THE EFFECT OF ACUTE FAT INGESTION ON GLUCOSE METABOLISM

INVESTIGATING THE EFFECT OF ACUTE FAT INGESTION ON GLUCOSE METABOLISM IN YOUNG, HEALTHY ADULTS: A DOSE RESPONSE STUDY

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TITLE: Investigating the Effect of Acute Fat Ingestion on Glucose Metabolism in Young, Healthy Adults: A Dose Response Study

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Lay Abstract

Regularly consuming a high-fat diet for several weeks-to-months, or even just a few days, can negatively impact blood sugar regulation which can lead to metabolic diseases such as type 2 diabetes. However, it is unknown whether a single high-fat meal may potentially play a role in the early development of metabolic disease. Therefore, the goal of this study was to understand the immediate effects of a single high-fat meal on blood sugar regulation. Participants consumed three meals of different fat quantity on separate occasions. After each meal, we took regular blood samples for seven hours to measure various molecules related to metabolism. We found that higher fat meals led to higher concentrations of triglycerides following the meal, and increased concentrations of glucose and insulin when a secondary meal was provided. This suggests that a single high-fat meal can disrupt metabolism, especially metabolic functioning of a secondary meal, and therefore could play a role in the early development of metabolic dysfunction and disease.

Abstract

The long-term consumption (several weeks) of a high-fat diet has been shown to disrupt glucose metabolism and give rise to metabolic diseases, such as type 2 diabetes. Shorter-term high-fat diets (e.g., 3-7 days) also lead to metabolic dysfunction; however, there is a lack of information regarding whether a single high-fat meal can disrupt metabolism in the hours immediately following consumption. Therefore, we investigated the initial metabolic changes that occur during the acute (7-hour) postprandial period following isocaloric meals of differing fat quantity in young, healthy adults. We compared the postprandial availability of metabolites during the five hours following consumption of a 25, 50, or 75% fat meal, and then initiated a two-hour 75 g oral glucose tolerance test (OGTT) to assess glucose handling. Nine recreationally active participants (n=7 male/n=2 female, age = 21 ± 6 years, BMI = 24.1 ± 2.7 kg/m^2, VO_2peak = 43.3 ± 2.9 and 38.3 ± 4.5 mL/min/kg for males and females, respectively) participated in this three-way, randomized, cross-over study. We found that postprandial area under the curve for glucose and insulin during the first five hours post-consumption was inversely related to the fat quantity of the meal; specifically, the meal with the highest fat content had the lowest postprandial glucose and insulin concentrations (1183 mM*300 minutes and 7,090 µIU/mL*300 minutes, respectively). However, meals with higher fat content were associated with reduced glucose tolerance during the OGTT. Over the entire seven-hour period, triglyceride concentrations were higher following the 75% (599 ± 251 mM*420 min) compared to the 25% fat meal (372 ± 150 mM*420 min, p=0.023). These results suggest that a single high-fat meal differentially affects the postprandial availability of glucose and lipid metabolites and acutely reduces glucose tolerance in young, healthy adults. This study has deepened the understanding of the early metabolic changes that follow high-fat consumption which could lead to the emergence of early-stage interventions that prevent or delay the development of metabolic diseases.

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List of Abbreviations

T2D	Type 2 diabetes
DAG	Diacylglycerol
GLUT4	Glucose transporter type 4
ATP	Adenosine triphosphate
IRS	Insulin receptor substrate
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol-4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-triphosphate
PKB	Protein kinase B
AGEs	Advanced glycation end products
HbA1c	Hemoglobin A1c
LDL-c	Low-density lipoprotein cholesterol
PKC	Protein kinase C
NEFA	Non-esterified fatty acid
AUC	Area under the curve
VO_2peak	Peak oxygen consumption
OGTT	Oral glucose tolerance test
HDL-c	High-density lipoprotein cholesterol
BMI	Body mass index
NAT	Naturally cycling females
DXA	Dual energy x-ray absorptiometry
RPM	Revolutions per minute
CV	Coefficient of variation
ELISA	Enzyme-linked immunosorbent assay
METs	Metabolic equivalent of task
HOMA-IR	Homeostatic model assessment of insulin resistance
OGIS	OGTT-based insulin sensitivity index
HR	Heart rate
RER	Respiratory exchange ratio

Declaration of Academic Achievement

The main investigator (AL) obtained ethics approval from the Hamilton Integrated Research Ethics Board; conceptualized and designed study protocol; performed recruitment, data collection and analysis, and statistical analyses; and responsible for writing, reviewing an editing.

The co-author (NS) assisted with data collection.

The co-author (KEB) assisted with conceptualization and design of study protocol, statistical analysis, and the writing process.

Chapter 1: Literature Review

1.0 Overview

Insulin resistance, a hallmark of health conditions such as prediabetes and type 2 diabetes (T2D), occurs when the body becomes resistant to the effects of insulin and requires higher insulin concentrations to achieve a normal homeostatic response. Over time, this may eventually lead to a loss of glucose regulation and subsequent hyperglycemia. Fatty acid metabolites (e.g., diacylglycerol (DAG) and ceramides) play a role in the development of insulin resistance by inhibiting the intramuscular insulin signaling pathway— thereby reducing glucose transporter type 4 (GLUT4) translocation to the sarcolemma and reducing glucose uptake from the bloodstream—and have been found to accumulate in excess due to obesity and/or the ingestion of large quantities of dietary fat. Several days of high-fat overfeeding (~50-90% energy from fat; ~40-50% excess energy) reliably disrupts glucose metabolism and reduces insulin sensitivity, but the acute (i.e., several hours post-ingestion) impact of high-fat feeding is inadequately studied. Understanding the early metabolic changes that occur after consuming a single high-fat meal may provide critical insight into the initial perturbations that underpin the development of metabolic disorders such as insulin resistance and T2D.

1.1 The Growing Burden of Metabolic Disease in Canada

Insulin resistance — a condition characterised by the body's inability to react appropriately in response to normal levels of insulin — is a highly prevalent underlying component of prediabetes, T2D, and metabolic syndrome (*Your Guide to Diabetes - Canada.Ca*, n.d.). T2D is an insidious condition that reduces lifespan, increases the risk of serious health complications such as kidney failure, vision loss, amputations, and heart attacks, and results in an all-cause

mortality rate twice as high compared to individuals without diabetes *(Diabetes Rates Continue to Climb in Canada - Diabetes Canada*, 2022; *Diabetes in Canada - Diabetes Canada*, n.d.). Currently, approximately 12 million Canadians live with T2D or prediabetes (*Diabetes Rates Continue to Climb in Canada - Diabetes Canada*, 2022). Prediabetes is diagnosed when blood glucose levels are elevated compared to normal concentrations (see **Table 1**) and affects approximately 6 million Canadians. Many individuals with prediabetes will eventually develop overt T2D (see **Table 1**) in addition to other health complications such as cardiovascular disease (*Prediabetes - Diabetes Canada*, n.d.; Punthakee et al., 2018). Metabolic syndrome is a cluster of risk factors for heart disease, stroke, and T2D (Riediger & Clara, 2011), and is diagnosed based on waist circumference, blood pressure, lipid panel, and fasting blood glucose (see **Table 2**). Currently, approximately 1 in 5 Canadian adults, and ~40% of individuals above the age of 65, have metabolic syndrome (*Metabolic Syndrome — Metabolic Syndrome Canada*, n.d.). Each year, the Canadian healthcare system spends $30 billion to help treat individuals with diabetes and given that the prevalence of prediabetes, T2D, and metabolic syndrome continues to rise, the growing burden of these metabolic diseases needs to be addressed (LeBlanc et al., 2019; *Diabetes Rates Continue to Climb in Canada - Diabetes Canada*, 2022).

Table 1 – Diagnostic criteria for prediabetes and type 2 diabetes (Punthakee et al., 2018).

Criteria	Prediabetes	Type 2 Diabetes
Fasting blood glucose (mM)	6.1 – 6.9	≥ 7.0
Two-hour blood glucose levels (mM) following a 75 g oral glucose tolerance test	7.8 – 11.0	≥ 11.1
Glycated hemoglobin (HbA1c) levels (%)	6.0 – 6.4	≥ 6.5

Table 2– Diagnostic criteria for metabolic syndrome (Swarup et al., 2024).

Criteria	Metabolic Syndrome*
Waist circumference (cm) *Men* *Women*	 > 102 > 88
Blood pressure (mmHg) *Systolic* *Diastolic*	 > 130 > 85
Fasting blood glucose (mM)	> 5.5
Serum triglyceride concentrations (mM)	> 1.7
High density lipoprotein concentrations (mM) *Men* *Women*	 < 1.0 < 1.3

*Diagnosed when ≥ 3 criteria are met.

1.1.1 Effective Prevention and Treatment of Metabolic Disease Through Lifestyle Interventions

While diabetes has a high incidence rate of 200,000 new cases each year and the prevalence of metabolic syndrome continues to climb, the progression of both of these conditions can be largely prevented or delayed (*Diabetes Rates Continue to Climb in Canada - Diabetes Canada*, 2022; LeBlanc et al., 2019). Individuals with T2D are often prescribed anti-diabetic drugs, but lifestyle interventions, such as regular physical activity, unmedicated weight loss, and eating a healthier diet, can help regulate blood glucose concentrations, significantly reduce the risk of metabolic syndrome and T2D development, and in some cases, lead to the complete remission of T2D (*Public Health Agency of Canada*, 2023; *Diabetes among Canadian Adults - Statistics Canada*, n.d.). For instance, a 12-week parallel group study in prediabetic individuals found that metformin (a drug used to lower blood glucose), exercise training, and exercise training while taking metformin all improved inulin sensitivity to the same extent (Malin et al., 2012). Another study found that a diet intervention was more effective than metformin at stimulating weight loss and preventing the diagnosis of overt T2D in prediabetic individuals (Knowler et al., 2002).

These studies provide support for both diet and exercise as potent, independent strategies to manage glycemia in metabolic disease.

Current guidelines for preventing or managing metabolic disease suggest a combination of both physical activity and healthy eating. However, diet alone may be a more attractive target since many individuals with metabolic disease face numerous real and perceived barriers to regular exercise. While some cite physical barriers (such as fatigue, low energy levels, and physical discomfort), others report concerns related to their metabolic condition, such as difficulty managing diabetes, risk of hypo- or hyperglycemia, and the fear of suffering a cardiac event (Alobaid et al., 2023; Bytyci Katanolli et al., 2022; Deshpande et al., 2024). Diet, on the other hand, offers multiple opportunities for intervention throughout the day. Specifically, meals that are low in fat may serve as a strategy to minimize metabolic dysfunction.

Current recommendations from the World Health Organization and the Canadian food guide advise obtaining less than 10% of daily energy intake from saturated fats (Organization, 2023); however, in 2015, total daily saturated fat intake in Canadians was slightly above this cutoff with 10.4% of daily energy being obtained from saturated fat (Harrison et al., 2019). It is critical that dietary changes are established to improve the health and quality of life of millions of Canadians.

1.2 Glucose Handling in Health and Metabolic Disease

Glucose plays a major role in the provision of energy and the maintenance of good health in humans; the regulation of glucose is largely controlled by insulin and glucagon. While glucagon increases blood glucose concentrations during periods of fasting, such as the time between meals, insulin promotes glucose uptake into tissues following feeding through a complex insulin-

signaling pathway. Metabolic complications, including but not limited to, insulin resistance and hyperglycemia can occur if disruptions to the insulin signaling pathway develop and, over time, these complications may progress to more serious health conditions such as metabolic syndrome and T2D.

1.2.1 Healthy Glucose Metabolism

Glucose is an important energy source for every organism in the world (Hantzidiamantis et al., 2024). Dietary carbohydrates are broken down into glucose molecules which can either be subsequently used to produce energy in the form of adenosine triphosphate (ATP) or stored in the form of glycogen, primarily in skeletal muscle, the liver, and adipose tissue (Hantzidiamantis et al., 2024). Driven by the high energy demands of the tissue, skeletal muscle takes up ~40% of glucose following a meal and is aptly referred to as a "glucose sink" (Shrayyef & Gerich, 2010). Successful glucose transport from the blood and into tissues requiring energy production is therefore of utmost importance for the proper functioning of the body.

Insulin, a hormone secreted from the islet beta cells of the pancreas in response to a meal (specifically, in response to increasing blood glucose concentrations), is a major player in glucose regulation (Giugliano et al., 2008). Insulin is an anabolic hormone and thus plays a role in the synthesis and storage of complex molecules: it promotes the storage of glucose molecules as glycogen by promoting glycogen synthesis and inhibiting glycogen breakdown in both the skeletal muscle and the liver (Petersen &f Shulman, 2018). In the adipose tissue, insulin promotes fat storage (by increasing lipogenesis) while inhibiting lipolysis and β-oxidation, and further reduces the production of glucose molecules by inhibiting gluconeogenesis in the liver (Petersen & Shulman, 2018). **Figure 1** depicts the action of insulin on adipose tissue, skeletal

muscle, and the liver. While insulin has many functions in the body, a major role of this hormone is glucose transport. Glucose is taken up into skeletal muscle and adipose tissue via GLUT4, and translocation of GLUT4 from intracellular vesicles to the membrane of cells is mediated by insulin and the insulin signalling pathway (Klip & Pâquet, 1990; Saltiel & Kahn, 2001). When insulin binds to the insulin receptor (a tyrosine kinase receptor), the receptor undergoes autophosphorylation of its tyrosine residues, creating a binding site for a protein known as the insulin receptor substrate (IRS) (Saltiel & Kahn, 2001). Once bound, IRS becomes activated via phosphorylation and recruits and binds a phosphoinositide 3-kinase (PI3K) molecule (Zheng & Wang, 2021). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to create phosphatidylinositol-3, 4, 5-triphosphate (PIP3), and PIP3 recruits and activates protein kinase B (PKB, also known as Akt). When PKB/Akt is activated, this molecule phosphorylates AS160 (also known as TBC1D1) and its homolog TBC1D4 (Mann et al., 2022). This activation results in the inhibition of activity from Rab-GTPase activating protein, ultimately resulting in the translocation of GLUT4 transporters from intracellular vesicles to the cellular membrane where they can transport glucose into the cell (Mann et al., 2022). A schematic of the insulin signalling pathway can be seen in **Figure 2.**

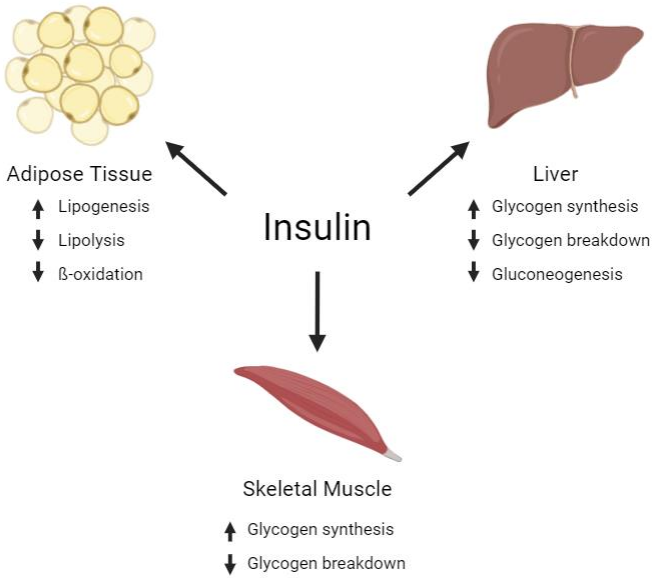

Figure 1: Depiction of the action of insulin on adipose tissue, skeletal muscle, and liver. In the adipose tissue, insulin increases lipogenesis and inhibits lipolysis and β-oxidation; insulin increases glycogen synthesis and inhibits glycogen breakdown in the skeletal muscle and liver and additionally inhibits gluconeogenesis in the liver.

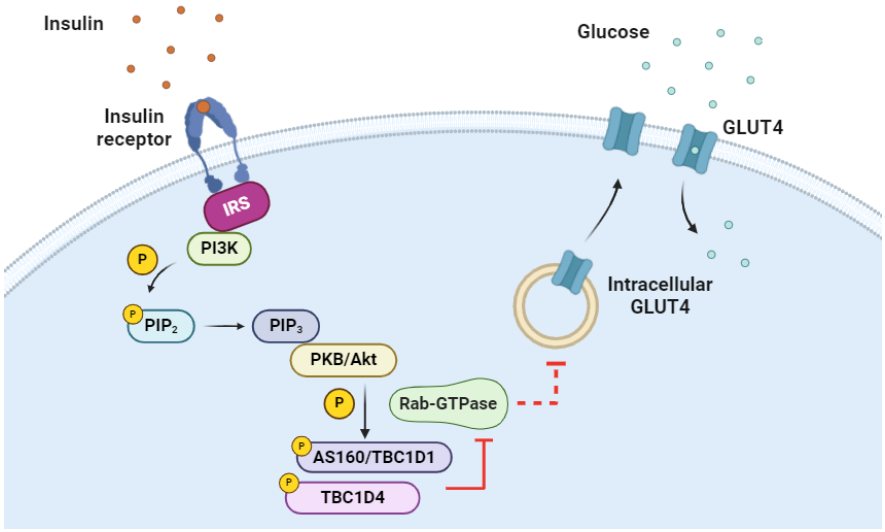

Figure 2: Schematic of the insulin signalling pathway. Insulin molecules bind to the insulin receptor leading to recruitment of PI3K, phosphorylation of PIP_2, and conversation of PIP_2 to PIP_3. Recruitment and activation of PKB/Akt occurs, leading to the phosphorylation of AS160/TBC1D1 and TBC1D4 which inhibits Rab-GTPase and allows for GLUT4 translocation from intracellular vesicles to the cellular membrane.

1.2.2 Perturbations to Glucose Metabolism

Individuals with elevated (>5.5 mM) and impaired (6.1 – 6.9 mM) fasting glucose have been shown to display reduced basal insulin secretion and glucose-stimulated first-phase insulin section but normal second-phase insulin secretion and peripheral insulin sensitivity (Meyer et al., 2006; McGraw & Lee, 2023). This suggests that β-cell function and insulin secretion are impaired at this stage, but de novo insulin synthesis and peripheral glucose uptake are functioning sufficiently (Pietropaolo & Le Roith, 2001). Impaired glucose tolerance, on the other hand, is defined as glucose levels of 7.8 – 11.0 mM following a two-hour glucose load and results in reduced stimulated first- and second-phase insulin secretion and peripheral insulin sensitivity but normal basal insulin secretion (Punthakee et al., 2018; Meyer et al., 2006). In both cases of impaired fasting glucose and impaired glucose tolerance, β-cell dysfunction impairs insulin secretion and continuous declines in β-cell function can lead to pancreatic exhaustion and β-cell death (Cerf, 2013).

While impaired fasting glucose and glucose intolerance manifest due to impaired insulin secretion, insulin resistance is not related to insulin secretion impairments, but rather develops due to impaired insulin action (Cerf, 2013). Insulin resistance occurs when there is an impairment in the body's natural response to insulin signaling (Insulin Resistance - StatPearls - NCBI Bookshelf, n.d.). The anabolic effect of insulin is reduced, leading to improper regulation of glucose concentrations as a result of reduced glucose uptake and glycogen synthesis in addition to increased glycogen breakdown, gluconeogenesis, and lipolysis (Petersen & Shulman, 2018). Altogether, this results in increased levels of glucose in the blood (hyperglycemia). Hyperglycemia is defined as blood glucose concentrations >7.0 mM while fasting or >10.0 mM two hours following a meal and chronic hyperglycemia can lead to serious health conditions

(Mouri & Badireddy, 2023; *Mean Fasting Blood Glucose*, n.d.). Chronic hyperglycemia causes a nonenzymatic glycation reaction to occur with proteins, forming advanced glycation end products (AGEs) which prevent normal functioning, conformation, and enzymatic activity of these glycated proteins (Rungratanawanich et al., 2021). AGEs have been shown to play a major role in various health complications including retinopathy, nephropathy, neuropathy, and cardiomyopathy due to the interruption of intracellular signaling cascades and gene expression (Singh et al., 2014).

