drop*)

insulin N5 (secret* OR level* OR measure* OR chang* OR output* OR concentr* OR profile* OR respon* OR rise* OR incr* OR releas*)

OGTT OR oral glucose tolerance test OR IVGTT OR intravenous glucose tolerance test

(MH "Glucose Tolerance Test")

(MH "Postprandial Period")

area under the curve OR area under curve OR AUC

Web of Science

Searched: 26/09/2022

((((((((((((((((((((((((TS=(diet*fat*)) OR TS=(oil NEAR/5 (intake or ingestion or load* or administ* or add* or supplement* or consum* or challeng*))))) OR TS=(lipid* NEAR/5 (intake or ingestion or load* or administ* or add* or supplement* or consum* or challeng* or toleran*)))))) OR TS=(fat* NEAR/5 (intake or ingestion or load* or administ* or add* or supplement* or consum* or challeng* or toleran*)))) OR TS=(fatty acid* NEAR/5 (intake or ingestion or load* or administ* or add* or supplement* or consum* or challeng* or toleran*)))))) OR TS=(*unsaturated* NEAR/5 fat*)) OR TS=(*saturated* NEAR/5 fat*)) OR TS=(fat emulsion* or fat toleran* test or intravenous fat* or FTT)))))) OR TS=((low* or modifi* or animal* or vegetable* or acid* or monounsaturat* or polyunsaturat* or saturat* or unsaturat*) NEAR/5 fat*)) OR TS=(((*unsaturat* NEAR/5 fatty acid*)) OR TS=(((*unsaturat* NEAR/5 oil*))))))))))

AND

(TS=(healthy NEAR/5 (adult* or volunteer* or subject* or participant* or population or patient*))) OR TS=(normoglyc* or nonobes* or nondiabet* or non-diabet* or non-obes* or NGT or NG or normal glucose toleran*)

AND

(((((TS=((glucose NEAR/5 (toleran* or test* or level* or measur* or index or reading* or profile* or output* or concentrat*)))) OR TS=((postprandial or post-prandial*) NEAR/5 glucose*)) OR TS=(IVGTT or intravenous glucose tolerance test or OGTT or oral glucose tolerance test)) OR TS=(AUC or area under the curve or area under curve)) OR TS=(insulin NEAR/5 (level* or measur* or index or secretion* or test* or release or profile* or output* or concentrat* or respon* or rise* or incre*))) OR TS=(postprandial or post-prandial* NEAR/5 insulin*)

Appendix 4. Funnel plots to assess publication bias in randomized controlled trials

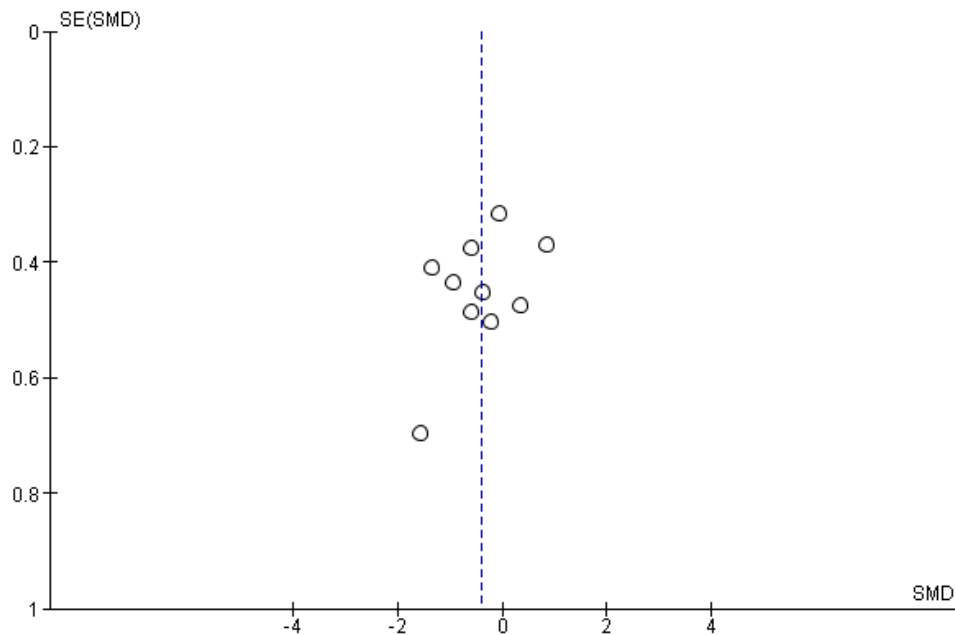


Figure 3.6A: Funnel plot of pooled $iAUC_{0-120 \text{ mins}}$ of glucose for addition of fat to carbohydrate vs carbohydrate alone. SE, standard error. SMD, standardised mean difference.

