Summer 2024

Effects of Ordered Eating on Various Postprandial Measures

Brian K. Ferguson
Old Dominion University, fergubk@pba.edu

EFFECTS OF ORDERED EATING ON VARIOUS POSTPRANDIAL MEASURES

by

Brian K. Ferguson
B.S. August 2018, Auburn University
M.S. August 2020, Auburn University

A Dissertation Submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

EDUCATION WITH A CONCENTRATION IN HUMAN MOVEMENT SCIENCES AND APPLIED KINESIOLOGY

OLD DOMINION UNIVERSITY
August 2024

Approved By:

Patrick Wilson (Director)

Leryn Reynolds (Member)

Cody Haun (Member)

ABSTRACT

EFFECTS OF ORDERED EATING ON VARIOUS POSTPRANDIAL MEASURES

Brian Keith Ferguson
Old Dominion University, 2024
Director: Dr. Patrick B. Wilson

Postprandial glucose (PPG) is an indicator of acute and chronic overall health, and aberrations in glucose, insulin, and other postprandial (PP) markers are common in obesity and cardiometabolic disorders. Chronically elevated glucose can lead to a number of health problems such as type 2 diabetes mellitus. Additionally, PPG responses can impact substrate utilization responses to exercise, which may have implications for both healthy and diseased populations. One potentially simple lifestyle modification to alter PPG and related markers is to change the order in which foods are consumed within meals. The purpose of this dissertation was to further explore the impact of ordered eating on a variety of PP measures and its possible effects on exercise responses in an acute setting. Study 1 was a systematic review of existing literature to assess the effect of ordered eating on a variety of PP measures including glucose, insulin, C-peptide, hunger, and satiety. Study 2 was a randomized crossover laboratory-based experiment that examined the effects of ordered eating on PPG, substrate utilization, hunger, satiety, and other variables surrounding an exercise bout. For Study 1, three databases were searched, reference lists of identified reports were searched, and an author of several studies was consulted to verify that relevant literature was included. The review included acute interventions that administered isocaloric meals of the same foods but with foods eaten in different orders. Participants for Study 2 were recreationally active, generally healthy adults aged 18 to 60 years old and had fasting blood glucose measured as well as resting gaseous exchange before being given a standard meal consisting of chicken, broccoli, and rice, which was ordered in a rice first

(RF) condition on one day and a rice last (RL) condition on a different day. Following the meal, participants rested for 60 minutes prior to beginning a 30-minute exercise bout at 70% of maximum heart rate. Throughout rest and exercise, participants rated hunger, appetite, satiety, and fullness and had gaseous exchange and blood glucose measured regularly. The main statistical analysis for study 2 was a two-way ANOVA with time and condition as within-subject factors to compare the RL and RF conditions. Results, in brief, showed that there was a significant effect of meal order throughout the literature, on PPG and PP insulin—consuming carbohydrate-dense foods last in meal sequence leads to lower glucose and insulin excursions on average. Glucagon like peptide-1 area under the curve was also generally higher when carbohydrate was consumed at the end of a meal. Still, evidence around incretin, gut hormone responses, and perceptual measures was generally of low or very low quality, leaving gaps for further research. Within the lab study, the impact on PPG was successfully replicated, and there was also an effect of meal sequence on substrate utilization—a RF sequence led to higher utilization of carbohydrate both at rest and during exercise. In sum, this dissertation demonstrates that the relatively novel approach of altering the meal sequence may have significant implications relating to blood glucose and substrate utilization.

Without the unwavering support of my wife, there is absolutely no way I would be in this position. This dissertation and its work are dedicated to her.

ACKNOWLEDGMENTS

To the great number of people who have helped and supported me along the way in this journey, it would not have been possible without all of you. Dr. Wilson, your ability to play the roles of advisor, mentor, boss, colleague, and friend somehow all at the same time is something that I can only hope to strive for. Your ability to be all these things to me and others is nothing short of amazing, so thank you. Thank you to the rest of my committee, Dr. Haun and Dr. Reynolds for taking the time to be mentors to me. Dr. Reynolds, your professional advice and insight into the business of academia has been invaluable. To Dr. Haun, it has been a while since that serendipitous airplane ride, but I certainly would not have guessed then that we would be in this position. Thank you for your insight and encouragement from that time in my life and then coming back now to help wrap up this chapter—War Eagle buddy. Dr. Branch, a special thank you for sitting on my comprehensive exam committee and for your passion for high-quality research and never cutting corners. Lastly, but certainly not least, a major thank you to everyone who volunteered large chunks of their valuable time to participate in the laboratory study included in this dissertation—hopefully, the world may be a little bit better off from our time together.