1.3 Impact of Dietary Fat on Glucose Metabolism

Low-carbohydrate diets are often prescribed to individuals with T2D as they are highly effective at improving glycemic control and reducing HbA1c levels due to the reduction of dietary carbohydrate-derived glucose molecules present in circulation (Evert et al., 2019). However, low-carbohydrate diets are often accompanied by higher intakes of dietary fat to compensate for the reduced caloric intake that occurs when lowering carbohydrate consumption. In individuals without overt T2Ds, this increased fat consumption could play a role in metabolic dysfunction. While fat is an essential component of a healthy diet, fat consumption can result in both positive and negative health impacts depending on both the quality and quantity of fat that is consumed. Modern day dietary habits typically reflect an overconsumption of fat, especially saturated fat (which is often associated with negative health implications), which can result in various metabolic complication such as insulin resistance.

1.3.1 Macronutrient Requirements and Dietary Fat Consumption Patterns

Canadian guidelines recommend that adults over the age of 19 obtain 45-65% of their total energy intake from carbohydrates, 10-35% from protein, and 20-35% from fat (*Dietary Reference Intakes Tables: Reference Values for Macronutrients - Canada.Ca*, n.d.). Dietary fats are an essential part of a healthy diet as they play a role in cellular function, hormone production, and the absorption of key nutrients, such as vitamin A, D, E, and K (*Fats and Oils | Heart and Stroke Foundation*, n.d.; *Dietary Fats | American Heart Association*, n.d.). Sources of unsaturated fats, such as olive oil, avocados, nuts, and salmon, have been shown to reduce low-density lipoprotein cholesterol (LDL-c) levels and reduce the risk of heart disease, while sources of saturated fats, such as butter, coconut oil, and full-fat dairy products, play a role raising LDL-c cholesterol levels (*Fats and Oils | Heart and Stroke Foundation*, n.d.; Grundy, 1989). Additionally, diets enriched in unsaturated fats, specifically omega-3, have been shown to prevent cardiovascular disease, myocardial infarction, bowel disease, and several types of cancer (Kaur et al., 2014). Based on these findings, the Canadian food guide recommends limiting saturated fat consumption and choosing foods containing healthy, unsaturated fats (*Choose Foods with Healthy Fats - Canada's Food Guide*, n.d.). Despite this recommendation, diets high in saturated fats and low in unsaturated fats are quite prevalent. Current omega-3 consumption is less than 20% of the consumption that took place over 100 years ago and ~95% of the population does not consume omega-3 in a quantity needed for good health (Kaur et al., 2014).

Fat consumption has increased in recent years, regardless of fat quality. In the United States, over the past twenty years, average fat consumption has increased to 33.2% (Shan et al., 2019) and in 2021, North America consumed the second highest amount of fats and oils with an average consumption of 673 kcal/capita/day (*Food Balance Sheets 2010-2021 Global, Regional*

and Country Trends FAOSTAT Analytical Brief 72, n.d.). This is unsurprising given that North America had an average daily per capita dietary fat supply of 160 g/person/day with over 40% of total dietary energy supply coming from fats alone in both Canada and the United States (Roser et al., 2024). Globally, saturated fat intake in over 40 countries exceeds the recommended 10% of total energy with average daily intake reaching up to 35% of total energy in some countries (*Fat Intake*, n.d.) Overconsumption of fat has been linked to increased risk of developing obesity, coronary heart disease, and certain types of cancer (*Fat Intake*, n.d.) and a high-calorie diet providing fat on the higher end of the daily recommended amount (35%) was shown to lead to reduced insulin sensitivity in just two weeks (Cornford et al., 2012). Despite these health complications, individuals around the world continue to consume dietary fat in high quantities.

1.3.2 The Role of Fatty Acid Metabolites in Insulin Resistance

Dietary fats have long been implicated in the development of metabolic disease; early epidemiological studies have found correlations between fat consumption and rates of metabolic disorder incidence and death. Data from thirty-seven countries found a strong, positive correlation between total and saturated fat intake with death rates related to arteriosclerotic heart disease and another study found that breast cancer mortality rates were significantly correlated to fat intake values (Masironi, 1970; Sasaki et al., 1993). Of specific interest, dietary fat intake has also been correlated with reduced insulin sensitivity and insulin resistance (J. C. Lovejoy, 2002; J. Lovejoy & DiGirolamo, 1992). While obesity has been implicated as a driver of the relationship between dietary fat intake and insulin resistance (Mayer-Davis et al., 1997), several studies have found positive correlations between dietary fat intake and insulin resistance, independent of obesity (Marshall et al., 1994; Mayer et al., 1993). Based on the long-standing

correlations between fat intake and metabolic disease, specifically insulin resistance, studies have taken place to understand the physiology of this relationship.

Dietary fats may induce insulin resistance through interference with intracellular insulin signalling. In rats fed a high-fat diet, GLUT4 expression in the muscle was normal but GLUT4 translocation to the plasma membrane was reduced by 50% (Zierath et al., 1997). This inhibition of GLUT4 translocation was mediated due to a reduction in insulin-stimulated IRS-associated activation of PI3K (Zierath et al., 1997). Another study found that rats fed a diet high in saturated fats showed increased muscle DAG accumulation and insulin resistance compared to rats fed a diet high in unsaturated fats, who only saw a small increase in muscle DAG and displayed improved insulin sensitivity (Lee et al., 2006). These results corroborate later findings that showed a 16-week exercise program in humans improved insulin sensitivity by preferentially partitioning lipid towards triglyceride storage as opposed to storage as DAGs or ceramides (Dubé et al., 2008). This can be explained by an association between increased DAG concentration resulting in increased activation in protein kinase C (PKC)-θ. Activation of PKC-θ results in increased serine phosphorylation of IRS, which reduces tyrosine phosphorylation, decreases IRS-1 activity, and reduces insulin-stimulated glucose uptake as a result (Yu et al., 2002). Ceramide, on the other hand, has been shown to inhibit PKB/Akt activity, thereby preventing GLUT4 translocation and thus playing a role in insulin resistance (Stratford et al., 2004). Additionally, a study conducted in human muscle cells found that cells incubated with saturated fats (palmitate and stearate), but not unsaturated fat (oleate), showed an impaired glucose uptake response to insulin (Montell et al., 2001). This occurred due to saturated fats accumulating as DAG and unsaturated fats accumulating as triglycerides (Montell et al., 2001).

A summary of these fatty-acid induced alterations in the insulin signalling pathway can be seen in **Figure 3**.

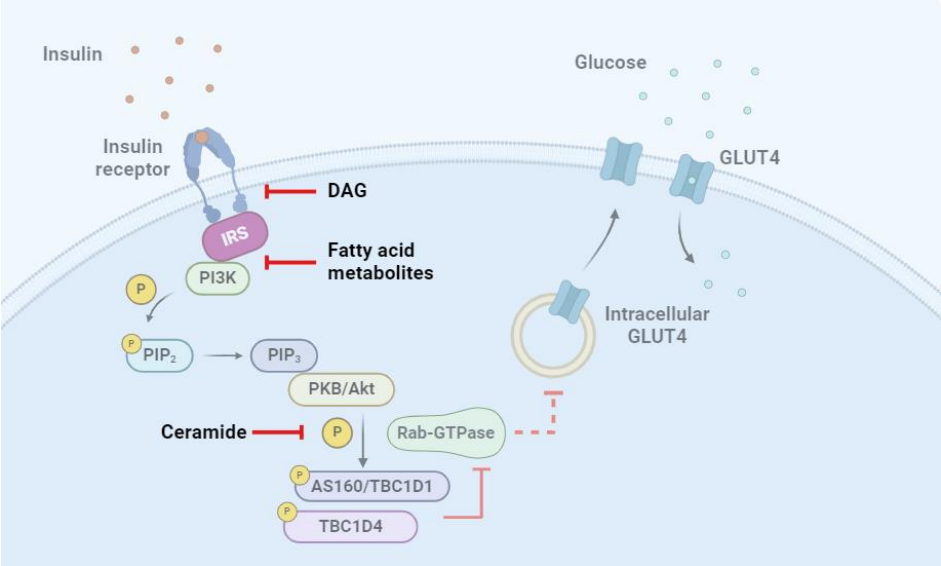

Figure 3: Fatty acid metabolites play a role in insulin resistance by inhibiting various molecules in the insulin signaling pathway. DAG prevents IRS recruitment, fatty acid metabolites inhibit PI3K activation, and ceramide inhibits PKB/Akt-induced activation of AS160/TBC1D4.

1.4 High-Fat Feeding Interventions Disrupt Glucose Metabolism

Several days (~5-14 days) of high-fat feeding reliably disrupts glucose metabolism in healthy adults and even very short lipid infusions (several hours of supraphysiological non-esterified fatty acid [NEFA] concentrations) can induce insulin resistance in healthy young adults. However, the early changes in glucose metabolism that occur following a physiological increase in fat (i.e., a single high-fat meal) are not fully understood.

1.4.1 Short-To-Medium Term Interventions (Several Days)

Short and medium-term high-fat diets, ranging from approximately 5 to 14 days, are often used in research as models to investigate lipid-induced insulin resistance. In addition to providing participants with excess fat (~50-95% fat), these diets also often provide excess calories (~40-50% excess energy). In one study, healthy men with a BMI between 21 and 26 kg/m^2 were provided with three 11-day eucaloric diets separated by an 8–10-week washout (Bisschop et al., 2001). All three diets were identical in energy and protein content (15% energy) but differed in fat and carbohydrate intake. The diet highest in fat (83% fat, 2% carbohydrate) resulted in increased endogenous glucose production and reduced insulin-stimulated glucose oxidation during a hyperinsulinemic euglycemic clamp compared to the intermediate-fat (41% fat, 44% carbohydrate) and low-fat (0% fat, 85% carbohydrate) diet. This suggests that a high-fat diet reduces the ability of insulin to suppress hepatic glucose production while suppressing the stimulatory effect of insulin on glucose oxidation (Bisschop et al., 2001). In another study, seven days of high-fat overfeeding (65% fat, +50% energy) in nine healthy, young adults was shown to reduce glucose tolerance by almost 12% and increase insulin area under the curve (AUC) by 26% following a mixed-macronutrient meal test compared to when the same meal was consumed prior to the high-fat diet (Parry et al., 2017).

Brøns et al. (2009) found that as few as five days of high-fat overfeeding (60% fat, +50% energy) reduced hepatic insulin sensitivity, assessed using a hyperinsulinemic euglycemic clamp, by 65% (resulting in increased hepatic glucose production) and increased fasting glucose levels in young, healthy men. Bakker et al. (2014) found that five days of high-fat overfeeding (+94% fat, +1275 calories, in addition to habitual diet) reduced peripheral insulin sensitivity by 20% during a hyperinsulinemic euglycemic clamp in a group of healthy, young, South Asian men.

Additionally, while Adochoi et al. (2009) saw no significant changes in whole body insulin sensitivity following five days of high-fat overfeeding (50% fat, +40% energy) or high-carbohydrate overfeeding (60% carbohydrate, +40% energy) in healthy, lean adults, the high-fat diet resulted in cellular changes compatible with reduced insulin sensitivity (increased serine phosphorylation of IRS-1 and reduced PI3K activity) while the high-carbohydrate diet induced changes compatible with increased insulin sensitivity (increased tyrosine phosphorylation of IRS-1 and increased PI3K activity). Given that both diets provided the same amount of energy, these results suggest that it is specifically the high-fat content of the diet, and not necessarily the high-energy content, that results in impaired peripheral insulin signalling pathways. Finally, Lundsgaard et al. (2017) showed that only three days of high-fat overfeeding diet (78% fat, +75% energy) was sufficient to significantly reduce whole-body insulin sensitivity and insulin-stimulated leg glucose uptake in healthy, young adults compared to a eucaloric control diet. The results of these studies indicate that high-fat diets as short as three to five days are sufficient to induce both hepatic and peripheral insulin resistance.

While a majority of the above studies used young, healthy, non-obese participants, physical fitness was not controlled for or reported. Physical fitness may protect against metabolic perturbations induced by a period of high-fat overfeeding. One study investigated seven days of high-fat overfeeding (65% fat, +50% energy) in young, lean, healthy adults with high cardio-respiratory fitness (average peak oxygen consumption [VO_2peak] = 49.59 and 39.28 mL/min/kg for males and females, respectively; Whytock et al., 2021). Following the intervention, the authors observed no change in insulin sensitivity (as assessed by an oral glucose tolerance test) in these participants. The researchers indicated that the physically active nature of their participants likely played a large role in their ability to tolerate a short-term high-fat overfeeding

diet, especially given that previous studies have found that high cardio-respiratory fitness reduces T2D risk and all-cause mortality (Syeda et al., 2023; Blair et al., 1989). The findings in the former study are corroborated by another study that showed no change in DAG or total ceramide concentration but increased intramuscular triglyceride concentration in type 1 muscle fibres, following seven days of high-fat overfeeding (64% fat, +47% energy) in young, healthy adults participating in at least 90 minutes of moderate-intensity physical activity each week (K. L. Whytock et al., 2020). This preferential storage of excess lipid as intramuscular triglycerides, (as opposed to metabolites, such as DAGs and ceramides, known to interfere with insulin signaling), provides further support for the protective effect of physical activity against to short-term high-fat feeding (K. L. Whytock et al., 2020).

The above studies suggest that several days (3-14 days) of high-fat feeding can result in metabolic changes linked to insulin resistance. Whether these changes are still seen after even shorter periods, such as after the consumption of a single high-fat meal, has not yet been fully researched. Given that as humans, we spend ~75% of our lives in the postprandial state and this dynamic, complex state is associated with high concentrations of molecules that can impact metabolism, inflammation, and our overall health (Meessen et al., 2019), it is of critical importance that metabolic changes that occur in the few hours following a meal are fully understood.

1.4.2 Acute Interventions (Several Hours)

Lipid-induced disruptions to glucose metabolism are apparent after several hours of increased lipid bioavailability. Studies involving intralipid infusions are often used to model the postprandial period as they involve the continuous infusion of an intravenous fat emulsion over a

short period of time (~4-6 hours), resulting in the relatively rapid onset of hyperlipidemia. One study, looking to investigate the impact of increased lipid availability on skeletal muscle functioning in healthy, young men, performed a six-hour hyperinsulinemic-euglycemic clamp on participants with or without the addition of lipid infusion (Brehm et al., 2006). The infusion increased plasma free fatty acid concentrations by ~83-fold and reduced whole body glucose metabolism by ~46% compared to when the clamp procedure was conducted without the addition of lipids (Brehm et al., 2006). Another study investigated the dose-response effect of plasma free fatty acid levels and insulin signalling in healthy, young adults by using a euglycemic insulin clamp and lipid infusion at either 30, 60, or 90 ml/h (Belfort et al., 2005). Increasing the rate of lipid infusion led to increased plasma free fatty acid availability which subsequently resulted in a reduction of insulin-stimulated glucose disposal by 22, 30, and 34%, respectively (Belfort et al., 2005). While the lowest infusion rate was sufficient to impair tyrosine phosphorylation of IRS-1, PI3K activity, and Akt/PKB activation, the highest infusion rate resulted in further significant impairments (Belfort et al., 2005). Finally, another study performed a six-hour hyperinsulinemic euglycemic clamp with lipid infusion in twelve healthy subjects and found that intramyocellular lipid content increased and glucose infusion rate decreased over the six-hour period, suggesting that increased plasma free fatty acid concentrations reduced insulin sensitivity (Bachmann et al., 2001). Lipid infusions are an excellent method to elevate plasma free fatty acid concentrations in a short period of time in order to investigate the impact of dietary fat in a postprandial setting. However, many studies utilising lipid infusions raise concentrations to levels that are not physiologically relevant (1.5 mM or higher) (Karpe et al., 2011). Following an overnight fast, non-esterified fatty acid concentrations are typically around 0.3-0.6 mM, only reaching ~1.3 mM after 72-hours of fasting

(Karpe et al., 2011). This raises the question of whether the results from such studies can be extrapolated and applied to more real-world settings. A more suitable method to evaluate the impact of fatty acid metabolites on glucose metabolism in the postprandial period is to provide participants with a single high-fat meal.

To our knowledge, only four studies have investigated the acute glucose metabolic response to a single high-fat meal. One such study provided older (average age = 54 years) overweight (average BMI = 26.7 kg/m^2) but otherwise healthy individuals with a high-fat meal of milk cream (93% fat, 775 calories) and took blood samples from the participants over a four-hour postprandial period (Obeid et al., 2018). They found that four hours after consumption of the meal, glucose concentrations declined by 7.6% (Obeid et al., 2018). Another study compared the consumption of a high-fat meal consisting of bread, butter, and sweetened coffee (59% fat, 874 calories) in individuals with and without abdominal obesity (Alayón et al., 2018). The two groups, differing in BMI (23 vs 30 kg/m^2, respectively) but not in age (39 vs 41 years old, respectively), showed no significant differences in glucose AUC during the four-hour postprandial time period but the individuals with obesity had a significantly higher insulin AUC (Alayón et al., 2018). Another study compared the ingestion of a liquid high-fat meal in four different groups of adults: non-abdominally obese controls, and abdominally obese individuals with low-, middle-, or high-postprandial insulin resistance (Wang et al., 2017). The groups ranged in average age from 51 to 57 years old and each group was provided with a meal consisting of 20 calories/kg body mass, 60% of which was fat. Following the consumption of the meal, blood samples were taken for up to eight hours. The researchers found that the glucose AUC was significantly elevated in all groups of individuals with abdominal obesity compared to the controls (Wang et al., 2017). Among the groups with abdominal obesity, the highest insulin

AUC was observed in those with the highest postprandial insulin resistance which was significantly higher than that observed in individuals with middle-postprandial insulin resistance. There were no significant differences between the group with abdominal obesity group with the lowest postprandial insulin resistance and the controls but they both had a significantly lower insulin AUC compared to the middle-postprandial insulin resistance group (Wang et al., 2017). This suggests that higher levels of insulin resistance are associated with more adverse insulin profiles following a single high-fat meal.

While the above studies focused solely on the postprandial time period following a single high-fat meal, another study investigated the impact of diurnal variations in carbohydrate and fat composition on glucose metabolism in young, lean adults (Ando et al., 2018). Three different meals were used: one of balanced macronutrients (15% protein, 25% fat, 60% carbohydrate), one high in fat (15% protein, 50% fat, 35% carbohydrate), and one high in carbohydrates (15% protein, 15% fat, 70% carbohydrate). On three separate occasions, participants were provided with either a full day of macronutrient balanced meals; a high carbohydrate breakfast and dinner with a high fat lunch; or a high fat breakfast followed by a high carbohydrate lunch and dinner. The macronutrient composition for all three days was the same (15% protein, 25% fat, 60% carbohydrate). The researchers found that postprandial peak blood glucose concentrations were ~1.1 mM higher after a high carbohydrate meal when the previous meal was high in fat and that the preprandial respiratory quotient was negatively associated with postprandial glycemic response (Ando et al., 2018). This suggests that consuming a single meal high in fat can increase postprandial glucose concentrations of the following meal.

Altogether, the above studies show that consuming a single high-fat meal has the potential to disrupt glucose and lipid metabolism and can even impact the metabolic handling of

nutrients in a secondary meal. Additional research is required to gain a deeper understanding of the early metabolic detriments that occur following a single high-fat meal and may play a role in the initial stages of metabolic impairments that could lead to the development of metabolic disease. Specifically, more information is needed in a young, healthy population without weight- or age-related defects in metabolism.

1.5 Knowledge Gap Summary

In contrast to chronic high-fat diet research, there is a paucity of data on the acute glucose metabolic response to high-fat feeding in humans. The few studies that exist have produced discrepant results, with one study (Obeid et al., 2018) reporting reductions in blood glucose levels following a single high-fat meal, another (Alayón et al., 2018) reporting no change, and other studies reporting increases in glucose levels when a high-fat meal preceded a secondary meal (Ando et al., 2018) or was consumed by insulin resistant individuals with obesity (Wang et al., 2017).