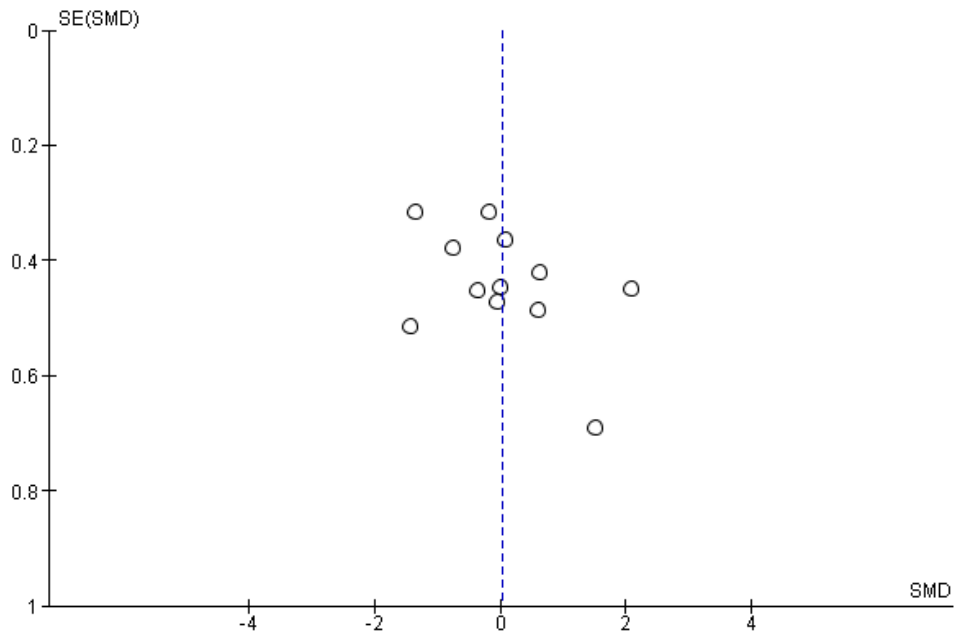


Figure 3.6B: Funnel plot of pooled change in glucose concentration at 120 minutes for addition of fat to carbohydrate vs carbohydrate alone. SE, standard error. SMD, standardised mean difference.

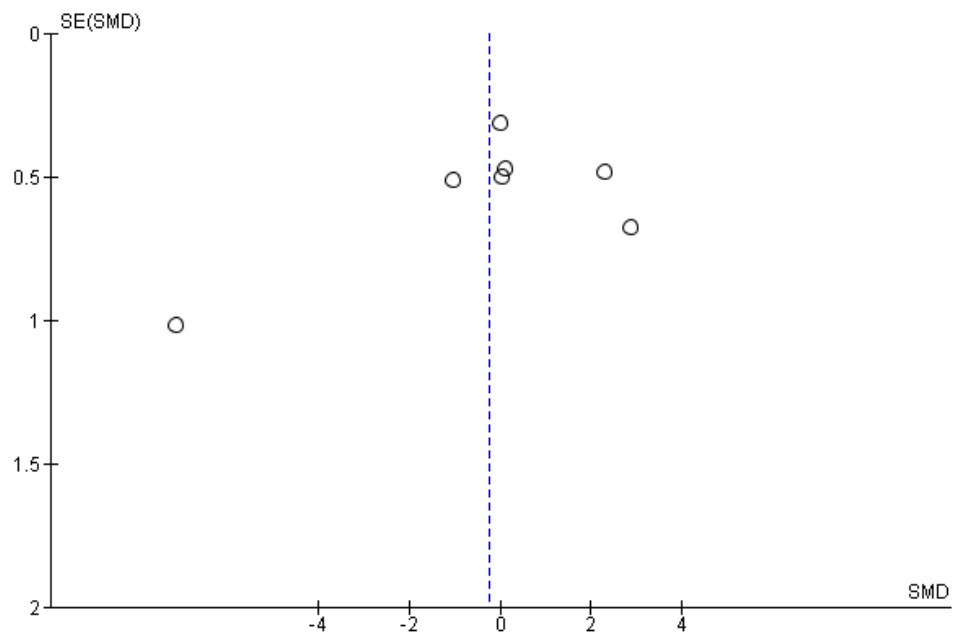


Figure 3.6C: Funnel plot of pooled iAUC_{0-120 mins} of insulin for addition of fat to carbohydrate vs carbohydrate alone. SE, standard error. SMD, standardised mean difference.

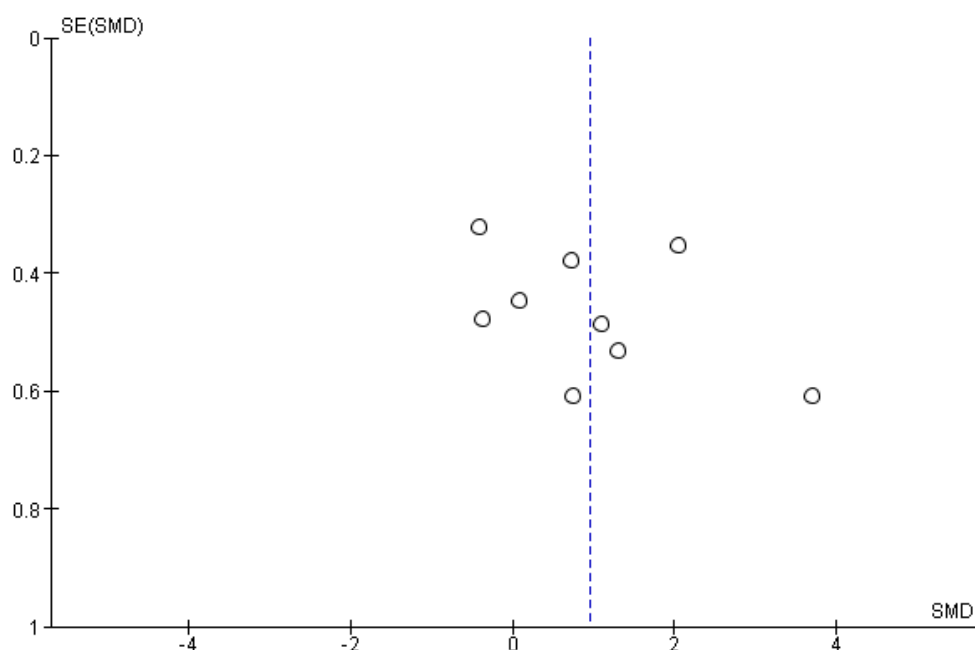


Figure 3.6D: Funnel plot of pooled change in insulin concentration at 120 minutes for addition of fat to carbohydrate vs carbohydrate alone. SE, standard error. SMD, standardised mean difference.