TABLE OF CONTENTS

LIST OF TABLES....viii

LIST OF FIGURES....ix

CHAPTER I....1

Current Pharmacological Interventions for Managing Hyperglycemia....2
Nutrition in Diabetes Patients....4
The Interactions of Glycemia and Exercise....6
Statement of the Problem....9
Statement of Purpose....10
Specific Aims and Hypotheses....11
Study Outcome Variables....11
Limitations....12
Delimitations....12

CHAPTER II....14

Introduction....14
Background Information and Contextual Literature....15
Ordered Eating and Its Effects on Blood Glucose and Related Markers....26
Carbohydrate and its Relationship to Exercise....30
Chapter Summary....38

CHAPTER III....41

Study 1 Methods....41
Study 2 Methods....44

CHAPTER IV....57

Introduction....57
Methods....59
Results....61
Discussion....73
Conclusions....79

CHAPTER V....80

Introduction....80
Methods....82

Recruitment and Participants....83
Data Processing....91
Statistical Analysis....94
Results....95
Discussion....105
CHAPTER VI....109

REFERENCES....114

VITA....129

LIST OF TABLES

Table 1. Systematic Review Results by Outcome.....65

Table 2. Grade Assessments.....73

Table 3. Actigraph Activity Levels.....96

Table 4. Actigraph T-Tests.....96

LIST OF FIGURES

Figure 1 Enrollment and Follow-up.....47

Figure 2. Order of Events for Laboratory Visits 2 and 3.....52

Figure 3. Systematic Review Screening Process.....62

Figure 4. Overview of Cochrane Risk of Bias.....64

Figure 5. Enrollment and Follow-up.....85

Figure 6. Order of Events for Laboratory Visits 2 and 3.....90

Figure 7. Blood Glucose vs Time (Based on Condition).....97

Figure 8. VO_2 in Resting Phase....98

Figure 9. VO_2 During Exercise....99

Figure 10. Carbohydrate (CHO) Oxidation at Rest....100

Figure 11. Carbohydrate (CHO) Oxidation During Exercise....100

Figure 12. Fat Oxidation at Rest....101

Figure 13. Fat Oxidation During Exercise....101

Figure 14. RER at Rest....102

Figure 15. RER During Exercise....103

Figure 16. FS by Condition....104

Figure 17. RPE by Condition....104

CHAPTER I

INTRODUCTION

Anecdotally, the concept of what we eat, and its health effects is something that has been around likely since the dawn of civilization. For example, the Greek physician Hippocrates of Kos is quoted as saying "let food be thy medicine," referring to the overall benefits of eating a healthy diet and as part of a healthy lifestyle (Trüeb, 2020). However, the idea that the order by which food is eaten in a meal could impact health is a relatively recent development (Imai et al., 2010). In short, the core concept is that the order of consumption of foods with different nutrient contents (carbohydrates, water, fiber, protein, etc.) during a meal significantly affects postprandial glucose (PPG), insulin, and other metabolites and may have long-term implications as well (Imai et al., 2010, 2013, 2014; Shukla et al., 2015, 2017, 2018). A prime example of this sort of strategy, which will be referred to subsequently as *ordered eating*, is to consume heavy carbohydrate-containing foods such as breads and pastas last in a meal while consuming low-carbohydrate containing foods such as vegetables or lean meats first.

The possible applications of such a simple approach range from lowering hyperglycemia in prediabetes and diabetes patients to possibly manipulating substrate utilization and perceptual responses during exercise. This multi-article dissertation provided a comprehensive systematic review of the literature concerning the concept of ordered eating. It also includes an acute, laboratory-based study of ordered eating and how it may affect PPG, substrate utilization, and perceptual responses during an exercise bout.