These discrepant findings may be explained by differences in diet duration, participant characteristics, and meal composition. While all acute high-fat studies focus on a single postprandial period, the duration of this period varied, with some postprandial periods lasting twice as long as others (i.e., 4-hours vs 8-hours). Participant characteristics were diverse as well, with average ages ranging from 22 to 57 years old. Some studies included individuals with overweight (based on BMI) or individuals with abdominal obesity (as defined by waist circumference), while others focused solely on lean, normal weight adults. Physical activity or cardio-respiratory fitness was not often assessed or controlled for, creating the potential for a wide range of fitness levels to be present in participants both within and between studies. This is

important because age, BMI, abdominal obesity, and physical activity levels each independently influence glucose metabolism, and could all impact how well individuals tolerate high-fat feeding. The total energy (as low as 643 kcal and as high as 874 kcal) and fat content (as low as 50% fat and as high as 93% fat) of the high-fat test meals varied greatly between studies, too. Some studies dosed meals based on body mass (20 calories/kg body mass), rather than providing a standard meal to each participant. Finally, the ingredients of meals ranged from more physiologically relevant meals (bread, butter, and coffee) to meals of less physiological relevance (milk cream).

Given the differences seen between studies, and lack of control of key variables, it is difficult to make accurate comparisons and draw definitive conclusions on the impact of dietary fat on postprandial glucose metabolism. My thesis project will address these concerns and knowledge gaps by investigating whether a dose-response relationship exists between the quantity of dietary fat and glucose and lipid metabolism. Young, healthy, adults with average aerobic fitness will be assessed to allow for this relationship to be investigated in individuals with minimal age-, weight-, or fitness-related detriments in metabolic health. Participants will consume three isocaloric (15 calories/kg body mass; 15% energy from protein) meals of varying fat quantities (25%, 50%, or 75% energy from fat) on three separate occasions. Providing meals of different fat quantity, but the same ingredients and amount of energy, will allow for better understanding of the impact of dietary fat on postprandial glucose and lipid metabolism in the absence of confounding variables that could influence results.

1.6 Objectives and Hypotheses

The objective of this double-blind, three-way cross-over dose response study was to investigate the acute impact of oral fat ingestion on glucose and lipid metabolism in young, healthy adults. On three separate occasions and in a randomized order, participants consumed a dairy-based liquid meal containing 25, 50, or 75% energy from fat and providing 15 kcal/kg body mass. Serial blood samples were drawn over the seven-hour postprandial period. After the first five-hours, a two-hour 75 g oral glucose tolerance test (OGTT) was conducted.

I aimed to compare the following between meals of increasing fat quantity:

1) Glucose tolerance five-hours after consuming a liquid meal (primary objective);
2) Postprandial availability of glucose metabolites (glucose and insulin); and
3) Postprandial availability of lipid metabolites (triglycerides, total cholesterol, high-density lipoprotein [HDL-c], and low-density lipoprotein [LDL-c]).

I hypothesised that, as fat quantity of the meals increased, I would observe:

1) A decrease in glucose tolerance as determined by an increase in glucose and insulin AUC;
2) Increased postprandial concentrations of glucose and insulin; and
3) Increased postprandial concentrations of triglycerides, total cholesterol, and LDL-c, and decreased postprandial concentrations of HDL-c.

Chapter 2: Investigating the Effect of Acute Fat Ingestion on Young, Healthy Adults: A Dose Response Study

2.0 Methods and Materials

2.1 Overview of Study Design

This double-blind three-way cross-over nutrition intervention study included a total of four visits to the laboratory: an initial baseline visit and three experimental visits where participants consumed a test meal containing 25, 50 or 75% energy from fat (**Figure 4**). The three experimental visits were conducted in a randomized order at least one week apart to ensure adequate washout of any residual metabolic effects from the previous visit. This study received clearance from the Hamilton Integrated Research Ethics Board (Research Ethics Board #: 16153) and all participants provided written informed consent prior to taking any part in the study (see **Appendix 1** for final approval letter).

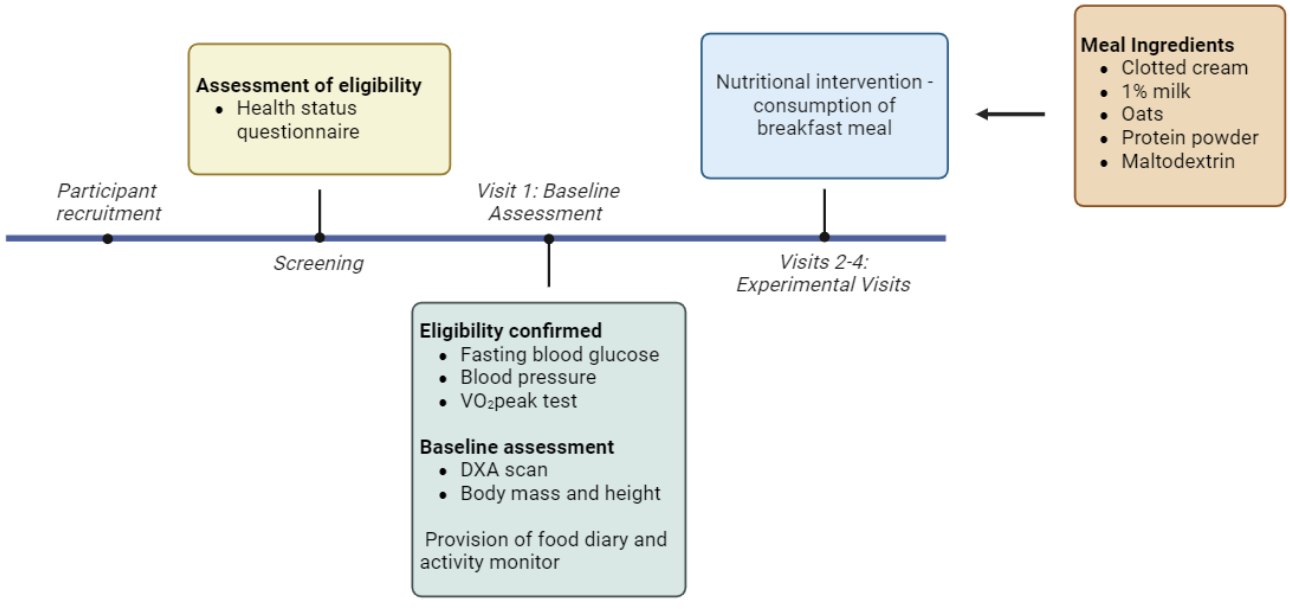

Figure 4: Overview of the experimental design.

2.2 Recruitment

Participants were recruited from Hamilton and the surrounding area using posters displayed around the McMaster University campus and online (e.g., Instagram). We also actively recruited from undergraduate classes (KINESIOL 1F03, KINESIOL 3Q03, and KINESIOL 4EE3) throughout the year. See **Figure 5** for a CONSORT diagram outlining the flow of participants through the study.

2.3 Participants

This study included healthy adults aged 18-35 years who were normal weight to overweight based on body mass index (BMI) (BMI: 18.5-30.0 kg/m^2) and weight stable (± 2 kg) for the past six months. Female participants used either second or third generation oral contraceptives.[1] **Table 3** provides a complete list of inclusion and exclusion criteria.

[1]This inclusion criterion is based on preliminary data from our lab showing that, compared with naturally cycling females (NAT) (NAT, 4.0 ± 0.5 mM), fasting glucose concentrations are modestly but significantly higher in young females taking second (4.4 ± 0.3 mM, $p<0.01$ vs. NAT) or third (4.5 ± 0.3 mM, $p<0.01$ vs. NAT) generation oral contraceptives (Karaguesian et al. *in preparation*). We observed no significant difference in fasting glucose concentrations between females using second or third generation oral contraceptives. For the present study, we chose to include oral contraceptive users over naturally cycling females to aid recruitment success and for ease of scheduling experimental visits for this three-way crossover study, which needed to be completed within approximately one year.

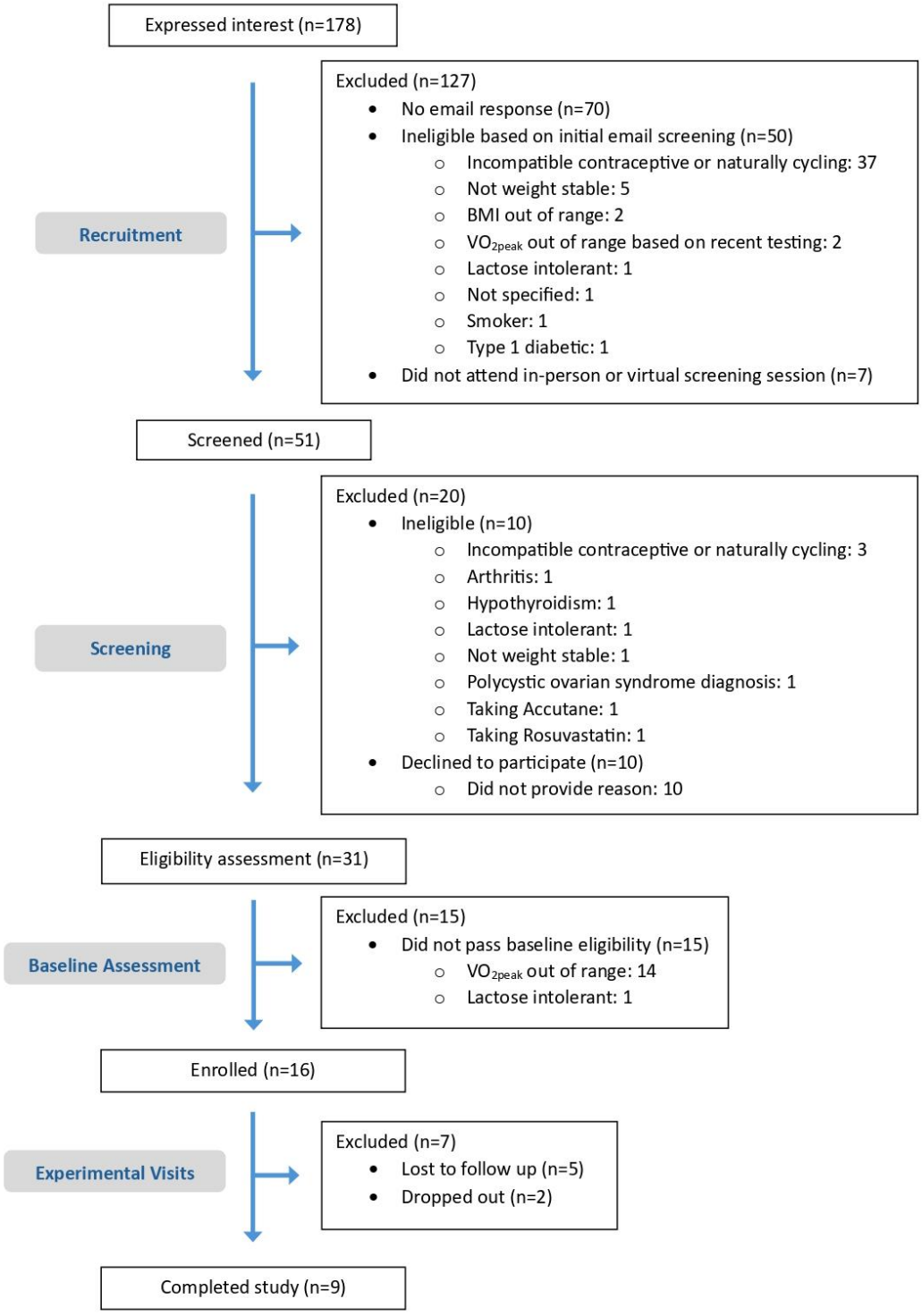

Figure 5: CONSORT flow diagram depicting the progression of participants through the study.

Table 3: Eligibility criteria

Inclusion criteria	Exclusion criteria
• BMI between 18.5 and 30.0 kg/m^2 • Weight stable for the previous six months (± 2 kg) • VO_2peak in the "below average" to "above average" range[a] • Fasting blood glucose <6.0 mM[b] • Resting blood pressure <140/90 mmHg • Taking second or third generation oral contraceptives for a minimum of three months[c]	• Smoking • Diabetes, cancer, or other metabolic disorders • Cardiac or gastrointestinal problems • Infectious disease • Barium swallow or nuclear medicine scan in the previous 3 weeks • Follow a strict vegan diet • Pregnant or breastfeeding[c] • Diagnosis of polycystic ovary syndrome[c]

Abbreviations: VO_2peak, peak oxygen consumption.
[a]Based on reference values set out by the American College of Sports Medicine, this equates to 38-50 mL/kg (males) and 35-47 mL/kg (females) for 18–25-year-olds; or 35-48 mL/kg (males) and 34-45 mL/kg (females) for 26–35-year-olds. (American College of Sports Medicine, 2017).
[b]Assessed via finger prick blood draw and glucometer.
[c]Females only.

2.4 Screening

Interested individuals were preliminarily screened over email for age, BMI, weight stability, and (for females) contraceptive use and those who were potentially eligible attended an in-person or virtual screening session (**Figure 4**) to provide additional detailed information about their health and physical activity. Eligible individuals were then scheduled for a baseline assessment at McMaster University. After an overnight fast (no food or drink except water for 8-12 hours), individuals underwent their assessments in the following order: height and weight measurements, resting blood pressure assessments (OMCRON Healthcare Inc.; Lake Forest, USA; GE Healthcare; Chicago, USA), a fasted blood glucose test (AccuChek Guide, Roche Diagnostics; Indianapolis, USA), a VO_2peak test on a stationary bike, and a dual-energy x-ray absorptiometry (DXA) scan. Individuals with impaired fasting glucose (>6.0 mM), hypertension (>140/90

mmHg), or VO_2peak values outside of a below average to above average range were considered ineligible.[2] A table of VO_2peak values separated by percent ranking and classification can be found in **Appendices 2** and **3**. Only previously screened individuals with unimpaired fasting blood glucose, healthy blood pressure, and below-to-above average VO_2peak continued were included in the study and underwent a DXA scan.

2.4.1 VO_2peak Test

All VO_2peak tests were conducted on either an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0; Groningen, The Netherlands) or a stationary exercise bike (Kettler; Ense, Germany) based on equipment availability and functionality (see **Appendix 4** for more information). Thirty minutes prior to each test, air calibration, metabolic ergonomics calibration, and mixing chamber calibration of the metabolic cart (COSMED, Quark CPET; Albano, Italy) took place. Before the test, participants were fitted with a facemask (Hans Rudolf; Shawnee, USA) which connected to the metabolic cart. To ensure the facemask was properly fitted over the face, investigators tested the seal by covering the mouthpiece with a palm, instructing the participant to breathe in, and checking that no air was able to flow between the edges of the mask and the participant's skin. A full seal around the mouth and nose was achieved before proceeding with the test. A heartrate monitor (Polar Electro; Kempele, Finland) was fitted with a strap around the participants chest with the monitor placed directly on the skin, against the participant's xiphoid process. The height and vertical alignment of the seat and handlebars on the bike were adjusted so that the knee was not locked when fully extended (at the bottom of the

[2]Although fasting glucose, blood pressure, and VO_2peak were screening measures, these tests were conducted during the baseline visit, after informed consent was provided by the participants in the screening meeting.

pedal stroke) and did not reach above the hips when flexed (at the top of the pedal stroke). Participants began with a two-minute seated rest period during which they remained stationary on the bike while resting VO_2 measures were monitored, followed by a three-minute warm-up at 50 watts of resistance at a self-selected pace. Following the warm-up, participants were instructed to maintain a cadence of 70-90 revolutions per minute (rpm) while the load increased at an incremental set rate (5 watts every 10 seconds on the Kettler bike or 1 watt every 2 seconds on the Lode bike). Participants continued to cycle against increasing resistance until their cadence dropped below 60 rpm for longer than 10 seconds. All participants reached at least 85% maximal heart rate and a respiratory exchange ratio >1.10. VO_2peak was calculated as the average of the highest three VO_2peak outputs from the OMNIA software of the metabolic cart using the mixing chamber CPET system; each output reflected a 10-second average during the test.

2.4.2 DXA Scan

A DXA scan (Lunar iDXA, GE HealthCare; Chicago, USA) was conducted to measure whole-body and regional lean soft tissue and fat mass. The main investigator (AL) calibrated the DXA scanner every morning using a Quality Assurance test and a QA Block Phantom (GE HealthCare; Chicago, USA) of known weight and density. Participants were instructed to wear loose and comfortable clothing with minimal metal attachments (e.g., zippers or buttons) to prevent interference with the x-ray images; a hospital gown was provided to participants if needed. For the scan, participants were asked to lay on the scanning table while the investigator positioned their arms alongside their body with palms facing inwards and fixed their legs together using a Velcro band to maintain a 45° internal rotation. Internal rotation of the legs

produces a clearer image of the femoral neck, which facilitates segmentation of the lower limbs and pelvis. The same investigator conducted and subsequently analyzed individual DXA scans; specifically, regions of interest were adjusted to ensure consistent segmentation of body parts across participants.

2.5 Experimental Visits

Females were scheduled for their experimental visits during days 3-7 of the placebo pill phase to allow for hormone levels to drop following cessation of active pill use. During these visits (see **Figure 4 "Visits 2-4 Experimental Visits"**), participants arrived at the lab following an overnight fast and having abstained from alcohol and moderate-to-vigorous exercise for 72 hours. Upon arrival to the lab, participant's body mass was reassessed for use in the preparation of test meals. An intravenous catheter was inserted into the antecubital vein and a fasted blood sample was obtained followed by the consumption of a dairy-based liquid meal containing either 25, 50, or 75% total energy from fat and equating to 15 kcal/kg body mass. Participants were randomised to the order of their study condition and were asked to consume the meal within 20 minutes. Randomization was conducted using https://www.randomizer.org/ by a research assistant who was not directly involved in participant recruitment or testing. The decoded randomization key was held by this research assistant who prepared the test meals for AL. Blood samples were taken every 15 minutes for the first hour following meal consumption to capture the rapid changes in metabolic concentrations following digestion and absorption (i.e., 15, 30 45, and 60 min) and every 30 minutes for the subsequent four hours (i.e., 90, 120, 150, 180, 210, 240, 270 and 300 min) (see **Figure 6**). After the 300 min (5-hour) blood sample was drawn, a 75 g two-hour OGTT (Trutol^{TM}, NERL Diagnostics Corporation; East Providence, USA) was

initiated to measure glucose tolerance. For the OGTT, blood samples were taken every 15 minutes for the first hour (i.e., 315, 330, 345 and 360 min) and every 30 minutes for the second hour (i.e., 390 and 420 min). Participants remained seated in the laboratory for the duration of the visit and were able to consume water ad libitum. Serum concentrations of glucose, insulin, triglycerides, total cholesterol, HDL-c, and LDL-c were analyzed at each timepoint.

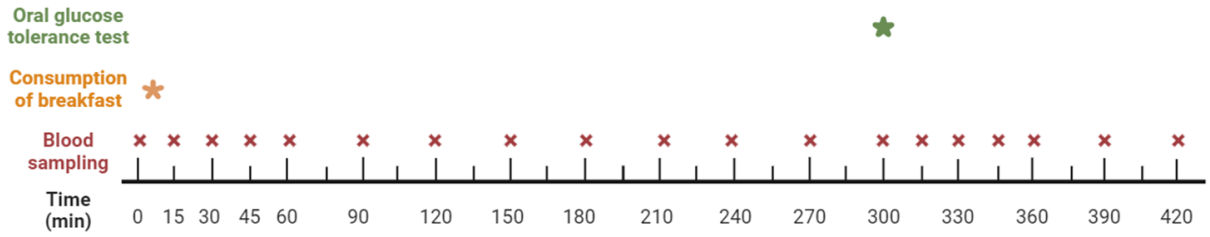

Figure 6: Schematic of each experimental visit, outlining the timing of blood sampling, consumption of the breakfast test meal, and the oral glucose tolerance test

2.5.1 Nutritional Composition of the Test Meals

All test meals were made from old-fashioned oats, 1% milk, clotted cream, protein powder, and maltodextrin blended together by the research team (see **Appendix 5** for brand information for each ingredient). The test meals were designed to have a similar taste and smell across the three conditions and were provided to the participants in opaque plastic water bottles. The test meals always provided 15% total energy from protein, with the remaining energy coming from varying amounts of carbohydrates and fats, depending on the condition. Detailed nutritional information for the meals can be found in **Table 4**.