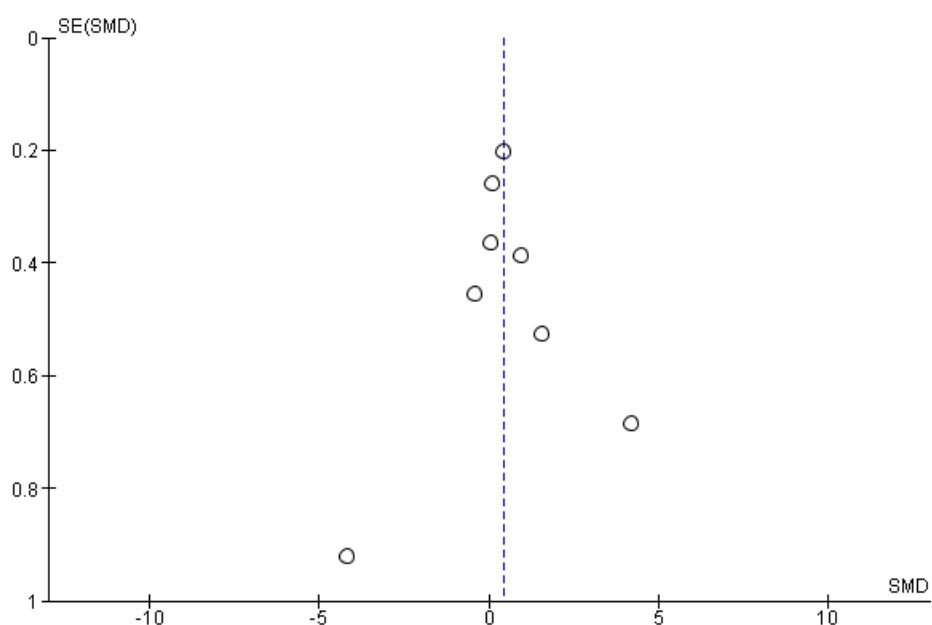


Figure 3.6E: Funnel plot of pooled $iAUC_{0-120 \text{ mins}}$ of glucose for studies comparing SFA vs MUFA. SE, standard error. SMD, standardised mean difference.

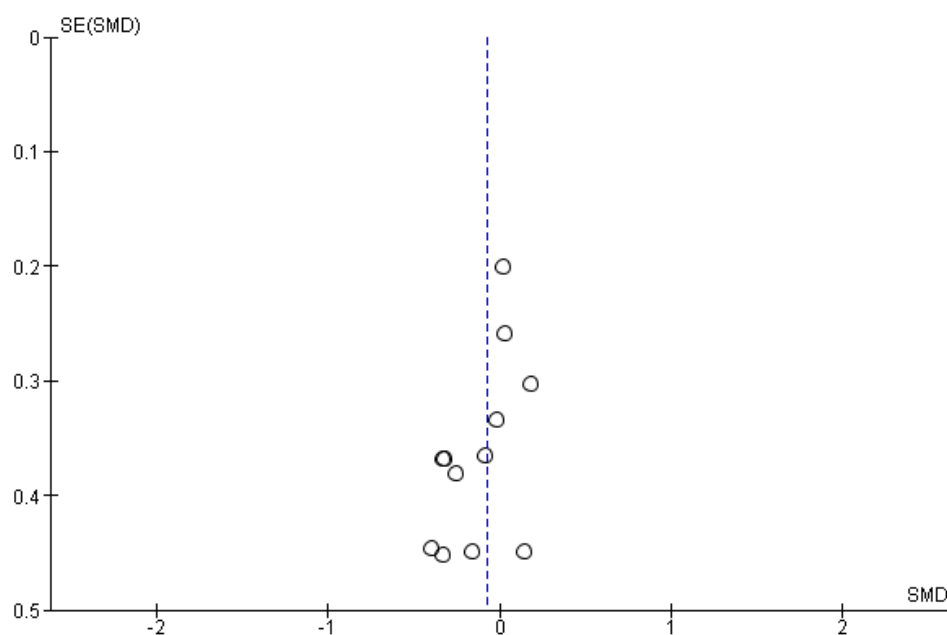


Figure 3.6F: Funnel plot of pooled change in glucose concentrations at 120 minutes for studies comparing SFA vs MUFA. SE, standard error. SMD, standardised mean difference.

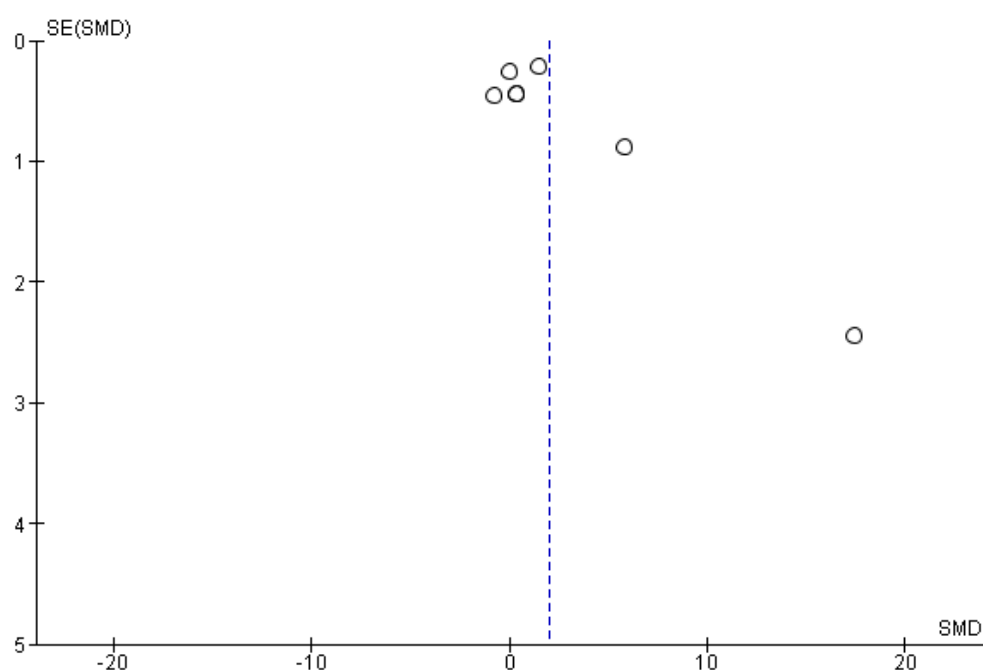


Figure 3.6G: Funnel plot of pooled $iAUC_{0-120 \text{ mins}}$ of insulin for studies comparing SFA vs MUFA. SE, standard error. SMD, standardised mean difference.