Current Pharmacological Interventions for Managing Hyperglycemia

One potential application of the ordered eating concept is the management of diseases characterized by hyperglycemia such as diabetes. In theory, the application of ordered eating principles could result in less reliance on medications, which can be costly and/or result in side effects. In order to make any sort of effort toward reducing the incidence and prevalence of any disease, we need to understand the mechanisms of action and etiology of that disease. Diabetes is characterized by elevated fasting plasma glucose (FPG), PPG, and/or (Hb)A1c. It is a state of chronic hyperglycemia either by way of decreased or impaired insulin secretion as in type one diabetes mellitus (T1DM) or by increased insulin secretion accompanied by insulin resistance and impaired glucose utilization as in type 2 diabetes mellitus (T2DM) (Blaslov et al., 2018). Interestingly, despite the increasing prevalence of T2DM, the overall burden of the disease, and the vast quantity of research funding the disease receives, the exact mechanism of action that results in insulin resistance is not fully understood (Blaslov et al., 2018). There are essentially two aspects of T2DM that do develop, which are an inability to suppress glucose production by the liver and an impaired ability to take up glucose by tissues, most notably the skeletal muscle (Blaslov et al., 2018; DeFronzo, 1988; Petersen & Shulman, 2018).

Unsurprisingly, most pharmacological interventions focus on targeting the mechanisms that we do understand. Metformin now is almost universally considered the first-line intervention or "pleiotropic agent" for T2DM (ADA, 2018). Metformin works by reducing hepatic gluconeogenesis and glycogenolysis secondary to enhanced insulin resistance (Rodbard et al., 2007). Of course, there are more pharmacological-based interventions for specific cases or more advanced disease stages such as the oral hypoglycemic agents' class of drugs that includes saxagliptin, vildagliptin, and others (ADA, 2018). These are more frequently referred to as

"gliptins," but these second-line interventions are beyond the scope of this dissertation. Other popular classes of drugs include thiazolidinediones and the "tides" that include glucagon-like peptide-1 (GLP-1) receptor agonists such as exenatide, dulagutide, and lixisenatide (Quianzon & Cheikh, 2012).

More recently, sodium-glucose-transporter-2 (SGLT2) inhibitor class of drugs have increased in utilization since canagliflozin was first approved by the FDA in 2013 (Blaslov et al., 2018). This class of medication inhibits the kidneys' reabsorption of glucose (Blaslov et al., 2018; Verma & McMurray, 2018). There does seem to be a shift toward these SGLT-2 inhibitors as a possible drug of choice, and they even show cardiovascular benefits (Verma & McMurray, 2018). As an example, in a cardiovascular event outcome trial that involved 7,020 T2DM patients, major adverse cardiovascular events such as myocardial infarction were significantly reduced by empagliflozin (SGLT-2 inhibitor), and reductions of 35% and 38% in hospitalization and death in heart failure patients were observed (Fitchett et al., 2018; Verma et al., 2018; Zinman et al., 2015). Unfortunately, as these drugs are still early in their market lifespan, they are not particularly cost efficient. According to a study published in 2022, total monthly expenditures for SGLT-2 inhibitors were over $400, and for uninsured patients, that burden could largely fall on them individually (Aggarwal et al., 2022). As such, for an enormous swath of the population, medications such as these are unaffordable.

In addition to cost issues, a major potential pitfall of pharmacological intervention for hyperglycemia management is medication-induced hypoglycemia. In patients with T1DM, severe hypoglycemia events are common–somewhere between 3.3% to 13.5% of patients experience them every year (Pettus et al., 2019). In patients with diabetes, the normal response of secreting glucagon, epinephrine, cortisol, and growth hormone is often blunted or impaired, and

hypoglycemia in response to exogenous insulin can lead to acute cognitive problems, general neurological effects, and even syncope (Nakhleh & Sheadeh, 2021). While the burden of hypoglycemia is generally most in T1DM, a review by Silbert et al. (2018) reported that somewhere between 46% and 58% of T2DM patients reported having hypoglycemia symptoms over the course of six months. Further, the authors found that up to 4% of individuals had symptoms severe enough to warrant medical intervention (Silbert et al., 2018).

To date, studies on the impact of ordered eating on hypoglycemia risk are limited, but other nutrition research suggests that, at least in theory, the concept of ordered eating could be used as a strategy mitigate hypoglycemia risk. As an example, in their study where T1DM patients were assigned to either a high-fiber or low-fiber group (diets were weight-maintaining and composed of natural foods), Giacco and colleagues found that the high-fiber group saw reduced hypoglycemic events compared to the low-fiber group (Giacco et al., 2000). Foods higher in fiber tend to result in a lower glycemic index (GI) and improved insulinemic response (Liese et al., 2003), which can reduce the risk of reactive hypoglycemia. In the existing literature on ordered eating, eating carbohydrate-rich foods last in a meal has often led to more gradual rises and falls in PPG and insulin (Imai et al., 2010, 2013, 2014; Shukla et al., 2015, 2017, 2018), which is akin to a low-GI pattern.