Table 4: Nutritional information for each breakfast meal

	Condition		
	25% fat	**50% fat**	**75% fat**
Calories	15 kcal/kg body mass	15 kcal/kg body mass	15 kcal/kg body mass
Fat	25% energy	50% energy	75% energy
Carbohydrate	60% energy	35% energy	10% energy
Protein	15% energy	15% energy	15% energy

The absolute amount of fat, carbohydrate, and protein in each meal varied per participant based on individual body mass.

2.6 Biochemical Analysis

Blood was collected into 4 mL red vacutainers and kept at room temperature to clot for a minimum of 30 minutes before being centrifuged at 1000 g for 10 minutes at 4°C. Serum was aliquoted into 2 mL Eppendorf tubes and stored at -80°C for batch analysis. To avoid excessive freeze-thaw cycles, we froze a separate aliquot for each analyte. The volumes aliquoted were as follows: 75 µl for glucose, insulin, triglycerides, and total cholesterol; 500 µl for HDL-c.

2.6.1 Glucose

Serum glucose was analyzed using an Accu-Check® glucometer (Roche; Basel, Switzerland), due to significant reagent backorder issues with our in-house spectrophotometric assay (more information can be found in **Appendix 6**). Fifteen µL of previously frozen serum was transferred to a weigh boat and then applied to the glucometer strips. For each timepoint, three 15 µL drops of serum were analyzed. Average glucose concentration was calculated between the two replicates with the lowest coefficient of variation (CV).

2.6.2 Insulin

Serum insulin was measured using an enzyme-linked immunosorbent assay (ELISA) (ALPCO Immunoassays; Salem, NH). A 96-well plate, pre-coated with a human insulin-specific antibody,

was provided in the kit. First, six standards, two controls, and the samples were added to the plate in duplicate (25 µL per well) followed by 100 µL of detection antibody. The plate was mixed and allowed to incubate for one hour at room temperature. Following the incubation period, the plate was washed six times with working strength wash buffer. One hundred µL of TMB substate (a chromogenic substance which undergoes an enzymatic reaction to produce a measurable colour) was then added to each well and the plate was left to incubate for 15 minutes at room temperature. Stop solution was added to the plate prior to the measurement of optical density using a spectrophotometer at 450 nm. A standard curve was created from the six standards provided and a trend line was then used to calculate the concentration of insulin in each sample, based on the average of the two replicate wells.

2.6.3 Triglycerides and Total Cholesterol

Serum triglycerides and total cholesterol were measured using a spectrophotometric assay (Sekisui Diagnostics; Burlington, USA); both assays used the same standard and controls. Depending on the assay, a triglyceride- or cholesterol-specific reagent was added to a borosilicate glass tube containing 10 µl of standard, control, or sample. Following an incubation period (37ºC for both triglycerides and cholesterol; 10 minutes for triglycerides and 20 minutes for cholesterol), each reaction mixture was allocated to a 96-well plate; standards, controls, and samples were run in triplicate. The optical density was measured using a spectrophotometer at 520 nm for triglyceride or 505 nm for cholesterol and the concentration of each analyte was determined based on the two replicates with the lowest CV.

2.6.4 High-Density Lipoprotein

HDL-c was analyzed by the Hamilton Regional Laboratory Medicine Program at McMaster University using a direct HDL-c slide assay (VITROS Chemistry Products) due to the inability to obtain a high-quality human serum HDL-c analysis kit to analyze HDL-c in-house. HDL-c concentration was only calculated for every other timepoint (0, 30, 60, 120, 180, 240, 300, 330, 360, and 420 minutes) due to resource constraints at the laboratory. Phosphotungstic acid and magnesium chloride were used to separate HDL-c from non-HDL-c precipitations and an HDL-c-specific surfactant (Emulgen B-66) was used to dissociate cholesterol and cholesterol ester from the HDL-c complexes. The HDL-derived cholesterol esters were hydrolysed to cholesterol by selective cholesterol ester hydrolase followed by oxidation of the free cholesterol to cholestenone and hydrogen peroxide using cholesterol oxidase. Finally, peroxidase was added to hydrogen peroxide in the presence of a leuco dye to produce a coloured dye. The density of the dye was measured by reflectance spectrophotometry and is proportional to the HDL-c concentration of the original sample.

2.6.5 Low-Density Lipoprotein

LDL-c was calculated using the following formula (Friedewald et al., 1972):

$$LDL\ Cholesterol\ (\frac{mmol}{L}) = Total\ Cholesterol - HDL - (\frac{Triglycerides}{2.2})$$

LDL-c concentrations are only calculated for time points where HDL-c concentration is available (i.e., every other timepoint).

2.6.6 Inter- and Intra-Assay Variability

The inter- and intra-assay CVs for assays completed by the main investigator (i.e., in-house) were < 9.5% and < 6.0%, respectively. The inter- and intra-assay CVs for each analyte can be found in **Appendix 7**.

2.7 Energy Intake

All participants completed a three-day food diary to measure habitual energy intake and macronutrient distribution. Participants were provided with written and oral instructions on how to properly record dietary intake over the course of three non-consecutive days (two weekdays and one weekend day), including information on how to specify quantity and type of food, drinks, and supplements. Participants were asked not to alter their habitual diet and to eat as normal during these days. Food records were analyzed using MyFitnessPal (Austin, USA) and data regarding total energy intake (kcals/day), carbohydrate intake (grams/day), fat intake (grams/day), and protein intake (grams/day) were extracted from each of the food diaries.

2.8 Energy Expenditure

Energy expenditure was assessed over the course of three consecutive days using arm-mounted accelerometers (BodyMedia SenseWear; Pittsburgh, USA) and analyzed using the BodyMedia SenseWear Software. Participants were instructed to wear the accelerometer on the back of their non-dominant upper arm using a banded strap and only remove it to shower, bathe, or swim. Total energy expenditure (kcals/day), average metabolic equivalent of tasks (METs) (METs/day), active energy expenditure (kcals/day), and average steps (steps/day) were extracted from each accelerometer.

2.9 Statistics

2.9.1 Calculations

Total AUC was calculated using GraphPad Prism (Boston, MA). Average peak analyte concentration and average time of peak analyte concentration were calculated from each participant's highest analyte concentration. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin concentrations and the following formula (Matthews et al., 1985):

$$HOMA - IR = \frac{fasting\ glucose\ \left(\frac{mmol}{L}\right) \times fasting\ insulin\ \left(\frac{\mu IU}{mL}\right)}{22.5}$$

The Matsuda insulin sensitivity index was calculated using the following formula (Matsuda & DeFronzo, 1999):

$$Matsuda\ index = \frac{10{,}000}{\sqrt{((FPG\ \times FPI) \times (\bar{G} \times \bar{I}))}}$$

where FPG = fasting plasma glucose, FPI = fasting plasma insulin, $\bar{G}$ = mean OGTT glucose concentration, and $\bar{I}$ = mean OGTT insulin concentration.

OGTT-based insulin sensitivity index (OGIS) was calculated using the following website:

http://webmet.pd.cnr.it/ogis/ogis.php

2.9.2 Sample Size Calculation

We based our sample size calculation on a previous study (Ando et al., 2018) that reported a significant difference in peak postprandial glucose concentrations following ingestion of a test meal containing 15% fat compared with a test meal containing 50% fat (5.6 ± 0.6 vs. 6.1 ± 0.8

mM, $p<0.05$) in young healthy males. Although the fat content of the meals in Ando et al. (2018) does not align perfectly with the conditions in this thesis, the magnitude of difference (50%-15% = 35%) is similar to the difference between our 25% and 50% fat conditions (50%-25% = 25%) and our 50% and 75% fat conditions (75%-50% = 25%). Given that we expected the difference in peak glucose to be more pronounced between the highest and lowest fat content meals (i.e., 25% vs. 75%), we believed this was a conservative and sound approach for ensuring that we would be able to detect a difference in peak glucose concentrations between at least two of our conditions. We used the means and standard deviations reported by Ando et al. and the variance approach in G*Power, to estimate an effect size f of 0.68 (small to medium) for a repeated measures ANOVA (within-subjects factor) with 1 group and 3 measurements. Setting power to 0.80 and alpha to 0.05, this yielded a sample size of 9.

2.9.3 Statistical Analysis

For all data, normality was assessed by Shapiro-Wilk's test and sphericity was assessed by Mauchly's Test of Sphericity. We used two-way repeated measures ANOVAs to evaluate the effects of dietary fat quantity (Factor 1; 25, 50, 75%) and time (Factor 2; 0-420 minutes) on glucose, insulin, triglyceride, total cholesterol, HDL-c, and LDL-c concentrations. Outliers were assessed by examination of studentized residuals for values greater than ±3. One-way repeated measures ANOVAs were used to evaluate the effect of dietary fat quantity (25, 50, and 75%) on insulin sensitivity indices (HOMA-IR, Matsuda index, and OGIS), AUCs, peak concentrations, and time to peak concentrations. Outliers were assessed by examination of a boxplot for values greater than or less than the 1.5 interquartile range value. In the case of significant F ratios, specific differences were identified using Least Significant Difference post hoc test. Statistical

analysis was completed using SPSS (v26.0, IBM Corporation; New York, USA) and significance was accepted as $\alpha < 0.05$. Data are presented as means ± standard deviations, unless otherwise stated.

When assessing AUC, peak concentrations, and timing of peak concentrations, the data were split into two separate periods (with the exception of the assessment of total AUC): the postprandial period (0-300 min) and the OGTT period (315-420 min). Separating these time periods enables the analysis of how different quantities of dietary fat affect postprandial AUC, peak concentrations, and time to peak concentration, independently of the effects observed following a glucose challenge. Statistical analyzes were run on these periods separately.

2.10 Missing Data

Due to technical issues during blood collection, insufficient blood volume was collected from a single participant for the 240-minute timepoint of the 50% fat meal experimental visit. Glucose, insulin, triglyceride, and total cholesterol analysis was conducted using the blood that was successfully collected; however, there was not enough blood leftover to perform HDL-c analysis or the subsequent LDL-c calculation. In this case, the average HDL-c and LDL-c concentration from the other eight participants was used in place of the missing data prior to statistical analysis (which was completed as normal).

Accelerometer data was not included for a single participant due to accelerometer malfunction and insufficient time to recollect data. Accelerometer data is presented for $n = 8$.

3.0 Results

3.1 Normality and Sphericity

For outcomes that violated the assumption of sphericity (two-way ANOVAs: glucose, insulin, triglyceride, total cholesterol, HDL-c, LDL-c; one-way ANOVAs: glucose total AUC, insulin OGTT AUC, triglyceride OGTT AUC, time to peak glucose postprandial concentration, time to peak insulin postprandial concentration, glucose two-hour OGTT concentration, Matsuda index, and OGIS), an epsilon correction was applied. A Greenhouse-Geisser correction was used when epsilon was less than 0.75 and the Huynh-Feldt correction was used when epsilon was greater than 0.75 (Two-Way Repeated Measures ANOVA in SPSS Statistics | Laerd Statistics Premium, 2015).

For outcomes that were not normally distributed (two-way ANOVAs: glucose, insulin, triglyceride, total cholesterol; one-way ANOVAs: glucose total AUC, insulin total AUC, triglyceride OGTT AUC, time to peak glucose postprandial concentration, time to peak insulin postprandial concentration, HOMA-IR, and Matsuda index), the ANOVA test was run as normal because in all cases, a majority of residuals were normally distributed and thus it could be assumed that the violation was not severe enough to require transformations (Two-Way Repeated Measures ANOVA in SPSS Statistics | Laerd Statistics Premium, 2015).

3.2 Recruitment Success

Recruitment and data collection for the present study took place over the course of eleven months with data analysis occurring over the latter six months. In total, 178 individuals expressed interest in the study. Of these interested individuals, 29% attended the screening

meeting and 17% performed the baseline assessment visit. The enrollment rate was 9% with an attrition rate of 44% (**Figure 5**).

3.3 Subject Characteristics

Nine participants (7 male, 2 female) were enrolled in the study and completed all three nutrition intervention visits. Their baseline characteristics are displayed in **Table 5**. Participants were considered normal weight according to BMI and both fasting blood glucose and blood pressure averages fell within a healthy range. The mean body fat percentages for males and females were 22.5 ± 5.9% and 30.8 ± 4.6%, respectively, and correspond to average amounts when compared to reference values for DXA (*Dexa Body Composition Scan - Accurate Imaging Diagnostics DEXA at Accurate Imaging Diagnostics*, n.d.; Anthropometric Measurements: When to Use This Assessment, n.d.)

VO_2peak was 43.3 ± 2.9 mL/min/kg for males and 38.3 ± 4.5 mL/min/kg for females, which correspond to average aerobic fitness levels for each sex. On average, at VO_2peak, participants achieved a respiratory exchange ratio of 1.19 ± 0.05, a maximum heart rate of 183 ± 9 bpm, and a peak power output of 251 ± 30 W.

All females arrived at the lab for the intervention visits on day 5 ± 2 of their placebo (i.e., non-active) pill week. During the intervention visits, participants consumed the 25%, 50%, and 75% fat meals in 7.1 ± 7.0 minutes, 7.6 ± 4.6 minutes, and 4.3 ± 3.6 minutes, respectively. Participants consumed the OGTT in 2.5 ± 1.3 minutes.

Table 5: Baseline subject characteristics for all participants (n=9)

Variable	Mean ± SD		Healthy reference values/ranges	
Age (years)	21 ± 6		N/A	
Height (m)	1.71 ± 0.06		N/A	
Weight (kg)	70.7 ± 9.0		N/A	
BMI (kg/m^2)	24.1 ± 2.7		18.5 – 24.9	
Body composition				
Whole-body fat (%)	Males:	22.5 ± 5.9	Males:	18 – 24
	Females:	30.8 ± 4.6	Females:	25 – 31
Fat-free mass (kg)	Males:	56.18 ± 6.19	N/A	
	Females:	45.89 ± 4.41		
Bone mineral content (kg)	Males:	2.82 ± 0.19	N/A	
	Females:	2.91 ± 0.17		
Aerobic fitness				
VO_2peak (mL/min/kg)	Males:	43.3 ± 2.9	Males:	38 – 50 (18-25 years old) 35 – 48 (26-35 years old)
	Females:	38.3 ± 4.5	Females:	35 – 47 (18-25 years old) 34 – 45 (26-35 years old)
HR at VO_2peak (bpm)	183 ± 9		N/A	
RER at VO_2peak	1.19 ± 0.05		N/A	
Peak power output (W)	251 ± 30		N/A	
Fasting blood glucose[a] (mM)	5.3 ± 0.2		<5.6	
Blood pressure (mmHg) Diastolic Systolic	 113 ± 9 66 ± 5		 <140 <90	

Values are represented as mean ± standard deviation. Abbreviations: BMI, body mass index; HR, heart rate; RER, respiratory exchange ratio
[a]Measured on whole blood using a glucometer

3.4 Energy Intake and Energy Expenditure

Total energy expenditure was 2572 ± 410 kcal/day, average METs were 1.7 ± 0.2 METs/day, and average wear time of accelerometer was 93 ± 15%. As determined by the 3-day food diary, total energy intake was 2688 kcals/day with an average consumption of 35% (104 g) fat, 45% (301 g) carbohydrates, and 21% (139 g) protein per day. Information is summarized in **Table 6**. The total energy expenditure is approximately equal to the total energy intake indicating that our participants were likely in energy balance.

Table 6: Energy expenditure and energy intake for all participants (n=9)

Variable	Mean ± SD
Energy expenditure (n=8)	
Total energy expenditure (kcals/day)	2572 ± 410
METs (METs/day)	1.7 ± 0.2
Time on body (%)	93 ± 15
Active energy expenditure (kcals/day)	699 ± 233
Daily steps (steps/day)	9406 ± 2229
Energy intake and macronutrient distribution (n=9)	
Total energy intake (kcals/day)	2688 ± 861
Protein	
g/d	139 ± 59
% energy	21 ± 6
Carbohydrate	
g/d	301 ± 113
% energy	45 ± 9
Fat	
g/d	104 ± 37
% energy	35 ± 8

Abbreviations: g/d, grams per day; METs, metabolic equivalent of task.

3.5 Blood Analysis

For the results reported below, the postprandial period will be defined as timepoints 0-300 minutes while the remaining two hours (315-420 minutes) will be used to define the period following ingestion of the OGTT. We have separated these time periods to better distinguish between postprandial availability of metabolites versus glucose tolerance.

3.5.1 Glucose

We observed a significant treatment-by-time interaction for glucose concentrations over the entire 7-hour period ($p<0.001$). The specific differences between treatments (i.e., 25% vs. 50% vs. 75%) can be seen in **Figure 7** and the specific differences over time for each treatment are shown in **Figure 8**—for clarity, each treatment is presented on a separate graph and only

significant differences from baseline (0 min) are shown. Total AUC values for the entire 7-hour period were 1935, 2031, and 2042 mM*420 minutes for the 25%, 50%, and 75% fat meals, respectively and were not statistically different (p=0.147).

During the postprandial period (0-300 min), we observed a significant effect of treatment on peak glucose concentrations (p<0.001, see **Table 7**). Average peak glucose concentrations for the 25% fat meal (6.1 ± 0.9 mM) were significantly higher than the 50% fat meal (5.5 ± 1.1 mM, p=0.019 vs. 25% fat meal) and the 75% fat meal (4.6 ± 0.6 mM, p<0.001 vs. 25% fat meal). Peak glucose concentrations following the 50% and 75% fat meals were not significantly different (p=0.051). We observed a significant effect of treatment on time to peak glucose concentrations. Average time to peak glucose concentrations for the 75% fat meal (150 ± 123 min) occurred significantly later than the 50% fat meal (23 ± 11 min, p=0.014 vs. 75% fat meal) and the 25% fat meal (30 ± 24, p=0.028 vs. 75% fat meal). Time to peak glucose concentrations between the 25% and 50% fat meal were not significantly different (p=0.466). Postprandial AUC values were 1309, 1238, and 1183 mM*300 minutes for the 25%, 50%, and 75% fat meals, respectively (**Figure 9A**). A significant effect of treatment was observed for postprandial AUC (p=0.039), with AUC following the 25% meal tending to be 11% higher than the 75% meal (p=0.054).

During the OGTT period (300-420 min), there was a significant effect of treatment on AUC (p<0.001, see **Table 8**). Specifically, AUC during the OGTT for the 25% fat meal (555 ± 64 mM*120 min) was significantly lower than the 50% fat meal (771 ± 120 mM*120 min, p<0.001) and 75% fat meal (779 ± 208 mM*120 min, p=0.005) (**Figure 9B**).

Prior to consumption of the OGTT beverage (300 min), serum glucose concentrations had returned to the fasting range (<5.6 mM). Average glucose concentrations at the end of the two-hour OGTT period (420 min) were 4.9, 5.8, and 6.3 mM for the 25%, 50%, and 75% fat

meals, respectively, with no significant differences between treatment (p=0.116). While no individual two-hour glucose concentrations were in the impaired range (>7.8 mM) following the 25% or 50% fat meal, three individuals met the criteria for impaired glucose tolerance following the 75% fat meal. Their two-hour glucose concentrations were 8.7, 9.4, and 8.7 mM.