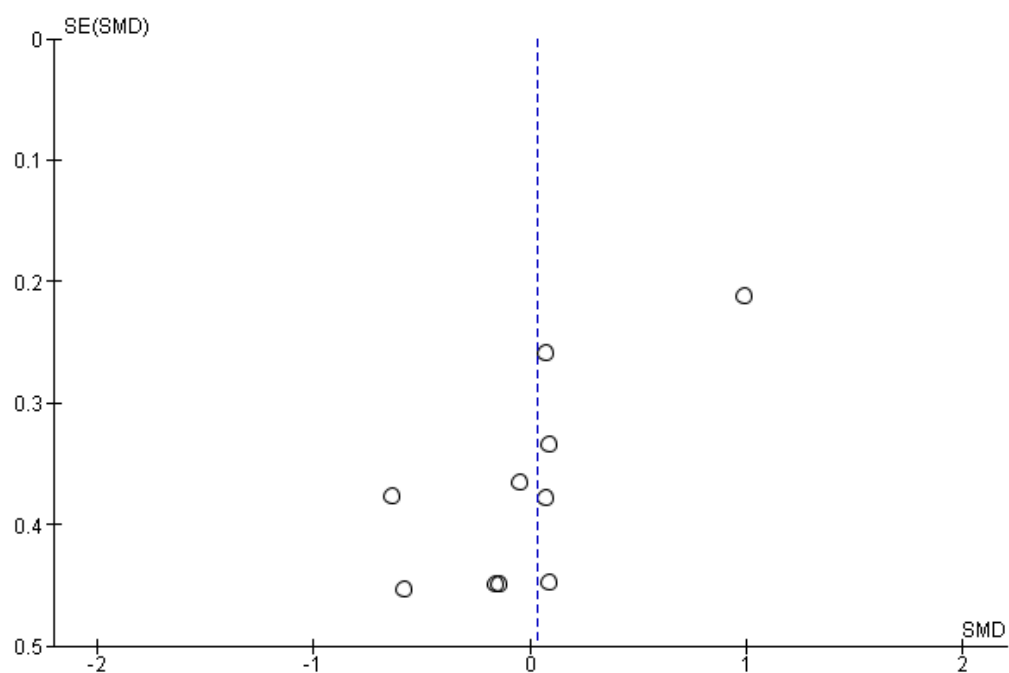


Figure 3.6H: Funnel plot of pooled change in insulin concentration at 120 minutes for studies comparing SFA vs MUFA. SE, standard error. SMD, standardised mean difference.

Appendix 5. Forest plots after leave one out sensitivity analyses

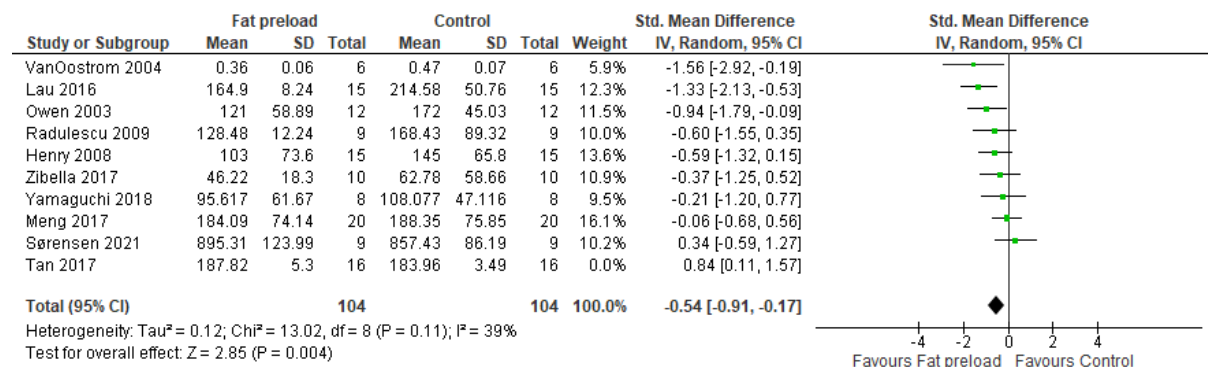


Figure 3.7A: Forest plot comparing the $iAUC_{0-120 \text{ mins}}$ for glucose after removing Tan et al., on addition of fat to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

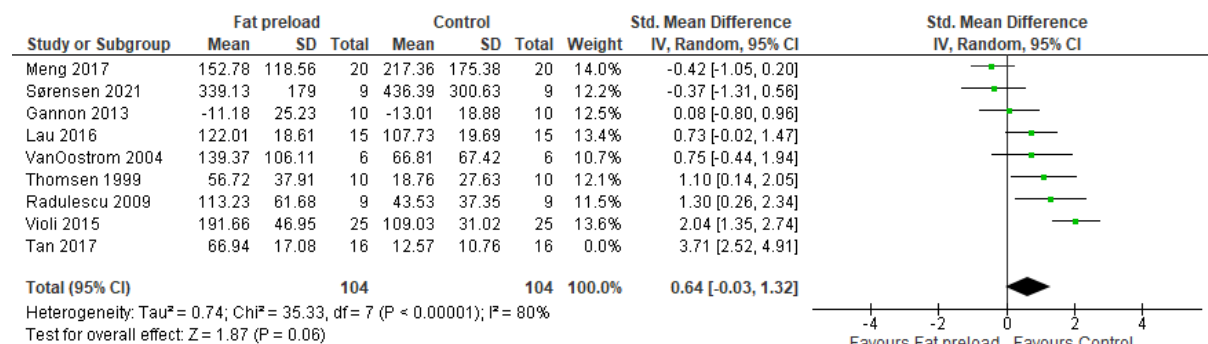


Figure 3.7B: Forest plot comparing the change in insulin concentration at 120 minutes after removing Tan et al., on addition of fat to carbohydrate versus carbohydrate alone. Data are presented as a standardized mean difference with 95% confidence intervals.