Nutrition in Diabetes Patients

Patients and stakeholders that are well educated regarding intervention(s) tend to perform better nutritional interventions than patients and stakeholders that are not well educated (Marzban et al., 2022). Having knowledge of diabetes and its general evolution seems to associate with improved overall quality of life and lower the burden on surrounding family. In

their 2015 cross-sectional study that included 291 T2DM patients, Kueh and colleagues found that knowledge of diabetes was a significant predictor for attitudes and self-management, which ultimately predicted a positive impact on quality of life for the patients (Kueh et al., 2015). Unfortunately, based on international studies, there is a general lack of awareness of diabetes and its mechanisms that disproportionately affects individuals and families of lower socioeconomic status (Bani, 2015; Khan & Khan, 2000; Kheir et al., 2011). As a result of poor knowledge and easily accessible poor-quality food, the "Western" diet has become popular in the United States and is at the forefront of causes for the poor glucose metabolism that leads to metabolic syndrome, obesity, and eventually diabetes (Rico-Campa et al., 2019; Schwartz & Porte, 2005).

Dietary change on its own, or in conjunction with some of the pharmacological interventions described earlier, is often cited as *the* treatment of choice for controlling blood glucose in T2DM patients (Guo et al., 2020; Ley et al., 2014). There is emerging evidence for single nucleotide polymorphism (SNP) moderated precision nutrition strategies that would allow diets to be customized to individuals, but such interventions require incredible investment and are, at this stage, still in their infancy despite what promise they may show (Guo et al., 2020). More regular and accessible dietary interventions are the norm, and they are supposed to be the first-line intervention before even a Metformin intervention (Samson et al., 2023). However, many physicians demonstrate a reluctance to educate patients regarding nutrition or related concepts as they are not typically educated in these domains directly (Forouhi et al., 2018). In the settings where dietary interventions of some capacity are prescribed, there is debate as to what interventions are appropriate. In a 2013 systematic review, Ajala and colleagues considered different dietary interventions and their effects on (Hb)A1c, blood lipid profile, overall weight, and some quality measures (Ajala et al., 2013). These interventions did not necessarily

exclusively include diabetes patients (though the majority of participants did have diabetes), and the interventions were all implemented over a period of at least six months. In short, the authors found that low-carbohydrate, low-GI, Mediterranean, and high-protein diets all had positive effects on glycemic control. Considering the variability of these dietary interventions and their respective success, one can conclude that a large variety of dietary interventions can mitigate some of the effects of diabetes and hyperglycemia. Ordered eating provides a novel approach to add to the possible dietary interventions and has shown some evidence as a means of managing glucose levels (Imai et al., 2010, 2013, 2014; Shukla et al., 2015, 2017, 2018). Furthermore, order eating may offer some attraction to patients given that it relies less on altering *what* they eat but rather the *sequence* by which they eat foods.

The Interactions of Glycemia and Exercise

Along with pharmacological intervention and possible general nutritional advice, exercise is considered to be a first-line treatment for management of diabetes (Kirwan et al., 2017). Exercise is an approach that can be implemented for almost any patient who is not severely disabled, and it is efficacious, scalable, and affordable (Kirwan et al., 2017; Wing et al., 2013). In a 2017 meta-analysis, Grace and colleagues reviewed 27 studies (38 intervention groups) and found that (Hb)A1c was appreciably lowered with exercise (Grace et al., 2017). Additionally, there was an improved response on average with increased exercise intensity (Grace et al., 2017). This review included studies that were conducted chronically, over a period of 20 weeks of regular exercise. Additionally, they found that homeostatic model assessment of insulin resistance (HOMA-IR) and FPG also improved with exercise. Further, the authors found that exercise improved (Hb)A1c and peak VO_2 at higher intensities more effectively than at lower

and moderate intensities. In short, higher intensity exercise is generally more useful for improving these markers of health compared to moderate and lower intensity exercise (Grace et al., 2017). Certainly, these are improvements that are noteworthy, but what about in an acute setting?