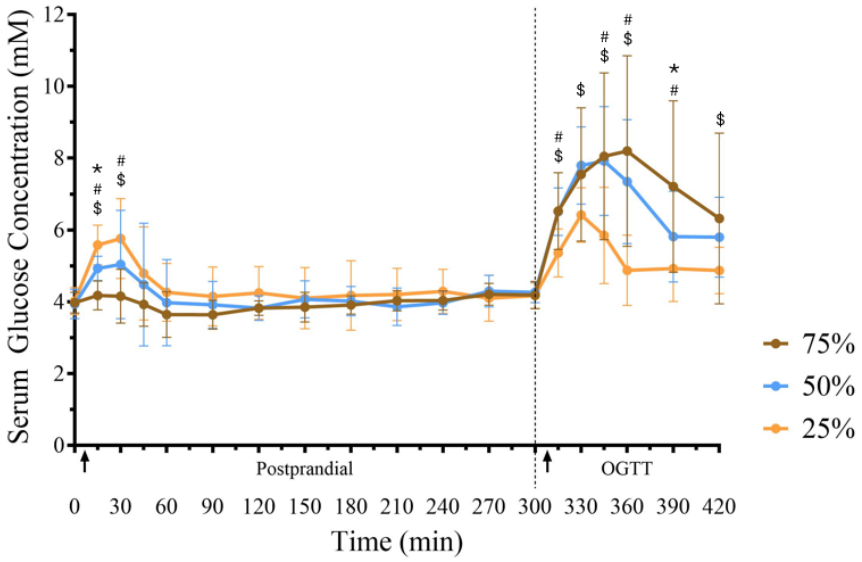

Figure 7: Differences between treatment on average serum glucose concentration for meals containing 25%, 50%, and 75% fat. $p<0.05 between meals containing 25% and 50% fat, *p<0.05 between meals containing 50% and 75% fat, #p<0.05 between meals containing 25% and 75% fat. Arrows indicate the consumption of the liquid meal and the OGTT, respectively. Data are mean ± SD. Data were analyzed using a two-way ANOVA, but for clarity, only specific differences between treatment are presented. See Figure 8 for differences over time.

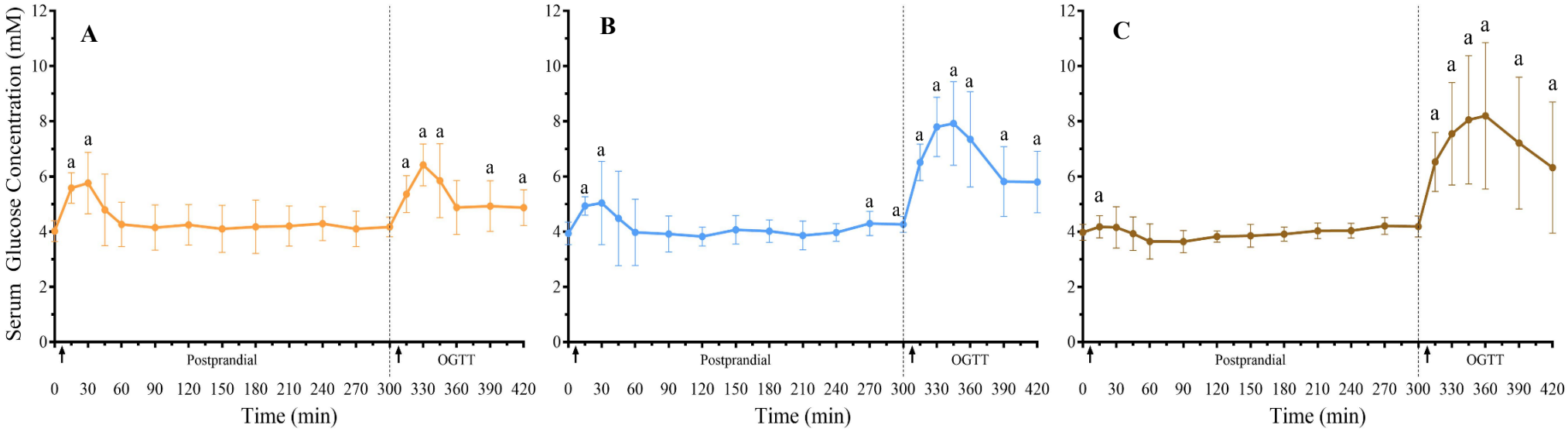

Figure 8: Differences over time on average serum glucose concentration for meals containing 25% **(A)**, 50% **(B)**, and 75% fat **(C)**. "a" indicates significant difference from baseline (0 min) within each treatment ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD. Data were analyzed using a two-way ANOVA, but for clarity, each treatment is presented on a separate graph and only specific differences over time are presented. See Figure 7 for differences between treatment

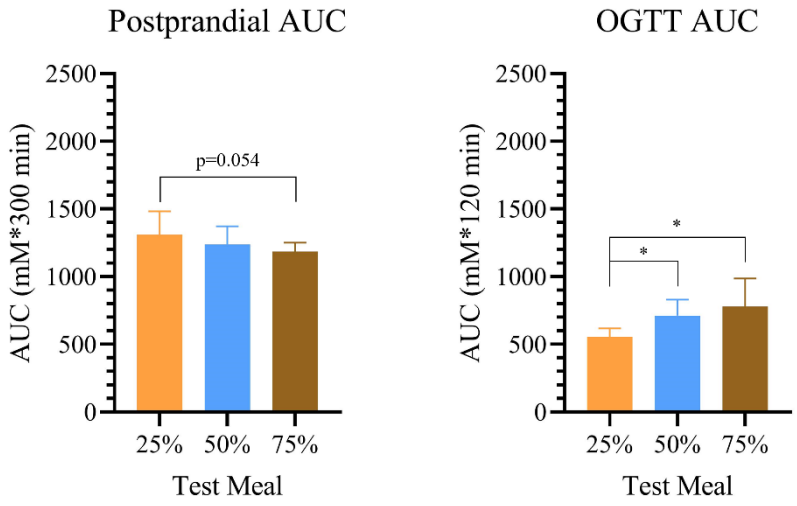

Figure 9: Glucose AUC values for the 5-hour postprandial period **(A)**, and the 2-hour OGTT period **(B)**. *p<0.05. Data are mean ± SD.

Table 7: Summary of postprandial data for glucose and insulin (n=9)

	25% fat	**50% fat**	**75% fat**
	Glucose		
Peak glucose (mM)	6.1 ± 0.9$^{\$,\#}$	5.5 ± 1.1	4.6 ± 0.6
Time of peak glucose (min)	30 ± 24$^{\#}$	23 ± 11$^{\#}$	150 ± 123
	Insulin		
Peak insulin (µIU/mL)	135.44 ± 55.50$^{\#}$	112.91 ± 44.75$^{\#}$	53.09 ± 22.58
Time of peak insulin (min)	43 ± 19	38 ± 13	70 ± 59

Data are mean ± SD.
$p<0.05 compared to 50% fat meal
#p<0.05 compared to the 75% fat meal

Table 8: Summary of data following the OGTT for glucose and insulin (n=9)

	25% fat	**50% fat**	**75% fat**
	Glucose		
OGTT 0 min (µIU/mL; 300 min)	4.2 ± 0.4	4.3 ± 0.3	4.2 ± 0.4
OGTT 2-hour (µIU/mL;420 min)	4.9 ± 0.7^{a}	5.8 ± 1.1^{a}	6.3 ± 2.4^{a}
	Insulin		
OGTT 0 min (µIU/mL; 300 min)	23.80 ± 16.37	12.08 ± 5.64	14.30 ± 8.91
OGTT 2-hour (µIU/mL;420 min)	38.60 ± 27.73$^{\$,\#,a}$	59.49 ± 32.26^{a}	80.71 ± 45.82^{a}

Data are mean ± SD.
^{a}p<0.05 compared to the OGTT 0 min concentration within each condition
$p<0.05 compared to 50% fat meal
#p<0.05 compared to the 75% fat meal

3.5.2 Insulin

We observed a significant treatment-by-time interaction for insulin concentration during the entire 7-hour period (p=0.042). The specific differences between treatments can be seen in **Figure 10** and the specific differences over time for each treatment in **Figure 11**— for clarity, each treatment is presented on a separate graph and only significant differences from baseline (0 min) are shown. Total AUC values for the entire 7-hour period were 25,344, 22,432, and 19,829 μIU/mL*420 minutes for the 25%, 50%, and 75% fat meals, respectively, and no significant differences were observed (p=0.101).

During the postprandial period, we observed a significant effect of treatment on average peak insulin concentrations (p<0.001, see **Table 7**). Average peak insulin concentration for the 75% fat meal (53.09 ± 22.58 μIU/mL) was significantly lower than the 50% fat meal (112.91 ± 44.75, p<0.001) and the 25% fat meal (135.44 ± 55.50, p<0.001). Average time to peak insulin concentrations were 43, 38, and 70 min for the 25%, 50%, and 75% fat meals, respectively, with no significant differences between any of the three meals. A significant effect of treatment was observed for postprandial AUC (p<0.001) with values of 18,313, 13,462, and 7,090 μIU/mL*300 minutes for the 25%, 50%, and 75% fat meals, respectively (**Figure 12A**; p<0.05 for all pairwise comparisons).

During the OGTT period, a significant effect of treatment (p=0.004, see **Table 8**) was observed for average insulin concentration at the end of the two-hour period (420 min) with significant differences between the 25% fat meal (38.60 ± 27.73 μIU/mL) with the 50% (59.49 ± 32.26, p=0.04 vs. 25% fat meal) and 75% fat meals (80.71 ± 45.82, p=0.01 vs. 25% fat meal). There were no significant differences in average insulin concentration at the end of the OGTT between the 50% and 75% fat meal (p=0.063). OGTT AUC values were 6,424, 8,405, and

12,112 µIU/mL*120 minutes for the 25%, 50%, and 75% fat meals, respectively, with no significant differences (**Figure 12B**; p=0.117).

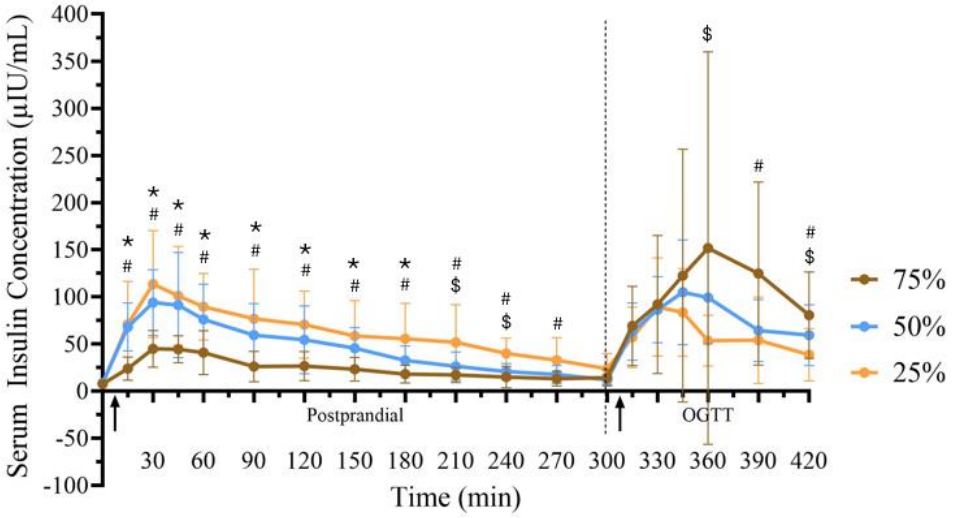

Figure 10: Differences between treatment on average insulin glucose concentration for meals containing 25%, 50%, and 75% fat. $p<0.05 between meals containing 25% and 50% fat, *p<0.05 between meals containing 50% and 75% fat, #p<0.05 between meals containing 25% and 75% fat. Arrows indicate the consumption of the liquid meal and the OGTT, respectively. Data are mean ± SD. Data were analyzed using a two-way ANOVA, but for clarity, only specific differences between treatment are presented. See Figure 11 for differences over time.

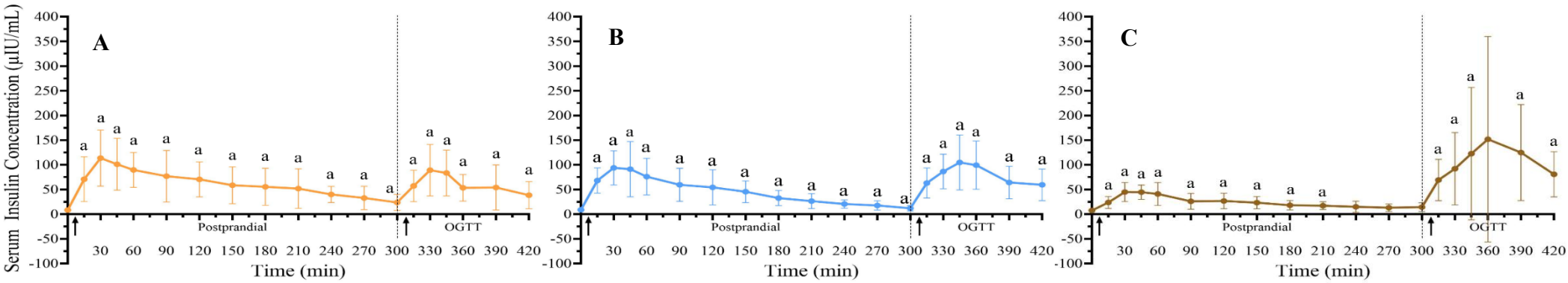

Figure 11: Differences over time on average serum insulin concentration for meals containing 25% **(A)**, 50% **(B)**, and 75% fat **(C)**. "a" indicates significant difference from baseline (0 min) within each treatment ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD. Data were analyzed using a two-way ANOVA, but for clarity, each treatment is presented on a separate graph and only specific differences over time are presented. See Figure 10 for differences between treatment.

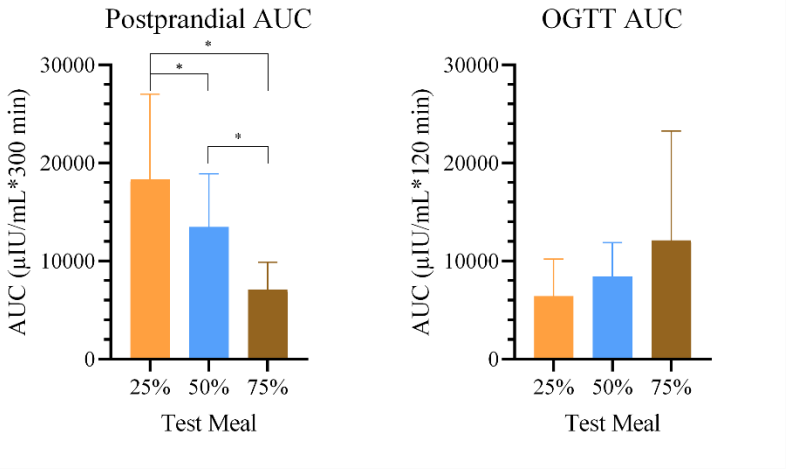

Figure 12: Insulin AUC values for the 5-hour postprandial period **(A)**, and the 2-hour OGTT period **(B)**. *p<0.05. Data are mean ± SD.

3.5.3 Insulin Sensitivity Indices

The average HOMA-IR, Matsuda index, and OGIS values calculated during each of the three visits can be seen in **Table 9**. All average HOMA-IR values fall below the cut-off for early insulin resistance (1.9); however, five individual HOMA-IR values (from three different participants) were elevated above this cut-off. Four of the values fell into the cut-off for early insulin resistance (prior to 25% fat meal: 2.1 and 2.3; prior to the 50% fat meal: 2.0; prior to the 75% fat meal: 2.4) and one value indicated insulin resistance (prior to the 50% fat meal: 3.1). There were no significant differences between treatments for average HOMA-IR or Matsuda index. A significant effect of treatment was seen for OGIS ($p=0.012$); specifically, the OGIS value for the 25% fat meal was significantly higher compared to the other two meals ($p<0.05$) indicating greater insulin sensitivity following the 25% fat meal.

Table 9: Average HOMA-IR, Matsuda index, and OGIS values for all participants (n=9)

	25% fat	**50% fat**	**75% fat**
HOMA-IR	1.4 ± 0.6	1.6 ± 0.7	1.3 ± 0.5
Matsuda index	4.73 ± 4.05	3.94 ± 1.75	3.52 ± 1.87
OGIS (ml/min/m^2)	521± 66$^{\$,\#}$	470 ± 41	433 ± 77

Data are mean ± SD.
$p<0.05 compared to 50% fat meal
#p<0.05 compared to the 75% fat meal

3.5.4 Triglycerides

We observed a main effect of treatment on serum triglyceride concentration during the entire 7-hour period (p=0.01), such that concentrations were higher following the 75% fat meal compared to the 25% fat meal (p=0.023) and the 50% fat meal (p=0.045); there was no difference in triglyceride concentrations between the 50% and 25% fat meals (p=0.102). We also observed a main effect of time on serum triglyceride concentrations (p=0.008, seen in **Figure 13)**; for clarity, only significant differences from baseline (0 min) are shown. NOTE: The Greenhouse-Giesser correction was used to determine the significance of the interaction effect, which might explain why significant main effects for treatment and time were observed in the absence of an interaction effect.

A main effect of treatment on total AUC was observed (p=0.012); specifically, the total AUC for the 25% fat meal (372 ± 150 mM*420 min) was significantly lower than the total AUC for the 75% fat meal (599 ± 251 mM*420 min, p=0.023) but no differences were seen compared to the 50% fat meal (491 ± 208 mM*420 min, p=0.086). No significant differences were observed for total AUC between the 50% and 75% fat meal (p=0.072). Prior to the consumption of the OGTT (300 min), triglyceride concentrations were significantly elevated compared to fasting values (p=0.006) (**Figure 13**). A summary of postprandial and OGTT data for triglycerides can be found in **Appendix 8**.

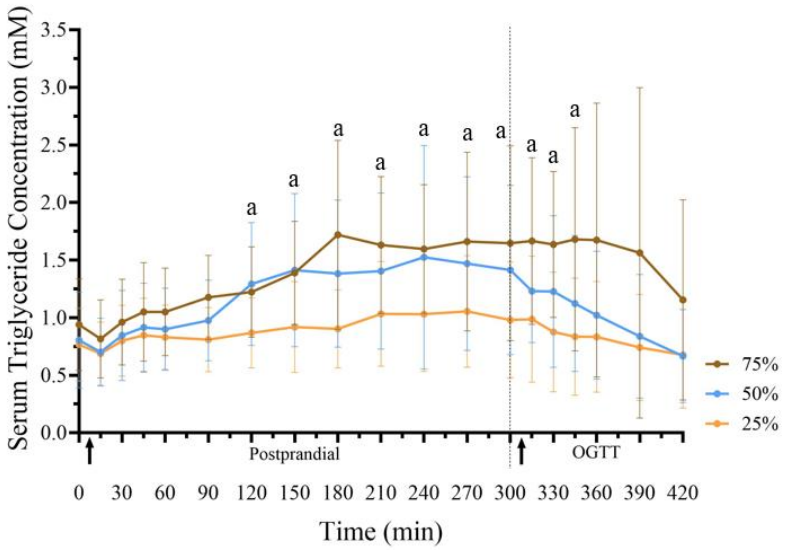

Figure 13: Average serum triglyceride concentration for meals containing 25%, 50%, and 75% fat. We observed a main effect of treatment and a main effect of time. The letter "a" indicates timepoints that were significantly different from baseline (0 min) ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD.

3.5.5 Total Cholesterol

We observed a main effect of time on serum total cholesterol concentration during the entire 7-hour period ($p<0.001$), seen in **Figure 14**—only significant differences of postprandial concentrations from baseline (0 min) are shown. Total cholesterol concentrations decreased ~5-7% from baseline at 15, 30, 60, 90, 120, and 210 mins, and then increased ~ 6% above fasting at 315 min. Total AUC for the entire 7-hour period was 1753, 1703, and 1741 mM*420 minutes for the 25%, 50%, and 75% fat meals, respectively (no significant differences). A summary of postprandial and OGTT data for total cholesterol can be found in **Appendix 8**.