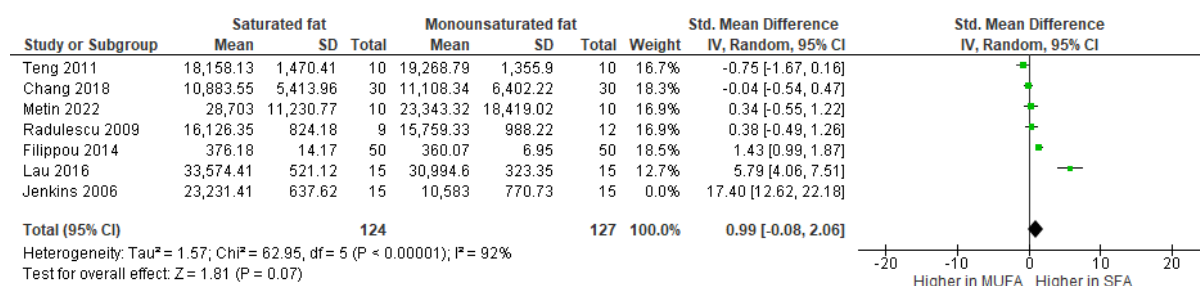


Figure 3.7C: Forest plot comparing the $iAUC_{0-120 \text{ mins}}$ of insulin, after removing Jenkins et al., between SFA and MUFA. Data are presented as a standardized mean difference with 95% confidence intervals.

Appendix 6: Results of meta-regression for glucose and insulin outcomes

Incremental AUC (0 – 120 mins) Glucose

Covariate	Coefficient	Standard error	P value	95% CI
% males	-0.002	0.009	0.829	-1.456 to 0.912
Age	0.020	0.017	0.239	-0.013 to 0.052
BMI	0.074	0.057	0.199	-0.039 to 0.186
Fat dose	-0.037	0.019	0.049	-0.073 to -1.238×10^{-4}

Incremental AUC (0 – 120 mins) Insulin

Covariate	Coefficient	Standard error	P value	95% CI
% males	-7.699×10^{-4}	0.029	0.979	-0.058 to 0.056
Age	0.017	0.059	0.774	-0.098 to 0.132
BMI	0.070	0.177	0.694	-0.278 to 0.417
Fat dose	0.081	0.063	0.194	-0.041 to 0.204

Appendix 7. Participant Information Sheet and Consent form

LIPID INGESTION AND GLUCOSE TOLERANCE IN HEALTHY VOLUNTEERS AND PATIENTS WITH TYPE 2 DIABETES



مركز إمبريال كوليدج لندن للسكري
Imperial College London Diabetes Centre

A Mubadala Health Partner

PROJECT INFORMATION

INTRODUCTION

You are being invited to take part in a research study. Please read the following information carefully; it is important that you understand why the research is being done and what it is you are being asked to do. Please ask us if you do not understand or need more information.

WHY IS THE STUDY BEING DONE?

Type 2 Diabetes is a very common illness in the UAE. People with type 2 diabetes have difficulty controlling their blood glucose which can often rise too high after a meal. We think that olive oil taken with a meal may cause the body to release hormones which could reduce or prevent this rise. If this is the case, we would be able to offer our patients a diet which could improve their diabetes control and possibly reduce the number of medications they need to take.

WHY HAVE I BEEN CHOSEN?

We are inviting patients who attend the Imperial College London Diabetes Centre (ICLDC) to take part in this study, whether they have diabetes or not.

DO I HAVE TO TAKE PART?

It is your choice. If you do not take part, your care will not be affected in any way. If you do enter the study, you can withdraw at any time; you do not have to give any reason and there will be no penalty or disadvantage to you for doing so. If you want to withdraw a long time after taking part, or after the study is over, your information might already have been used and it might not be possible to take it out of the study.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

You will be asked to sign this consent form, so that we have a written record of your agreement to participate. You will be asked questions about your normal diet. We may take a blood test, and will measure weight, height and blood pressure. We will also look at your electronic medical records to find out about your general health. You may be asked to temporarily discontinue certain diabetes medications at least a week before the study day. If you are asked to do this, a researcher will explain what to do if your blood sugar becomes too high, and you will be given contact details for somebody to call if you have any questions.

The study is being performed at the ICLDC Research Centre at the Khaleej al Arabi Branch. You would be required to attend on two separate days at least a week apart after an overnight fast. We will insert a small plastic tube into your arm in order to take blood samples. You will be asked to drink about two tablespoons of oil, followed by a glucose drink 15 minutes later. Alternatively, the glucose will be administered through a drip. One of our researchers will ask you some questions about how palatable the oil is and if you feel any sickness during the study. The total volume of blood sampled during the study will be up to 90 millilitres. This is approximately one fourth of the amount of blood taken if a person donates blood and you are not expected to feel any ill effects.

You will not be able to eat anything else during the study, but you can drink water freely. You will be able to eat as soon as the study is finished.

WILL I NEED TO TAKE ANY NEW MEDICINES AS PART OF THE STUDY?

No. You will not be asked to take any new medication as part of this study.

WILL THERE BE ANY RISKS TO TAKING PART?