Acute exercise bouts have even shown to increase glucose uptake by the skeletal muscle by increasing insulin sensitivity (Holloszy, 2005). This is comparable to a transient hypertrophy of skeletal muscle, and it is followed by an overall increase in insulin sensitivity which leads to overall improvement in glucose management (Holloszy, 2005; Magkos et al., 2008). This improvement in glucose uptake and utilization is likely a result of adenosine monophosphate-activated protein kinase (AMPK) activation which enhances glucose transport, protein synthesis, overall nutrient metabolism, and lipid synthesis (Hawley et al., 2006).

While exercise clearly impacts glycemic control and related outcomes, there is also evidence that the glycemic response to feeding plays a significant role in the metabolic responses to exercise. Specifically, altering glucose and insulin levels during the postprandial (PP) period can impact the regulation of blood glucose during subsequent exercise as well as the body's selection of substrate (fat versus carbohydrate). For example, ingesting carbohydrate-dense meals prior to the onset of exercise causes significant secretion of insulin by the pancreas–so much so that it may lead to exercise-induced hypoglycemia (Coyle et al., 1985). The risk of hypoglycemia in this situation—often referred to as rebound hypoglycemia, which was probably initially described in a 1940 paper (Boje, 1940)—is most pronounced when carbohydrate is ingested 30-60 minutes prior to exercise (Jeukendrup & Killer, 2011). Theoretically, a pre-exercise meal with carbohydrate-heavy foods eaten first would increase the risk of rebound hypoglycemia or exercise-induced hypoglycemia due to higher levels of circulating insulin.

As it relates to substrate utilization during exercise, manipulating the GI of feedings prior to exercise has been an active area of research for several decades (Ormsbee et al., 2014). In one example crossover study that included 10 trained cyclists completing three exercise-to-exhaustion trials, researchers at San Jose State University compared a high GI pre-exercise meal to a low GI pre-exercise meal and a control condition (DeMarco et al., 1999). Of primary interest for the purposes of this dissertation is respiratory exchange ratio (RER). The research team found that in the low-GI pre-exercise meal condition, RER was statistically significantly lower out to 120 minutes when measured at 20-minute intervals. This indicates that out to 120 minutes, fat utilization was significantly higher in the low-GI pre-exercise meal. In line with these findings, a review by Ormsbee et al. (2014) reported that multiple studies have found increased fat oxidation and lower reliance on carbohydrate with lower-GI meals, which could theoretically translate to sparing of muscle glycogen stores.

In terms of performance, Ormsbee and colleagues found in their 2014 review that, in some studies, ingesting low-GI meals before exercise led to improved performance during endurance exercise bouts, though the results were not consistent in all studies (Ormsbee et al., 2014). Likewise, in a systematic review and meta-analysis that included 19 studies totaling 188 participants, Burdon and colleagues found largely neutral results in terms of endurance performance. Among the studies that included exogenous carbohydrate ingestion during exercise, impact on performance was equivocal (Burdon et al., 2017). There was slight improvement with no during-exercise carbohydrate ingestion compared with low GI pre-exercise meals, but there were no pooled effects (Burdon et al., 2017). Essentially, the effect of manipulating pre-exercise meal GI on performance has shown mixed results to this point.

Statement of the Problem

Obesity, hyperglycemia, and indeed T2DM are unfortunately common within the United States. As such, they are often at the forefront of medical research, and as a result, there is an abundance of literature regarding possible pharmacological interventions, dietary interventions, and exercise interventions. There still, however, clearly remains something to be found, because rates of obesity and T2DM are continuously on the rise and are at the highest recorded rates in human history (CDC, 2022). A primary concern and issue facing physicians, patients, and stakeholders is not necessarily a lack of efficacy but a lack of accessibility, education, and most importantly, a lack of adherence (Forouhi et al., 2018). In order to fill this gap, a completely new intervention may not be necessary rather, it becomes more important to ensure that a new intervention may work synergistically with those already in place so as not to inherently disenfranchise patients or to discredit current interventions. The question now becomes: is it possible to have the elements of healthcare working synergistically? In many cases, exercise, nutrition, and pharmacological interventions are considered independently from one another, but none of them exist in a vacuum.