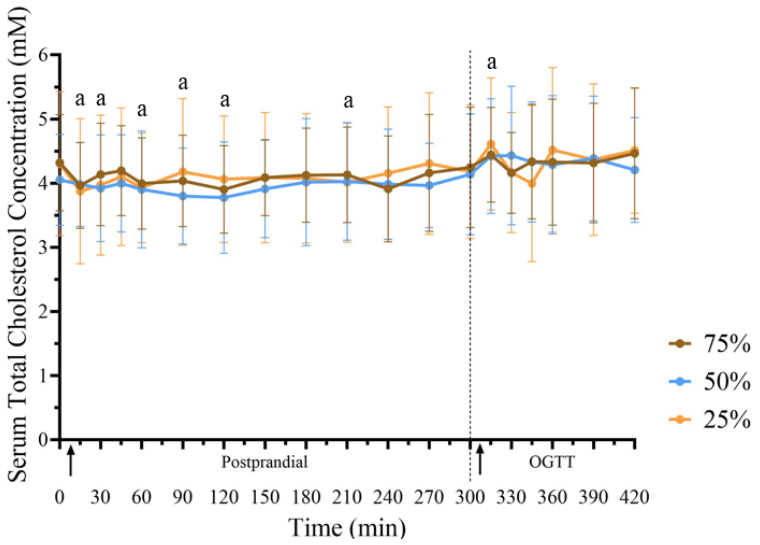

Figure 14: Average serum total cholesterol concentration for meals containing 25%, 50%, and 75% fat. "a" indicates the significant main effect of time from baseline (0 min) ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD.

3.5.6 High-Density Lipoprotein

We observed a main effect of time on serum HDL-c concentration during the entire 7-hour period ($p=0.012$), seen in **Figure 15**—only significant differences of postprandial concentrations from baseline (0 min) are shown. An initial ~8% decrease from baseline occurred at 30 min but postprandial concentrations returned to fasting concentrations by 300 min. Total AUC for the entire 7-hour period was 543, 533, and 531 mM*420 minutes for the 25%, 50%, and 75% fat meals, respectively (no significant differences). A summary of postprandial and OGTT data for HDL-c can be found in **Appendix 8**.

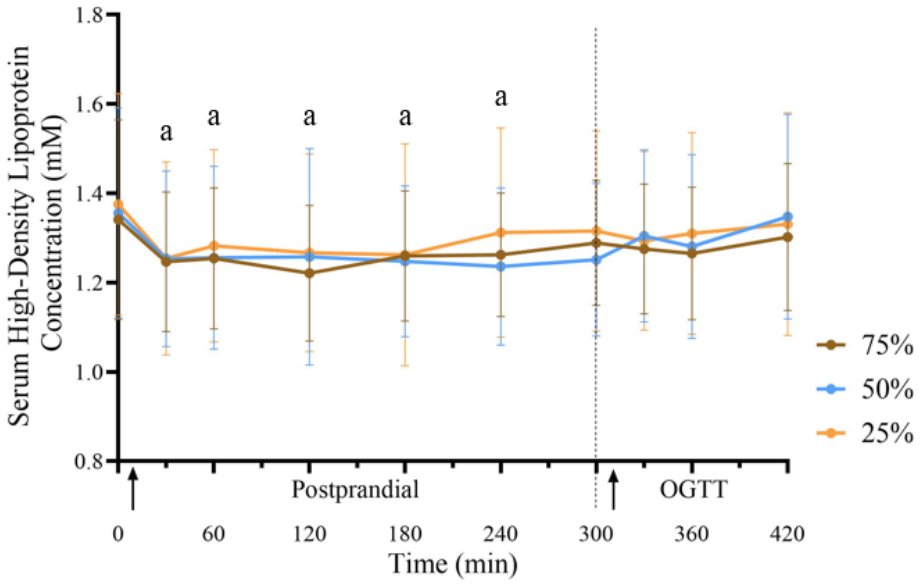

Figure 15: Average serum HDL-c concentration for meals containing 25%, 50%, and 75% fat. "a" indicates the significant main effect of time from baseline (0 min) ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD.

3.5.7 Low-Density Lipoprotein

We observed a main effect of time on serum LDL-c ($p=0.002$), seen in **Figure 16**—only significant differences of postprandial concentrations from baseline (0 min) are shown. Postprandial concentrations decreased ~10-15% from fasting between 60-300 min and then increased ~8% above fasting at 420 min. Total AUC for the entire 7-hour period was 1039, 941, and 928 mM*420 minutes for the 25%, 50%, and 75% fat meals, respectively (no significant difference). Prior to the consumption of the OGTT (300 min) LDL-c concentrations were significantly elevated compared to fasting values ($p=0.028$) (**Figure 16**). A summary of postprandial and OGTT data for LDL-c can be found in **Appendix 8**.

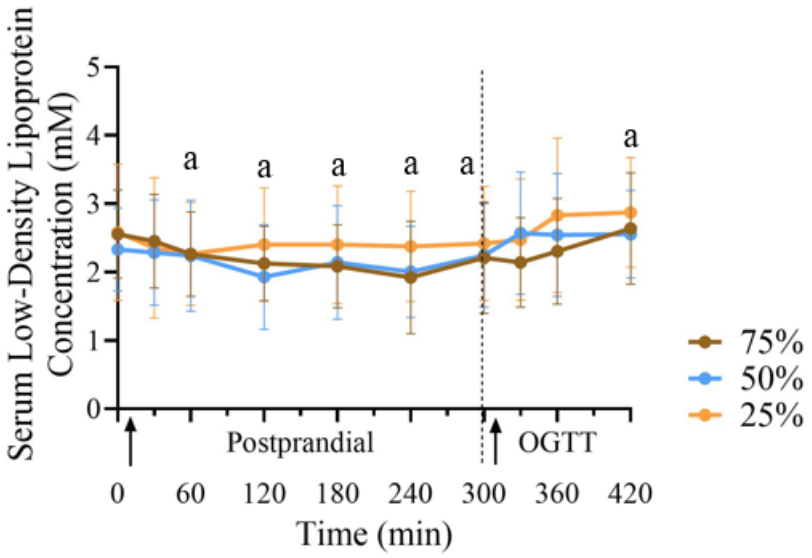

Figure 16: Average serum LDL-c concentration for meals containing 25%, 50%, and 75% fat. "a" indicates the significant main effect of time from baseline (0 min) ($p<0.05$). Arrows indicate the consumption of the meal and the OGTT, respectively. Data are mean ± SD.

4.0 Discussion

4.1 Summary of Results

This thesis examined the impact of increasing quantities of fat on: a) glucose tolerance five-hours post-meal consumption, and b) postprandial availability of glucose and lipid metabolites. We observed that, as the fat quantity of an isocaloric meal increased, so did glucose and insulin concentrations during an OGTT initiated five-hours post-consumption. This suggests that glucose tolerance is reduced with increasing fat quantity and aligns with our original hypothesis. We also hypothesized that the postprandial availability of glucose, insulin, triglycerides, total cholesterol, and LDL-c would increase alongside increasing quantities of fat, while postprandial HDL-c concentrations would decrease. However, contrary to our hypothesis, we observed an inverse relationship between postprandial concentrations of glucose and insulin and fat quantity, with the lowest fat meal inducing the highest postprandial concentrations of these metabolites. Postprandial triglyceride concentrations increased with increasing quantities of fat, which aligns with our original hypothesis, while total cholesterol, HDL-c, and LDL-c concentrations remained unaffected by the fat quantity of an isocaloric meal.

4.2 The Fat Load of a Mixed Macronutrient Meal Differentially Affects the Postprandial Availability of Glucose and Lipid Metabolites

Over the five hours following the consumption of a liquid isocaloric meal, the postprandial availability of key glucose (glucose, insulin) and lipid (triglyceride, total cholesterol, HDL-c, and LDL-c) metabolites was dependent on the fat quantity of the meal.

4.2.1 Increasing Fat Quantity is Associated with Lower Postprandial Glucose and Insulin Availability

Circulating concentrations of glucose and insulin varied during the postprandial period based on the quantity of fat present in the mixed macronutrient meal. In our study, we saw average peak postprandial glucose concentrations of 6.1, 5.5, and 4.6 mM following the 25%, 50%, and 75% fat meals, respectively. These values are on the lower end of those seen in previous research with a meal provided by Wang et al. (20 kcal/kg body mass, 57% fat, 31% carbohydrate) resulting in an average peak glucose concentration of approximately 6.1 mM during an eight-hour postprandial period. Another study, conducted by Ando et al., provided participants with high fat (643 calories, 50% fat, 35% carbohydrate) and high carbohydrate (677 calories, 15% fat, 70% carbohydrate) meals. The high fat meals resulted in average peak glucose concentrations of approximately 5.7 and 5.5 mM while the high carbohydrate meals resulted in average peak glucose concentrations of approximately 7.3, 6.1, 6.6, and 8.0 mM. While some of the average peak glucose concentrations seen in the present study are similar to those observed in previous literature, for the most part, our values are lower than those previously reported.

The lower postprandial availability of glucose with increasing fat quantity is likely due to the difference in carbohydrate content between the meals. Dietary carbohydrates are a major contributor to variations in postprandial glucose concentrations because of their subsequent breakdown into glucose molecules; therefore, increased consumption of dietary carbohydrates leads to higher postprandial glucose concentrations (Association, 2019; Hantzidiamantis et al., 2024). The carbohydrate quantity of the 25%, 50%, and 75% fat meals in the present study was 60%, 35%, and 10%, respectively, and the average absolute amount of carbohydrate provided by each meal was 161, 93, and 27 g, respectively. Therefore, the reduction in carbohydrate quantity

likely played a large role in the lower postprandial glucose concentrations that were observed when fat quantity was increased. However, the absolute quantity of carbohydrates in the meals of the present study were higher than those seen in previous literature. Ando et al. provided participants with meals containing 35% (56.3 grams) and 70% (118.5 grams) of carbohydrates, both of which are lower than the carbohydrate quantity of the two meals with the highest carbohydrate content in the present study (35%, 93.1 grams; 60%, 160.5 grams). Given that our peak glucose concentrations were, for the most part, lower than those reported by Ando et al. despite the higher amount of carbohydrates, dietary carbohydrate quantity alone is likely not the sole factor involved in the lower postprandial glucose concentrations seen in the present study compared to previous literature.

Another factor that plays a role in postprandial glucose concentration is postprandial insulin concentration. Peak postprandial insulin concentrations for the current study were 135.44, 112.91, and 44.92 μIU/mL for the 25%, 50%, and 75% fat meals, respectively. In previous literature of individuals with normal weight, peak postprandial insulin concentrations of approximately 30 μIU/mL were reported by two independent research groups after the provision of a high fat meal [20 kcal/kg body mass, 57% fat, and 31% carbohydrates (Wang et al., 2017); 874 calories, 59% fat, and 37% carbohydrates (Alayón et al., 2018)]. These peak concentrations are over 3-fold lower than the value that resulted from our meal containing a similar amount of carbohydrates (112.91 μIU/mL; 35% carbohydrates). Additionally, both authors reported higher peak postprandial insulin concentrations when individuals with obesity consumed the same meal; Wang et al. observed a peak insulin concentration of approximately 90 μIU/mL in individuals with abdominal obesity and high levels of postprandial insulin resistance and Alayón et al. observed a peak insulin concentration of 71.6 μIU/mL in individuals with obesity. When

comparing the peak postprandial insulin concentration of 112.91 μIU/mL that we observed following the meal most similar to those mentioned above (50% fat, 35% carbohydrates), our value is closer to the ones observed in individuals with obesity, despite our participants having normal weight. Given that insulin is secreted in response to increases in blood glucose concentrations to facilitate glucose uptake (Giugliano et al., 2008) and the absolute quantity of carbohydrates consumed in the present study were higher than those seen in previous literature, higher-than-normal insulin concentrations likely occurred to maintain glucose within a euglycemic range following the large quantity of carbohydrates consumed. Concomitantly, the reduced postprandial availability of insulin that occurred following meals of increasing fat quantity can also be explained by the decreased consumption of dietary carbohydrates and thus the reduced quantity of glucose in the blood and a reduced requirement for uptake facilitated by insulin.

While the absolute quantity of dietary carbohydrates present in the meals and the high levels of insulin could partially explain why postprandial glucose concentrations decreased when dietary fat quantity increased, this may also be explained by the increased quantity of dietary fat itself. Gastric emptying is delayed when dietary fat is consumed due to the presence of receptors in the small intestine that respond to fat in a dose-response manner; higher quantities of dietary fat delay gastric emptying to a higher extent (Cooke, 1977; Lin et al., 1990). The current study contained meals with average absolute fat quantities of 29.7, 59.1, and 88.5 grams for meals containing 25%, 50%, and 75% fat, respectively. Compared to previous literature, Ando et al. provided meals containing 15% (11.3 grams) or 50% (35.7 grams) fat. This corresponds to a 65% increase in absolute fat quantity from the 50% fat meal presented by Ando et al. to the 50% fat meal in the current study. Due to the high quantity of fat in the meals of the present study, it is

possible that greater inhibition of gastric emptying occurred when fat quantity increased, resulting in the delayed appearance of glucose and insulin in circulation and therefore resulting in lower postprandial peak glucose and insulin concentrations as dietary fat quantity increased. However, this is speculation, and future research should be conducted to confirm the relationship between gastric emptying and postprandial glucose and insulin concentrations following high-fat meals.

Altogether, reduced dietary carbohydrate quantities and potential fat-induced inhibition of gastric emptying could explain the reduction in postprandial glucose and insulin concentrations seen when meals with increasing quantities of fat were consumed. Additionally, while postprandial insulin availability was reduced as fat quantity increased, the concentrations seen were higher than those reported in previous literature and therefore could further explain the reduction in postprandial glucose concentrations that were observed as well.

4.2.2 As Fat Content Increases, Postprandial Availability of Triglycerides Increases

Our study found that the postprandial availability of triglycerides, as assessed by postprandial AUC, was significantly elevated following the 75% fat meal compared to the 25% fat meal and triglyceride concentrations at the final postprandial timepoint (300 min) were significantly elevated ($p<0.05$, main effect of time) compared to fasting (0 min). These findings confirm that the consumption of increasing quantities of dietary fat results in the increased presence of triglycerides in the blood, and therefore confirms that our intervention was successful in respect to fat ingestion. These findings are supported by a previous study that found a dose-response relationship between serum triglyceride concentration and fat quantity of a single meal (Cohen et

al., 1988).

Peak postprandial triglyceride concentrations for the 25%, 50%, and 75% fat meals were 1.27, 1.79, and 2.00 mM. While the peak values seen in the present are similar to those observed in previous literature (peak postprandial triglyceride concentration of 1.5 mM following a meal containing 57% fat, Wang et al., 2017), the peak concentration following the 75% fat meal is above the current 1.98 mM cutoff for postprandial hypertriglyceridemia (Wilson et al., 2021). This suggests that in our young, healthy population, postprandial triglyceride concentrations were healthy following the 25% and 50% fat meals but exceeded the cutoff for hypertriglyceridemia following the meal containing the highest amount of fat.

When considering total cholesterol, the postprandial AUC concentrations did not differ significantly following any of the three meals. Although the average postprandial total cholesterol concentrations across the three treatments were decreased from fasting on multiple occasions, the reductions were never greater than 7%. Given that HDL-c and LDL-c concentrations are two major contributors to total cholesterol, these findings are consistent with the small changes observed in HDL-c and LDL-c postprandial concentrations of the present study. While some studies have also found minor decreases in postprandial total cholesterol concentration following a single high-fat meal (Wang et al., 2017), other studies have found no significant differences (Rifai et al., 1990; Averill et al., 2020). The non-remarkable variations seen in total cholesterol levels following a single meal prompted the Canadian Cardiovascular Society to update their Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult to state that non-fasting lipid testing is a suitable alternative to fasting tests in individuals without a history of hypertriglyceridemia (Anderson et al., 2016). Given that our participants were young, healthy and did not have a history of

hypertriglyceridemia, it can be assumed that the minor changes seen in postprandial total cholesterol concentrations following any of the three meals is representative of a normal response for this population.

4.3 A Single High-Fat Meal Acutely Reduces Glucose Tolerance

Glucose concentrations at the two-hour timepoint of an OGTT are used for the diagnosis of prediabetes and T2D. The average glucose concentrations at the end of the two-hour OGTT period (420 min) were 4.9, 5.8, and 6.3 mM for the 25%, 50%, and 75% fat meals respectively. While the glucose concentration tended to increase following the consumption of meals with increasing quantities of fat, there was no significant differences between the meals. The concentrations observed at the end of the OGTT correspond to normal glucose levels following a two-hour OGTT (<7.8 mM) and suggest that, on average, our participants had normal glucose tolerance. However, at the end of the OGTT that followed the 75% fat meal, three individuals presented with glucose concentrations indicative of impaired glucose tolerance (>7.8 mM) despite presenting with normal glucose concentrations at the end of the OGTT that followed the other two meals. This suggests that the fat quantity of the prior meal could result in impaired glucose concentrations of a secondary meal at an individual level.

The average insulin concentrations at the end of the OGTT were 38.60, 59.49, and 80.71 μIU/mL for the 25%, 50%, and 75% fat meal, respectively. Higher insulin concentrations were observed with increasing fat quantity of the preceding meal with significant differences in insulin concentration of the 25% fat meal condition compared to the other two meal conditions ($p<0.05$). While there are currently no standardized concentrations of insulin following an OGTT, previous

work conducted in middle-aged Japanese Americans without diabetes found insulin concentrations of 43.7, 71.0, 58.3, and 118.9 μIU/mL at the two-hour timepoint following a 75 g OGTT conducted after an overnight fast (Hayashi et al., 2013). Another study conducted a 75 g OGTT in three groups of middle-aged adults (94% male); the groups consisted of lean individuals without diabetes, individuals with obesity but without diabetes, and individuals with obesity and diabetes. Average insulin concentrations two hours following the OGTT were approximately 58, 136, and 15 μIU/mL for the control group, individuals with obesity, and individuals with obesity and diabetes, respectively (Tura et al., 2001). Therefore, the two-hour insulin concentrations observed in the present study seem to fall within the range of concentrations seen in previous literature.

While the two-hour glucose and insulin concentrations seen in the present study can be considered normal compared to reference values and previous literature, it is important to note that a typical OGTT is conducted following an overnight fast. This differs from the OGTT in the present study, which was conducted after a five-hour postprandial period. Despite the trend of concentrations to return to baseline as the postprandial period progressed, the final metabolite concentrations prior to the OGTT were significantly elevated compared to fasting (0 min) for both glucose and insulin following the 50% fat meal and elevated for insulin following the 25% fat meal. Therefore, the two-hour glucose and insulin concentrations seen in these conditions may be higher than if the OGTT was conducted in a true fasting state. Given that the concentrations for these conditions already fell within a normal and healthy range, this suggests that the values that resulted following an overnight fast would be lower and therefore still fall within the healthy range.

The peak OGTT glucose and insulin concentrations that occurred when the OGTT was

conducted following the 75% fat meal (8.8 mM, 175.28 μIU/mL, respectively) were greater than the peak postprandial glucose and insulin concentrations that were observed immediately following the meal with the highest carbohydrate content (60% carbohydrate; 6.1 mM and 135.44 μIU/mL, respectively). This suggests that a high-fat meal may disrupt glucose and insulin concentrations of a secondary meal to a greater extent than the metabolic disruption that occurs immediately following a single high-carbohydrate meal. This is supported by a study that observed approximately 1.1 mM higher postprandial peak glucose concentrations after a high-carbohydrate meal when the preceding meal was high-fat compared to high-carbohydrate (Ando et al., 2018). This study concluded that the postprandial glucose concentrations were directly impacted by the macronutrient content of the prior meal and confirmed that postprandial glucose responses are negatively associated with preprandial respiratory quotients (Ando et al., 2018).