You should not suffer any ill effects or disadvantages by taking part in the study. There will be no additional financial cost to you to participate in this study. As with all research, there is a chance that confidentiality could be compromised; however, we will take all necessary precautions to minimize this risk.

Form RES090 Version 1.3 Effective Date 05 July 2022

LIPID INGESTION AND GLUCOSE TOLERANCE IN HEALTHY VOLUNTEERS AND PATIENTS WITH TYPE 2 DIABETES



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A Mubadala Health Partner

WILL THERE BE ANY BENEFITS OF TAKING PART?

Because this is early research, we cannot promise that the study will help you, but the results might be used to improve the treatment of type 2 diabetes in future. Because we will remove your name, date of birth, and other personally identifying information before data processing, we will not be able to give you specific details of your study results.

You will not receive any financial benefits or gain access to extra medical care as part of the study.

WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

All information gathered in this study will be kept completely confidential. Medical researchers within ICLDC, who are trained in maintaining confidentiality, will access your medical records. Your details will be entered with a unique study identification number which will replace your hospital medical record number. Your details will not be shared with non-study personnel or anyone outside ICLDC. No reference will be made in written or oral form that could link you to this study. The study data will be accessible only to study investigators or members of the ICLDC research team.

All records including consent forms and research questionnaires will be stored in a secure facility for at least 3 years after completion of the study. The data gathered in this research study will be retained for at least 25 years.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Sometimes, new information becomes available during a study which changes the way we conduct that study. We do not anticipate that this will change your treatment.

WHAT HAPPENS WHEN THE STUDY STOPS?

You will restart any medicines that were stopped for the study and will continue your usual medical care at ICLDC.

WHAT IF THERE IS A PROBLEM?

ICLDC holds insurance policies that apply to this study. If you experience harm or injury from taking part in this study, you can claim compensation without having to prove that Imperial College 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 legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during this study then you should immediately inform the Principal Investigator. If you are still not satisfied with the response, you may contact the ICLDC Research Ethics Committee (REC) Coordinator. The normal national complaint mechanisms are also available to you.

WHAT WILL HAPPEN TO THE RESULTS OF THE STUDY?

The results will be published in a medical journal and may be presented at international medical conferences. Your name or other identifying information will not be used when discussing, reporting, or publishing these results. The results will be available in 3 years' time. If you wish to know the results of the study, please ask your doctor.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

The study is being organised by **Dr Nader Lessan**, Consultant Endocrinologist, in association with the Department of Investigative Medicine at Imperial College London. This research is funded by ICLDC, a Mubadala health partner.

WHO HAS REVIEWED THE STUDY?

The study was reviewed and approved by the ICLDC Research Ethics Committee (REC). It is possible that the REC may view this study's collected data for auditing purposes. The REC is responsible for the oversight of the protection of human subjects involved in research.

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CONTACT FOR FURTHER INFORMATION

If you would like to discuss your health care, please speak to your own doctor.

If you have any further questions about the study, Dr Mohsin Siddiqui can be contacted at: **02 4040 970** or by email at: **msiddiqui@icldc.ae**.

If you have any questions about the protection of human research participants regarding this study, you may contact, **Mrs Alia Al-Tikriti**, REC Coordinator at: **02 404 0892** or by email at: **aaltikriti@icldc.ae**.

Thank you for taking the time to read this information leaflet. If you would like to take part in the study, please read and sign the consent form.

**LIPID INGESTION AND GLUCOSE TOLERANCE IN
HEALTHY VOLUNTEERS AND PATIENTS
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INFORMED CONSENT FORM

Participant Full Name: _____

Participant ICLDC Number: _____ Participant Contact Number: _____

Participant Date of Birth: _____ Participant Gender: ☐ Male ☐ Female

If you would like to participate, please complete this section by ticking the appropriate boxes then signing at the bottom of the page.

1	I confirm that I have read and understood the relevant information sheet and have had the opportunity to ask questions.	☐ I agree
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.	☐ I agree
3	I understand that I will not benefit financially from taking part in the study (e.g. if research leads to commercial development of a new treatment).	☐ I agree
4	I understand that I may be re-contacted by ICLDC Clinical Research (e.g. to answer some more questions and/or attend another assessment visit), but this is optional.	☐ I agree
5	I give permission for access to my medical and other health-related records, and storage and use of this and other information about me, for health-related research purposes (even after my incapacity or death).	☐ I agree
6	I agree to take part in the study.	☐ I agree

I have read the information, or it has been read to me. I have had the opportunity to ask questions and my questions have been answered to my satisfaction. No promise or guarantee of benefits has been made to encourage me to participate. I consent voluntarily to participate in the study for the purposes indicated above.

Patient/Guardian Name: _____

Signature: _____ Date: _____

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the information.

I confirm that the participant was given an opportunity to ask questions and that all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this consent and/or information sheet may be provided to the participant (if requested).

Doctor Obtaining Consent: _____

Signature: _____ Date: _____

Witness/Interpreter: _____

Signature: _____ Date: _____

Appendix 8. Participant screening tool

Screening tool for
"Lipid ingestion and glucose homeostasis in
healthy volunteers and patients with type 2
diabetes"

Participant ID:



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Screening

Date: _____

Section 1: Participant information			
Name:			
ICLDC No:		DOB:	
Participant ID:		Date Recruited:	
Contact No:			
Ethnicity:		Blood Pressure:	
Weight:		Height:	
BMI:		Waist Circumference:	
Last COVID PCR date:		COVID vaccination	Yes / No

Section 2: Medical History	
Smoking: Current / Ex / Never	Diabetes: Yes / No
HTN: Yes / No	Obesity: Yes / No
Metabolic / Bariatric Surgery: Yes / No	GLP-1 analogue use: Yes / No
Insulin use: Yes/No	Olive Oil Supplements use: Yes /No
Pregnancy status: Pregnant / Not pregnant / NA	Urine Pregnancy test: Yes / No / NA
Other relevant Med Hx:	
Current Medications:	