A relatively new approach to the management of dysglycemia and improving health is altering the order of foods consumed within meals, which was previously termed ordered eating in this chapter. This approach involves eating foods in a particular sequence within a meal to alter downstream digestion and metabolism (Imai et al., 2010). There is some evidence that this simple addition to current interventions can yield improvements to blood lipid profile, weight, (Hb)A1c, and other measures both chronically and acutely (Imai et al., 2013, 2014; Shukla, 2017). Ordered eating also has potential in improving glucose maintenance in an acute exercise

setting and manipulating substrate utilization in such a way that could be advantageous during exercise. Among individuals with diabetes, utilizing ordered eating to reduce the magnitude of PPG and insulin excursions could theoretically reduce the risk of hypoglycemia during subsequent exercise. In addition, this same approach could potentially be used by athletes to prevent rebound hypoglycemia that results from pre-exercise carbohydrate feeding.

Statement of Purpose

The purpose of this dissertation was to determine the efficacy and ecological realities of ordered eating relating to the management of T2DM, its effects on blood glucose maintenance in an acute setting, and its effects on the glycemic, substrate utilization, and perceptual responses to exercise. This was accomplished through two separate studies; the first, a systematic review of the literature on the topic of ordered eating, and a second experimental intervention study. The systematic review focused on the current state of the literature regarding ordered eating and its potential efficacy as an acute intervention to improve PPG and related markers. The experimental intervention study involved examining the effects of ordered eating on responses to exercise. Outcomes of interest in the experimental study included PPG excursions, substrate utilization, and perceptual responses (hunger, satiety, gastrointestinal symptoms). Participants performed steady state exercise for 30 minutes, and these outcomes were measured before, during, and after exercise. This experiment took the form of a cross-over design with participants exercising under both a carbohydrate-first and a carbohydrate-last condition.

Specific Aims and Hypotheses

Specific Aim #1: To evaluate the current state of the literature related to ordered eating and its acute effects on postprandial measures well as its effects on other measures associated with T2DM.

Hypothesis: Altering meal order will result in variations in PPG and postprandial insulin (PPI) excursions. More specifically, the timing of carbohydrate-heavy foods relative to other foods will have the greatest effect on PPG and PPI.

Specific Aim #2: To evaluate the effects of ordered eating on PPG excursions, substrate utilization, and perceptual responses during an acute exercise bout.

Hypothesis 2a: PPG in terms of peak excursion will be lower in a carbohydrate-last meal condition compared to a carbohydrate-first condition.

Hypothesis 2b: There will be a larger delta change in peak-nadir glucose in the carbohydrate-first meal order.

Hypothesis 2c: Carbohydrate utilization at rest and during exercise will be higher and fat oxidation will be lower following a carbohydrate-heavy food first meal order.

Hypothesis 2d: Consuming carbohydrate-heavy foods early in meal order will result in a higher perception of satiety. There will be a minimal effect of meal order on gastrointestinal symptoms.

Study Outcome Variables

For Aim #1, the dependent variables included PPG, PPI, incretin hormones, C-peptide, and perceptual responses.

For Aim #2, the dependent variables included blood glucose, measured at 30-minute intervals; along with volumes of O_2 consumed and CO_2 produced, measured continuously; rates of carbohydrate and fat oxidation; perceived exertion; and perceptions of hunger, appetite, satiety, fullness, and gastrointestinal symptoms.

Limitations

1. The systematic review was inherently limited by the relatively small amount of literature that has been published on order of eating—the first study of this kind was published as recently as 2010 (Imai et al., 2010). Across most studies, the number of participants is also quite low in general, and most populations are also homogenous, east Asian populations. Additionally, systematic reviews, by their nature, often have the limitation of only examining a specific type of intervention—acute in this case.
2. Due to the inherent difficulty in recruitment for an intense protocol such as the crossover design utilized in the second study, the number of participants was relatively small. Additionally, utilizing subjective measures of gastrointestinal symptoms that vary substantially from one individual to another may make detecting small-to-moderate treatment effects for that outcome difficult.
3. Indirect measurement of fuel utilization and lack of measurement of insulin and fatty acids are further inherent limitations of the laboratory-based study.

Delimitations

Studies included in the systematic review were those that are acute interventions and those that administer isocaloric meals. Outcomes that were considered are PPG, PPI, C-peptide,

gut hormones, and perceptual responses. Databases included PubMed, Cochrane CENTRAL, and Web of Science. Lastly, studies were not excluded based on characteristics of participants.