4.3.1 Quantifying the Metabolic Response to an OGTT When the Preceding Meal Varies in Fat Content

Glucose tolerance and insulin sensitivity are closely related conditions that are associated with metabolic function. Indices such as the Matsuda index, the OGIS, and HOMA-IR can be used to quantify insulin sensitivity using fasting and/or OGTT glucose and insulin concentrations (Gutch et al., 2015; Mari et al., 2001). The Matsuda index is calculated using glucose and insulin concentrations from a fasted state and following an OGTT. This index represents the insulin sensitivity of hepatic and peripheral tissues, is highly correlated to the rate of whole-body glucose disposal during a euglycemic insulinemic clamp and predicts insulin resistance when values fall below 4.3 (Matsuda & DeFronzo, 1999; Gutch et al., 2015). The average Matsuda index values for the 25%, 50%, and 75% fat meals were 4.73, 3.94, and 3.52, respectively. These

values suggest that following the two meals with the highest fat quantity, insulin sensitivity was reduced. Given that standard OGTTs are performed following an overnight fast and the OGTT conducted in the present study was not, this could limit the interpretation of the Matsuda index values. The OGIS is another index used to assess insulin sensitivity from an OGTT (Mari et al., 2001). This index is calculated using participant weight and height in addition to glucose and insulin concentrations during an OGTT. The researchers who developed this index found that lean subjects, subjects with obesity, subjects with impaired glucose tolerance, and subjects with type 2 diabetes had average OGIS values of 440, 362, 302, and 239 ml/min/m^2, respectively (Mari et al., 2001). The OGIS values calculated for the 25%, 50%, and 75% fat meals of the present study were 521, 470, and 433 ml/min/m^2, respectively. These values align with those seen in the lean subjects of the original study and therefore indicate that our participants had normal glucose clearance and thus normal insulin sensitivity. However, the OGIS value calculated for the 25% fat meal was significantly elevated compared to the value from the 75% fat meal, suggesting that increasing quantities of fat resulted in significantly reduced, but still healthy, values of insulin sensitivity. The difference in the insulin sensitivity of our participants as determined by the Matsuda index and the OGIS likely comes down to performing the OGTT under non-fasting conditions in the present study. Further research should be conducted to determine reference values for the Matsuda index and OGIS when the OGTT is conducted in a postprandial state.

HOMA-IR values are calculated based on fasting glucose and insulin concentrations with values <1.0, >1.9, and >2.9 indicating insulin sensitivity, early insulin resistance, and insulin resistance, respectively (Jiménez-Maldonado et al., 2020). While the average values HOMA-IRs were all below the cut-off for early insulin resistance (1.4, 1.6, and 1.3, for the 25%, 50%, and

75% fat meals, respectively), individual HOMA-IR values varied. Prior to the 75% fat meal, one participant had a HOMA-IR value indicative of early insulin resistance (2.4) but a healthy average across all three meals (1.7) while two additional participants had high HOMA-IR values before the 50% fat (3.1 and 2.0) and the 25% fat meals (2.1 and 2.3) as well as high averages across all three conditions (2.2 and 2.0). While it is possible that the high HOMA-IR values present in these otherwise healthy participants occurred due to undisclosed breaking of the required overnight fast, all fasting glucose and insulin values were in the normal fasting range (<5.6 mM for glucose and <25 μIU/mL for insulin), however, the values were slightly elevated compared to the other participants. It is important to note that the two participants that displayed elevated HOMA-IR values on two of the three visits were the only two female participants who completed the study. All females presented to the lab during days 3-7 of their placebo pill week which corresponds to the week in which estrogen levels are lowest. While estrogen plays a protective role in insulin sensitivity, previous research is unclear whether insulin sensitivity changes throughout the menstrual cycle with some studies reporting significant changes to insulin sensitivity at different phases of the menstrual cycle (Yeung et al., 2010; Valdes & Elkind-Hirsch, 1991; González-Ortiz et al., 1998) and others reporting no change (Toth et al., 1987; Bingley et al., 2008). Despite these discrepant findings, another study found that adjusting for BMI and cardiorespiratory fitness resulted in variable changes in HOMA-IR across the menstrual cycle (Macgregor et al., 2021). Therefore, it is possible that the low levels of estrogen, as well as BMI and cardiorespiratory fitness, could influence the insulin sensitivity of females during their menstrual cycle, resulting in the high HOMA-IR values that were observed.

4.4 Increased Circulating Lipid Availability May Explain the Inverse Relationship Between Fat Content of a Meal and Glucose Tolerance

Understanding the fate of dietary fat can help explain the reductions in glucose tolerance that are seen when an OGTT follows a meal containing 75% fat. Following the ingestion of dietary fat, digestion takes place in the stomach and small intestine where fat is broken down into DAGs, monoglycerides, and NEFAs (Omer & Chiodi, 2024). These products are then absorbed into the intestinal cells, re-esterified into triglycerides and subsequently packaged into chylomicrons (Omer & Chiodi, 2024). These chylomicrons eventually enter the bloodstream, reaching peak concentrations approximately three to four hours post meal consumption, and deliver the dietary fat-derived products to various tissues of the body, including adipose and skeletal muscle, where they are broken down into NEFAs (Omer & Chiodi, 2024; Lambert & Parks, 2012). Compared to the first meal of the day, chylomicron concentrations are higher and contain larger amounts of lipids when formed after a secondary meal and therefore may play a role in the postprandial metabolism of a second meal (Lambet & Parks, 2012). Secondary meal effects following a high-fat meal have been reported in previous literature. One study found higher glucose concentrations following an OGTT when the previous meal was high-fat compared to high-carbohydrate (Denise Robertson et al., 2002) while another study found that consistently consuming a high-fat breakfast led to increased concentrations of NEFAs throughout the day which contributed to higher glucose and insulin concentrations during an OGTT compared to when a high-carbohydrate breakfast was regularly consumed (Frape et al., 1998). These results found in previous literature support the findings of the present study where higher concentrations of glucose and insulin were observed during an OGTT when the previous meal was high in fat. Given that increases in NEFAs have been implicated in reduced glucose oxidation (Randle et al.,

1963) and impairments in the insulin-signaling pathway (Zierath et al., 1997), it is possible that increasing the quantity of fat in the meals of the present study played a role in the reductions in glucose tolerance that were observed following an OGTT.

While glucose and insulin concentrations varied during the OGTT, of the lipid metabolites, only triglyceride AUC values increased with increasing quantities of fat while total cholesterol, HDL-c, and LDL-c AUC values did not. This suggests that when a secondary meal of high carbohydrate is consumed, only triglyceride concentration is impacted by the fat quantity of a preceding meal. This finding is corroborated by studies that have found triglycerides concentrations to increase progressively over the course of the day, peaking several hours following meal (Denise Robertson et al., 2002; Van Oostrom et al., 2000; Teff et al., 2004). The lack of significant differences in total cholesterol, HDL-c, and LDL-c AUC concentrations following an OGTT between meal conditions is supported by previously mentioned literature that found no significant changes in postprandial total cholesterol concentrations and new guidelines suggesting that healthy individuals without a history of hypertriglyceridemia do not experience remarkable changes in cholesterol levels following a meal (Rifai et al., 1990; Averill et al., 2020; Anderson et al., 2016). From these studies and the results of the current study, we can assume that cholesterol AUC values remain unchanged after ingestion of a high quantity of carbohydrates, regardless of the fat quantity of the previous meal.

4.5 Strengths and Limitations

The within-subjects design of this study is a key strength, given that previous acute fat ingestion studies have either provided participants with a single meal during a single laboratory visit or

employed a between-subjects design. Our within-subject design allowed us to control for individual variability in factors known to influence metabolism (such as age, BMI, and physical fitness), thereby increasing the likelihood that the observed results are due to the manipulations in dietary fat quantity. We also implemented other important controls to elevate this work above the existing literature and help tease apart the specific role of fat quantity on glucose tolerance. Firstly, we provided participants with isocaloric meals with protein clamped at 15% to control for the potential confounding effects of total energy intake or protein consumption. Secondly, participants remained seated at rest for the entire seven-hour protocol, since even a short walk has been shown to lower postprandial glucose concentrations (Engeroff et al., 2023). Finally, we selected stringent eligibility criteria in an effort to ensure a relatively homogeneous sample of young, healthy adults was enrolled. Previous acute fat ingestion studies have relied on qualitative measures of physical activity, which may be vulnerable to inaccurate reporting (Meh et al., 2023). Therefore, a VO_2peak test, the gold-standard assessment of cardiorespiratory fitness (Shephard et al., 1968), was used to quantitatively verify physical activity levels and remove any potential bias associated with subjective self-reports.

A potential limitation of the present study was our relatively small sample size of 9 participants. However, this number aligned with our a priori sample size calculation based on data from a similarly designed study that also investigated glycemic response following a high-fat meal. Furthermore, post hoc power calculations confirmed that we were sufficiently powered for most major outcomes. Another limitation is to the lack of standardized meals or tracking of meals in the days preceding the nutrition intervention visits, which may have impacted the metabolic response to our test meals, even though participants were always overnight fasted. However, we did characterize habitual diet by providing a three-day food diary to each

participant upon enrollment in the study, and participants' macronutrient distributions were in line with the acceptable macronutrient distribution ranges. Finally, while our postprandial period of five hours matched or exceeded those previously seen in the literature, some studies measure up to eight hours post-ingestion (Wang et al., 2017). It is possible that extending the postprandial period may have revealed additional changes in metabolism. However, given that Western culture promotes the consumption of three meals plus one-to-two snacks per day and a majority of Canadians report consuming 2-3 snacks daily (Paoli et al., 2019; Vatanparast et al., 2020), it is not likely that the postprandial period will reach, let alone exceed, five hours. Therefore, the postprandial period we established is likely an ideal length to observe potential metabolic changes that occur later in the postprandial period but still short enough to reflect a physiologically relevant postprandial period that would be observed in the real-world where meal frequency is high and thus uninterrupted postprandial states are short.

4.6 Future Directions

While the current study has begun to resolve previously unanswered questions regarding dietary fat quantity and the impact on glucose tolerance and postprandial metabolism, there remain important questions that future work should answer. Firstly, this research was conducted in a young, healthy population, which allowed us to characterize the initial metabolic response to a high-fat meal without the confounding effects of age- and health-related metabolic detriments. However, our findings remain to be explored in individuals with some degree of metabolic dysfunction (e.g., obesity, metabolic syndrome, polycystic ovary syndrome, older adults) (Pouliot et al., 1992; Rowe et al., n.d.; Moghetti & Tosi, 2021). These more sensitive populations would benefit from a deeper understanding of the early metabolic changes following a high-fat

meal that could lead to the emergence of early-stage interventions that prevent or delay the development of metabolic disorders.

The main fat type in our dairy-based meals was saturated fat, and future studies should explore the effect of fat type and source on postprandial glucose handling. Unsaturated fats have been shown to *improve* insulin sensitivity, fasting glucose, and insulin secretion (Imamura et al., 2016; McMacken & Shah, 2017), provided that total daily fat intake does not exceed 37% daily energy intake (Vessby et al., 2001). Therefore, further research is needed to confirm whether the same inverse relationship between fat quantity and glucose tolerance that we observed exists when the meal is primarily unsaturated fat. Fat source may also play an important role in metabolic health. The current study provided fat from dairy-based sources which may protect against glucose and insulin metabolic impairments, as well as T2D. Studies have found that higher intakes of skim milk, fermented dairy, buttermilk, and low-fat cheese were associated with lower risk of prediabetes and that T2D risk could be reduced by 5% for each one serving per day of total dairy products (Hirahatake et al., 2014; Eussen et al., 2016). Further research should be conducted to fully elucidate the impact of dairy and non-dairy based sources of fat on glucose metabolism.

The physical form of a meal (liquid vs. solid) may also play a role in the postprandial glucose and lipid metabolic changes. Both forms have been used in previous research on high-fat feeding and glucose metabolism, but questions remain regarding whether the differential digestion of a liquid vs solid meal may impact glucose and lipid outcomes. One study found that a high-fat solid meal resulted in prolonged gastric emptying compared to an energy-matched high-fat liquid meal (Achour et al., 2001) while another study found significant differences in insulin, but not glucose, response following a liquid meal compared to a solid meal (Brynes et

al., 1998). Given the potential role that the physical form of a meal has on postprandial metabolism, further research should be conducted to determine if lqiuid vs solid high-fat meal significantly influences glucose and lipid metabolism.

Finally, our test meals provided a relatively high amount of energy (average for all 9 participants = 1065 kcal/meal) and were designed to be a metabolic challenge. But it remains unknown whether "smaller" high-fat meals – that provide a similar amount of energy as the average breakfast or lunch meal – would induce similar decrements in glucose handling. Although high-calorie meal challenges are often used in high-fat feeding studies, not all high-fat consumption is accompanied with high-caloric intake and future research should investigate how "normal-sized" high-fat meals impact glucose metabolism

4.7 Impact and Significance

This study was the first to use a dose-response design to investigate the early perturbations that occur to glucose handling following high-fat ingestion. In contrast, the vast majority of previous research has focused on glucose homeostasis following chronic high-fat feeding or the acute impact of fat ingestion on lipid metabolism. Using a young, healthy population in a tightly controlled within-subject design allowed us to investigate how fat quantity impacts glucose tolerance and postprandial metabolism in the absence of age-related declines in health, caloric surpluses, or other metabolic detriments related to weight, fitness, or general health. This research has contributed to the limited knowledge on the initial changes in metabolism that occur following high-fat ingestion and could lead to the development of metabolic disease states such as type 2 diabetes and metabolic syndrome. This knowledge is of importance to individuals with

impaired metabolism who are looking to improve their glucose tolerance and homeostasis to prevent or delay the development of metabolic disease.

4.8 Conclusion

Increasing the quantity of fat in a single isocaloric meal led to reduced postprandial glucose and insulin concentrations, an increase in triglycerides, and no change in total cholesterol, HDL-c, and LDL-c. However, when an OGTT was initiated, higher fat quantity of the prior meal led to higher concentrations of glucose and insulin. This indicates that consumption of a single high-fat meal can disrupt glucose handling as early as five-hours post meal consumption. This study used a with-in subject design and implemented tight control throughout the study design and data collection process to ensure confounding variables were kept to an absolute minimum. However, this study is not without limitations which include a small sample size, a lack of control in meals prior to the nutrition intervention visit, and a short postprandial period. The present study has provided a deeper understanding of the early metabolic changes that follow high-fat consumption and could play an important role in the development of metabolic disease. Future studies should investigate the impact of high-fat consumption on glucose metabolism in more sensitive populations, using different fat types and sources, altering physical meal form, and reducing caloric intake to a more standardized quantity.

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Appendices

Appendix 1 – Final Approval Letter from the Hamilton Integrated Research Ethics Board

HiREB
Hamilton Integrated Research Ethics Board

Date: Jun-05-2023

Local Principal Investigator: Dr. Kirsten Bell

Participating HiREB Centre(s): McMaster University

Project ID: 16153

Project Title: Investigating the effect of acute fat ingestion on glucose metabolism in young healthy adults: a dose response study

Review Type: Full Board

Meeting Date: Apr-05-2023

Date of Final Approval: Jun-05-2023

Ethics Expiry Date: Jun-05-2024

The Hamilton Integrated Research Ethics Board (HiREB) Panel B has reviewed and approved the abovementioned study.

The following documents have been approved:

Document Name	Document Date	Document Version
Appendix 5 - Food Diary	Feb-20-2023	1.0
Appendix 3 - Case Record Form	Mar-07-2023	1.0
Appendix 6 - Current Health Status Questionnaire	Mar-07-2023	1.0
Appendix 7 - Participant Screening Form - V2 - Clean	May-22-2023	2.0
Appendix 4 - Participant Key - V2 - Clean	May-22-2023	2.0
Appendix 10 - Pre-Populated Text of Email from Recruitment Poster	May-22-2023	1.0
Appendix 1 - Recruitment Poster - V2 - Clean	May-22-2023	2.0
Appendix 11 - Social Media Version of Recruitment Poster	May-22-2023	1.0
Research Proposal - V2 - Clean	May-22-2023	2.0
Appendix 2 - Consent Form and Information Letter - V2 - Clean	May-22-2023	2.0
Appendix 8 - Script - V4 - Clean	Jun-05-2023	4.0

The following documents have been acknowledged:

Document Name	Document Date	Document Version
GCP Certificate - KBell	Apr-05-2022	Original
KBell - Phlebotomy certificate	Feb-20-2023	1.0
Autumn Lawrence Phlebotomy Training Letter of Recognition	Mar-10-2023	1.0
Appendix 9 - Budget - V2	May-22-2023	2.0

While HiREB has reviewed and approved this application, the research must be conducted in accordance with applicable regulations and institutional and/or public health requirements.

We are pleased to issue final approval for the above-named study until the expiry date noted above. Continuation beyond that date will require further review and renewal of HiREB approval. Any changes or revisions to the original submission must be submitted on a HiREB amendment form for review and approval by the

Hamilton Integrated Research Ethics Board.

REB members involved in the research project do not participate in the review, discussion or decision.

The Hamilton Integrated Research Ethics Board (HiREB) provides ethical review and ongoing ethical oversight on behalf of Hamilton Health Sciences, St. Joseph's Healthcare Hamilton, Research St. Joseph's-Hamilton, the Faculty of Health Sciences at McMaster University and Niagara Health. HiREB operates in compliance with and is constituted in accordance with the requirements of: The Tri-Council Policy Statement on Ethical Conduct of Research Involving Humans (TCPS 2); The International Conference on Harmonisation of Good Clinical Practices Guideline (ICH GCP); Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act 2004 and its applicable Regulations. For studies conducted at St. Joseph's Healthcare Hamilton, HiREB complies with the Health Ethics Guide of the Catholic Alliance of Canada. HiREB is qualified through the Clinical Trials Ontario (CTO) REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Sincerely,

Frederick A Spencer

Dr. Frederick A. Spencer, MD
Chair, Hamilton Integrated Research Ethics Board

Hamilton Integrated Research Ethics Board (HiREB)
237 Barton Street, Suite C1-205 Hamilton, ON L8L 2X2
Telephone: 905-521-2100, Ext. 42013

Appendix 2 – Estimated VO_{2max} from the YMCA Submaximal Cycle Ergometer Test by Age for Men; adapted from (American College of Sports Medicine, 2017)

Norms for Max VO_2 (mL/kg) - Men							
		Age (years)					
% Ranking	Classification	18-25	26-35	36-45	46-55	56-65	Over 65
100	Excellent	100	95	90	83	65	53
95		75	66	61	55	50	42
90		65	60	55	49	43	38
85	Good	60	55	49	45	40	34
80		56	52	47	43	38	33
75		53	50	45	40	37	32
70	Above average	50	48	43	39	35	31
65		49	45	41	38	34	30
60		48	44	40	36	33	29
55	Average	45	42	38	35	32	28
50		44	40	37	33	31	27
45		43	39	36	32	30	26
40	Below average	42	38	35	31	28	25
35		39	37	33	30	27	24
30		38	34	31	29	26	23
25	Poor	36	33	30	27	25	22
20		35	32	29	26	23	21
15		32	30	27	25	22	20
10	Very poor	30	27	24	24	21	18
5		26	24	21	20	18	16
0		20	15	14	13	12	10

Appendix 3 – Estimated VO_2max from the YMCA Submaximal Cycle Ergometer Test by Age for Women; adapted from (American College of Sports Medicine, 2017)

Norms for Max VO_2 (mL/kg) - Women							
		Age (years)					
% Ranking	Classification	18-25	26-35	36-45	46-55	56-65	Over 65
100	Excellent	95	95	75	72	58	55
95		69	65	56	51	44	48
90		59	58	50	45	40	34
85	Good	56	53	46	41	36	31
80		52	51	44	39	35	30
75		50	48	42	36	33	29
70	Above average	47	45	41	35	32	28
65		45	44	38	34	31	27
60		44	43	37	32	30	26
55	Average	42	41	36	31	28	25
50		40	40	34	30	27	24
45		39	37	33	29	26	23
40	Below average	38	36	32	28	25	22
35		37	35	30	27	24	21
30		35	34	29	26	23	20
25	Poor	33	32	28	25	22	19
20		32	30	26	23	20	18
15		30	28	25	22	19	17
10	Very poor	27	25	24	20	18	16
5		24	22	20	18	15	14
0		15	14	12	11	10	10

Appendix 4 – Summary of Results for the VO_2peak Tests Separated by Eligibility and Bike Model (Kettler or Lode Bike)

Due to technical issues on the Lode bike that prevented seat and handle bar adjustments, the Lode bike was not able to be used for every VO_{2max} test that was conducted as manual adjustments are not possible on this bike. The Kettler bike was used as an alternative when these technical difficulties arose.