Screening tool for
"Lipid ingestion and glucose homeostasis in
healthy volunteers and patients with type 2
diabetes"

Participant ID:



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Section 4: Food Frequency Questionnaire

1. Do you use olive oil as the principal source of fat for cooking? (use of olive oil for the preparation of at least 2 of the following groceries: salad, vegetable, meat/fish)	Yes	No	
هل تستخدم زيت الزيتون كمصدر رئيسي للدهون في الطبخ؟ (استخدام زيت الزيتون لتحضير 2 على الأقل من محلات البقالة التالية: سلطة ، خضروات ، لحم / سمك)	لا	نعم	
2. How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)?	Never or <1Tbsp	1 - 3 Tbsp	≥4 Tbsp
ما مقدار زيت الزيتون الذي تستهلكه يوميًا (بما في ذلك الزيت المستخدم في القلي ، والسلطات والوجبات التي تؤكل بعيدًا عن المنزل ، وما إلى ذلك)؟	كلا أو أقل من ملعقة واحدة	واحد إلى ثلاث ملاعق كبيرة	أربع ملاعق كبيرة أو أكثر

I have been given the opportunity to read the full patient information sheet and sign the consent form:

لقد أُتيحت لي الفرصة لقراءة ورقة معلومات المريض الكاملة والتوقيع على نموذج الموافقة

Name of participant _____ Date _____ Signature _____

Name of person taking consent _____ Date _____ Signature _____
(if different from researcher)

Researcher _____ Date _____ Signature _____

ORIGINAL TO BE KEPT IN PATIENT SITE FILE. ONE COPY FOR PATIENT, ONE FOR HOSPITAL NOTES AND ONE TO BE SENT TO TRIAL CENTRE.

Appendix 9. Visual Analogue Scales Questionnaire

LIPID INGESTION AND GLUCOSE TOLERANCE IN HEALTHY VOLUNTEERS AND
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ICLDC Number:

Participant Study ID:

Study time point (in minutes):

Date: Time:

1. How hungry do you feel right now?

Not hungry at all  Never been more hungry

2. How much do you think you can eat right now?

Nothing at all  A lot

3. How full do you feel right now?

Not at all full  Totally full

4. How sick do you feel right now?

Not at all sick  Extremely sick

LIPID INGESTION AND GLUCOSE TOLERANCE IN HEALTHY VOLUNTEERS AND
PATIENTS WITH TYPE 2 DIABETES



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5. How will you rate the palatability of the oil right now? (NB: not valid at -15 mins)

(a) Taste

Good  Bad

(b) Aftertaste

None  Much

(c) Palatability

Good  Bad

6. How irritable do you feel right now?

Not at all irritable  Extremely irritable

7. How anxious do you feel right now?

Not at all anxious  Extremely anxious

8. How sleepy do you feel right now?

Not at all sleepy  Extremely sleepy

Appendix 10. Visual analogue scores for irritability, anxiety and sleepiness

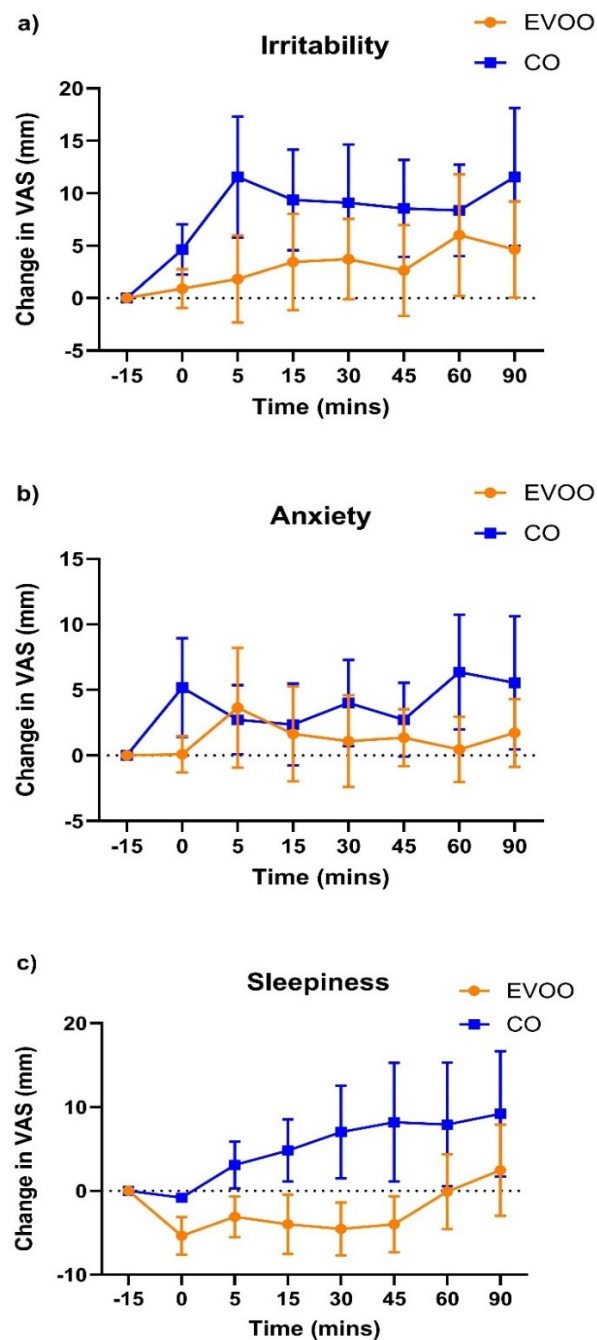


Figure 4.37: Change in VAS measured over 90 minutes following test oils administration, with oils administered immediately before intravenous glucose (Protocol 1): a) Irritability scores b) Anxiety c) Sleepiness. $n = 11$. Data presented as

mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.