Table A4.1 – Number of Participants and Average VO_2peak, Respiratory Exchange Ratio, Maximum Heart Rate, and Maximum Power Output Obtained from Eligible and Ineligible Participants on the Lode Bike

Lode Bike Stats				
		Eligible	**Ineligible**	**Total**
Participants	Male	5	4	**9**
	Female	3	1	**4**
	Total	**8**	**5**	**13**
VO_2peak	Male	42.84	43.74	**43.24**
	Female	37.70	37.27	**37.60**
	Total	**40.91**	**42.44**	**41.50**
Respiratory Exchange Ratio	Male	1.20	1.11	**1.15**
	Female	1.11	1.21	**1.14**
	Total	**1.17**	**1.13**	**1.15**
Maximum Heart Rate	Male	189	176	**183**
	Female	187	193	**189**
	Total	**188**	**180**	**185**
Maximum Wattage	Male	241	239	**240**
	Female	201	165	**192**
	Total	**226**	**220**	**224**

Table A4.2 – Number of Participants and Average VO_{2peak}, Respiratory Exchange Ratio, Maximum Heart Rate, and Maximum Power Output Obtained from Eligible and Ineligible Participants on the Kettler Bike

Kettler Bike Stats				
		Eligible	**Ineligible**	**Total**
Participants	Male	7	8	**15**
	Female	1	2	**3**
	Total	**8**	**10**	**18**
VO_2peak	Male	42.88	48.30	**45.77**
	Female	41.54	32.35	**35.41**
	Total	**42.71**	**45.11**	**44.04**
Respiratory Exchange Ratio	Male	1.18	1.16	**1.17**
	Female	1.19	1.17	**1.18**
	Total	**1.18**	**1.16**	**1.17**
Maximum Heart Rate	Male	186	191	**188**
	Female	N/A	182	**182**
	Total	**186**	**189**	**187**
Maximum Wattage	Male	298	289	**293**
	Female	245	180	**202**
	Total	**291**	**268**	**278**

Appendix 5 – Test Meal Ingredients and Nutrition Labels

Table A5.1 – Key Nutrition Information for each Test Meal Ingredient

Ingredient	Rolled Oats (Bulk Barn)	Clotted Cream (Devon's Company)	1% Milk (Neilson)	Whey Protein Isolate 90% - Unflavoured (Bulk Barn)	Maltodextrin (The Protein Company)
Serving size	100 grams	28 grams	250 mL	100 grams	30 grams
Energy (kcal)	379	150	110	390	116
Total fat (grams)	6.5	15	2.5	1.5	0
Saturated fat (grams)	1.1	10	1.5	0.3	0
Trans fat (grams)	0	0	0	0	0
Carbohydrate (grams)	68	0	12	4	29
Fiber (grams)	10	0	0	0	0
Sugar (grams)	1	0	12	1	1
Protein (grams)	13.2	0	9	90	0

Figure A5.2 – Nutrition Facts Label for Rolled Oats

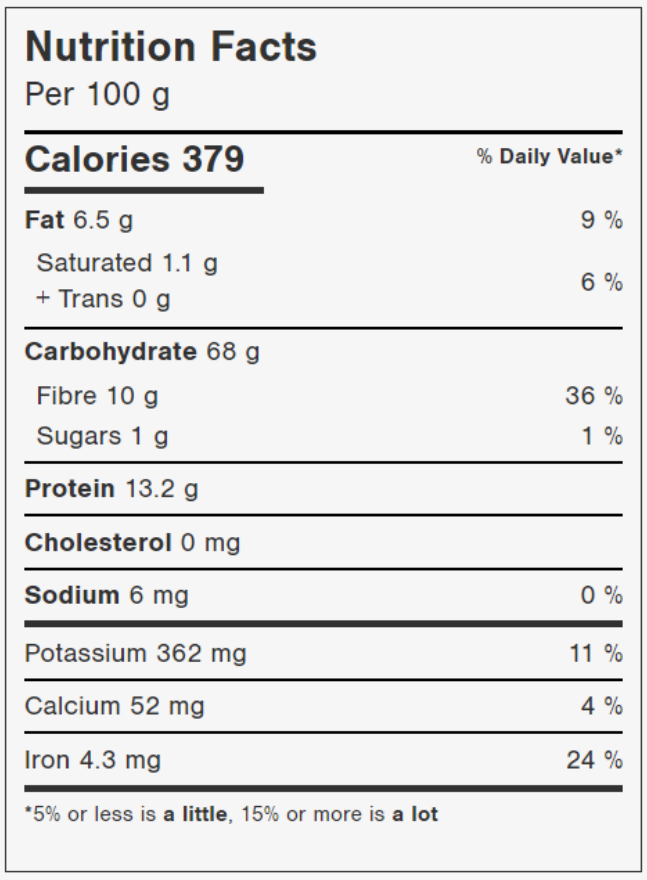


Figure A5.3 – Nutrition Facts Label for Clotted Cream

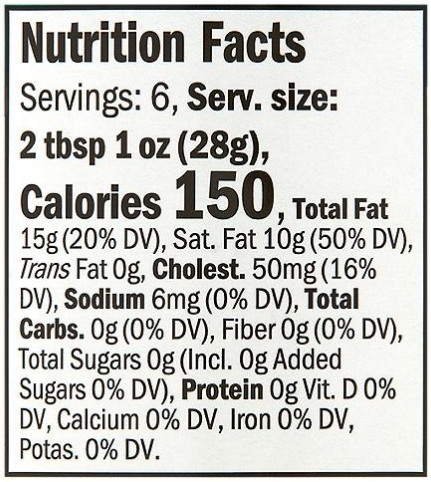


Figure A5.4 – Nutrition Facts Label for 1% Milk

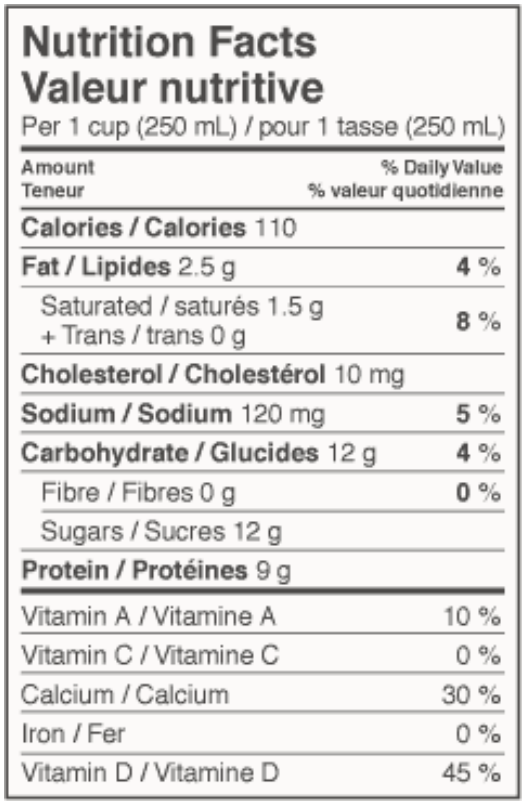
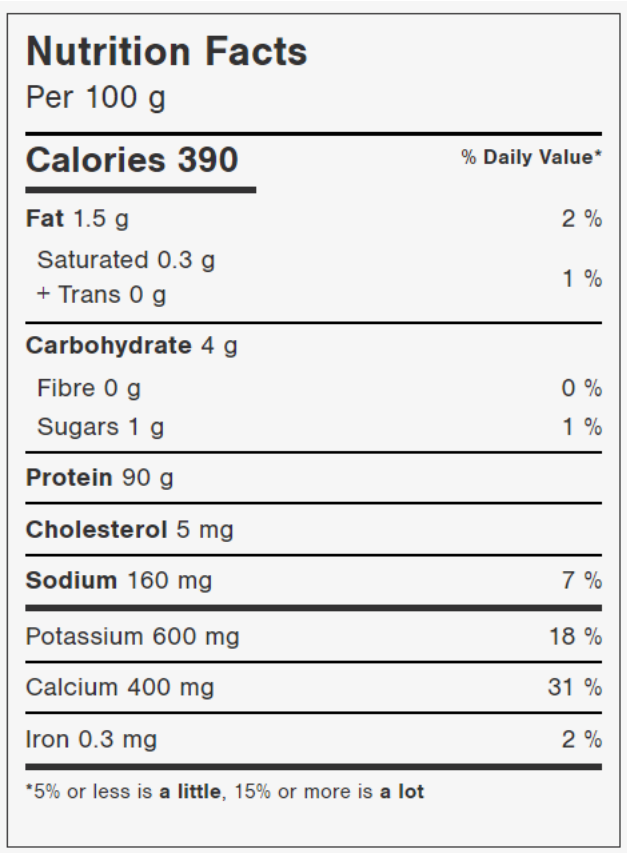


Figure A5.5 – Nutrition Facts Label for Whey Protein



Nutrition Facts

Per 100 g

Calories 390	% Daily Value*
Fat 1.5 g	2 %
Saturated 0.3 g + Trans 0 g	1 %
Carbohydrate 4 g	
Fibre 0 g	0 %
Sugars 1 g	1 %
Protein 90 g	
Cholesterol 5 mg	
Sodium 160 mg	7 %
Potassium 600 mg	18 %
Calcium 400 mg	31 %
Iron 0.3 mg	2 %

*5% or less is **a little**, 15% or more is **a lot**

Figure A5.6 – Nutrition Facts Label for Maltodextrin

Supplement Facts		
Serving Size: 30 g (1 scoop) Servings Per Container: 75		
	Amount Per Serving	%DV*
Calories	116	
Total Fat	0 g	0%
Saturated Fat	0 g	0%
Trans Fat	0 g	
Cholesterol	0 mg	0%
Sodium	0 mg	0%
Carbohydrates	29 g	10%
Fiber	0g	
Sugars	1g	
Protein	0g	0%
*Percent Daily Value based on 2000 calorie diet		

Appendix 6 – Graphic Comparison of Glucose Concentrations Analyzed using a Glucometer and a Spectrophotometric Enzymatic Assay

Due to heavy backorder issues involving PGO Enzyme Preparation Capsules (Sigma-Aldrich; St. Louis, MO), spectrophotometric quantification of glucose was not able to be conducted for the entire data set. Instead, serum glucose was analysed using an Accu-Check® glucometer (Roche; Basel, Switzerland) using a glucose oxidase reaction. Prior to backorder, glucose concentrations from a single participant were analysed using the spectrophotometric assay and compared to the glucose concentrations resulting from the glucometer. It was found that there was minimal difference between glucose concentrations determined using the assay in comparison to the glucometer.

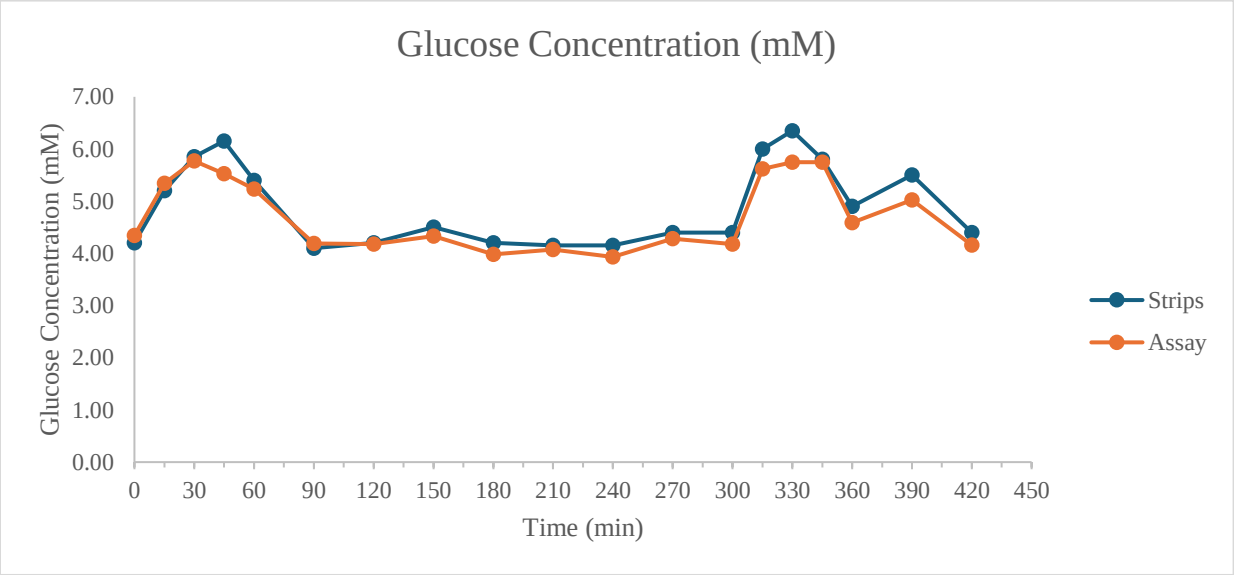


Figure A6.1 – Serum Glucose Concentration of a Single Participant During Visit 1 (Test Meal = 50% fat) Analysed Using a Glucometer and Blood Glucose Strips Compared to a Spectrophotometric Enzymatic Assay

Table A6.2 – Serum Glucose Concentration and Coefficient of Variation Values of a Single Participant During Visit 1 (Test Meal = 50% fat) Analyzed Using a Glucometer and Blood Glucose Strips Compared to a Spectrophotometric Enzymatic Assay

Time (min)	Glucose Concentration (mM)		Coefficient of Variation (%)
	Strips	Assay	
0	4.20	4.34	2.34
15	5.20	5.34	1.94
30	5.85	5.77	0.96
45	6.15	5.52	7.58
60	5.40	5.23	2.25
90	4.10	4.19	1.53
120	4.20	4.18	0.34
150	4.50	4.33	2.70
180	4.20	3.98	3.78
210	4.15	4.08	1.27
240	4.15	3.93	3.82
270	4.40	4.28	1.96
300	4.40	4.18	3.64
315	6.00	5.62	4.67
330	6.35	5.74	7.08
345	5.80	5.74	0.68
360	4.90	4.59	4.61
390	5.50	5.02	6.44
420	4.40	4.16	3.95

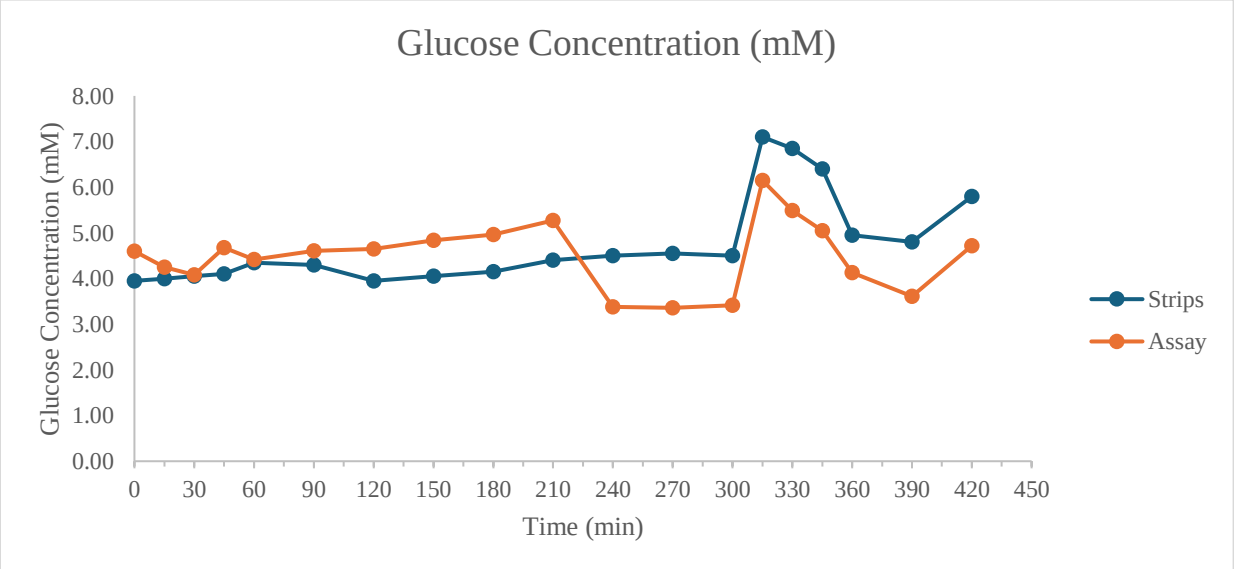


Figure A6.3 – Serum Glucose Concentration of a Single Participant During Visit 2 (Test Meal = 75% fat) Analysed Using a Glucometer and Blood Glucose Strips Compared to a Spectrophotometric Enzymatic Assay

Table A6.4 – Serum Glucose Concentration and Coefficient of Variation Values of a Single Participant During Visit 2 (Test Meal = 75% fat) Analyzed Using a Glucometer and Blood Glucose Strips Compared to a Spectrophotometric Enzymatic Assay

Time (min)	Glucose Concentration (mM)		Coefficient of Variation
	Strips	Assay	
0	3.95	4.60	10.73
15	4.00	4.25	4.21
30	4.05	4.08	0.59
45	4.10	4.68	9.33
60	4.35	4.42	1.08
90	4.30	4.61	4.90
120	3.95	4.65	11.50
150	4.05	4.84	12.58
180	4.15	4.96	12.60
210	4.40	5.27	12.79
240	4.50	3.38	20.14
270	4.55	3.36	21.29
300	4.50	3.42	19.37
315	7.10	6.15	10.16
330	6.85	5.49	15.60
345	6.40	5.05	16.73
360	4.95	4.13	12.74
390	4.80	3.61	19.94
420	5.80	4.72	14.58

Appendix 7 – Inter- and Intra-Assay Variability for Each Assay Conducted by the Main Investigator

	Inter-Assay (%CV)	Intra-Assay (%CV)
Glucose	N/A	1.13
Insulin	9.34	4.77
Triglycerides	8.10	5.92
Total Cholesterol	5.01	4.89

Appendix 8 – A Summary of the Postprandial and OGTT Data for Lipid Metabolites

Table A8.1 – Postprandial data for triglycerides, total cholesterol, HDL, and LDL (n=9)

	25% fat	**50% fat**	**75% fat**
	Triglycerides		
Peak triglyceride (mM)	1.27 ± 0.36#	1.79 ± 0.85	2.00 ± 0.83
Time of peak triglyceride (min)	180 ± 103	200 ± 87	200 ± 64
Triglyceride AUC (mM*300 min)	273 ± 98#	368 ± 153	410 ± 137
	Total Cholesterol		
Peak total cholesterol (mM)	4.48 ± 1.04	4.32 ± 0.86	4.48 ± 0.83
Time of peak total cholesterol (min)	148 ± 122	202 ± 112	122 ± 110
Total cholesterol AUC (mM*300 min)	1229 ± 302	1183 ± 230	1221 ± 208
	HDL		
Peak HDL (mM)	1.39 ± 0.25	1.38 ± 0.22	1.37 ± 0.202
Time of peak HDL (min)	200 ± 73	150 ± 122	183 ± 107
HDL AUC (mM*300 min)	386 ± 67	377 ± 53	377 ± 44
	LDL		
Peak LDL (mM)	2.91 ± 0.94	2.74 ± 0.87	2.85 ± 0.76
Time of peak LDL (min)	183 ± 111	157 ± 116	160 ± 115
LDL AUC (mM*300 min)	715 ± 246	639 ± 201	648 ± 167

Data are mean ± SD.
#p<0.05 compared to the 75% fat meal

Table 8.2 – Data for triglycerides, total cholesterol, HDL, and LDL during the OGTT (n=9)

	25% fat	**50% fat**	**75% fat**
	Triglycerides		
Triglyceride AUC (mM*120 min)	84 ± 51#	103 ± 55#	164 ± 106
	Total Cholesterol		
Total cholesterol AUC (mM*120 min)	457 ± 107	456 ± 100	455 ± 87
	HDL		
HDL AUC (mM*120 min)	118 ± 21	118 ± 19	115 ± 14
	LDL		
LDL AUC (mM*120 min)	251 ± 80	230 ± 71	215 ± 62

Data are mean ± SD.
#p<0.05 compared to the 75% fat meal