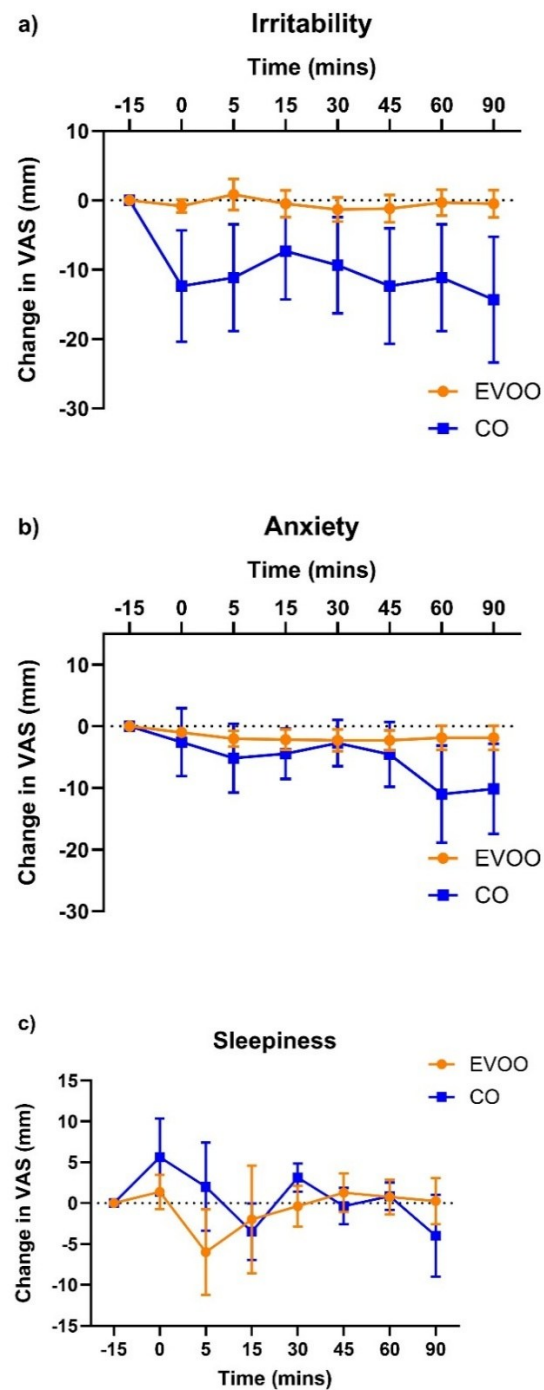


Figure 4.38: Change in VAS measured over 90 minutes following test oils administration, with oils administered 15 mins before oral glucose (Protocol 2):. a)

Irritability scores b) Anxiety c) Sleepiness. $n = 8$. Data presented as mean $\pm$ SEM. EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.

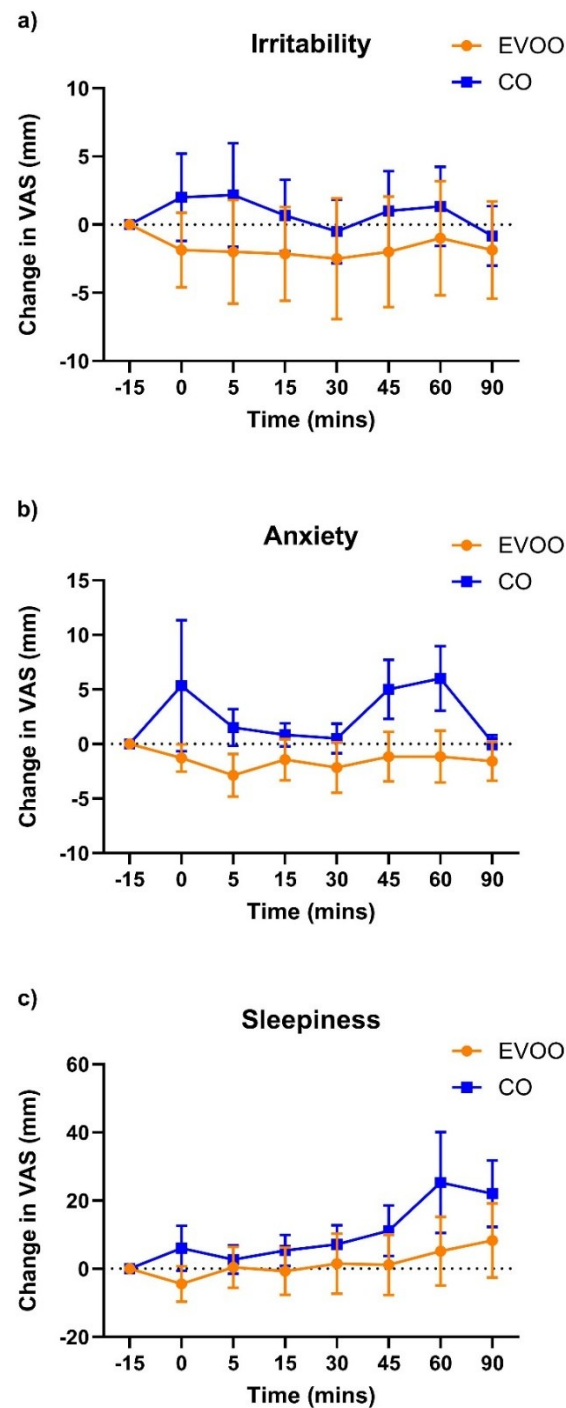


Figure 4.39: Change in VAS measured over 90 minutes following test oils administration, with oils administered 15 mins before intravenous glucose (Protocol

3):. a) Irritability scores b) Anxiety c) Sleepiness. $n = 6$. Data presented as mean $\pm$ SEM.
EVOO, Extra virgin olive oil, CO, Coconut oil, VAS, visual analogue score.