Participants included in the laboratory-based study were recreationally active individuals who were free from any metabolic disease or conditions that precluded exercise or may alter PPG or PPI. The questionnaires and other measures used are valid and have been used extensively in related literature.

CHAPTER II

LITERATURE REVIEW

Introduction

The purpose of this dissertation was to assess the potential efficacy of ordered eating–that is, consuming macronutrients in a prescribed order within the context of single meals–as it relates to reducing PPG acutely and explore how it may be related to glycemic responses, substrate utilization, and subjective perceptions during an exercise bout. Two separate studies were used. One systematic review was used to 1) evaluate the current state of the literature regarding ordered eating and its effects on acute glycemia and related biomarkers. An experimental study was used to 2) reproduce the previously studied acute PPG phenomenon related to ordered eating and examine its potential effects on glycemic responses, substrate utilization, and subjective perceptions during exercise.

The present literature review is broken down into three separate sections. The first section provides a broad overview of the background and motivation for this dissertation. The second section discusses, in short, the current state of the literature regarding ordered eating and demonstrate that there is likely not enough of said literature. The third and final section of the review discusses the importance of GI of pre-exercise meals and its potential impact on perceptual and physiological responses to exercise and speculate as to how it may be affected by changing the order of consumption of macronutrients in a meal.

Background Information and Contextual Literature

Blood Glucose, Its Importance, and Typical Regulation

Glucose and its derivatives play a fundamental role in many processes within the body to include production of usable energy. It is essential for providing sugar to the brain, muscles, and every other system in the body. All tissues that are within the central nervous system and peripheral nervous system require glucose in its base, free form to function (Cryer & Gerich, 1985). It naturally follows that regulation of glucose plays a vital role in not just survival, but overall well-being. Acute, prolonged hypoglycemia can, on its own, quickly lead to altered consciousness and death (Tirone & Brunicardi, 2001).

Because of its importance to overall health and physiological functioning, the body aims to regulate blood glucose within a narrow range (70-100 mg/dL). The body's goal of tightly regulating blood glucose relies on a system of organs that release hormones and neuropeptides in response to various stimuli (feeding, exercise, etc.). Of particular importance in this regulation of blood glucose are the pancreas and the liver. Through a negative feedback loop that relies on the balance of action between glucagon and insulin, the pancreas establishes and works to maintain "glucose homeostasis" (Röder et al., 2016). In a fasted state, blood glucose naturally drops. In these times of low blood glucose, alpha cells that co-occupy the islets of Langerhans with beta cells, release the peptide glucagon (Röder et al., 2016; Stanojevic & Habener, 2015). Glucagon raises blood glucose by stimulating hepatic glycogenolysis in addition to promoting renal and hepatic gluconeogenesis (Röder et al., 2016; Stanojevic & Habener, 2015). Conversely, insulin is secreted from the beta cells that occupy the islets of Langerhans on the pancreas and is secreted in the presence of elevated exogenous glucose (Röder et al., 2016). Insulin binds to insulin receptor sites on target peripheral tissues such as skeletal muscle where it triggers a signaling

cascade starting with the phosphorylation of the Cbl adaptor protein that ends with the inhibition of insulin stimulated GLUT4 translocation (Khan & Pessin, 2002).

Hyperglycemia and Associated Disorders

Hyperglycemia, as a word, is derived from ancient Greek meaning "high-sugar-blood" (Mouri & Badireddy, 2022). It is characterized as having a FPG higher than 125 mg/dL or a PPG of greater than 180 mg/dL two hours after eating and is, in and of itself, a disease (ADA, 2001; Mouri & Badireddy, 2022). As it relates to long-term cardiometabolic outcomes, hyperglycemia is frequently associated with obesity, T2DM, heart disease, and other poor outcomes (Monnier & Colette, 2015). Extended hyperglycemia fundamentally alters the physical structure and function of proteins throughout the body, which will lead to poor outcomes such as renal failure or retinopathy (Cerami et al., 1979; Pettitt et al., 1980; Tirone & Brunicardi, 2001).

Typically, healthcare providers diagnose hyperglycemia by measuring FPG and/or PPG, the latter being assessed during a two-hour oral glucose tolerance test (OGTT) (ADA, 2001; Mouri & Badireddy, 2022). While commonly confused for diabetes mellitus (DM), hyperglycemia refers only to blood sugar while DM is a condition of chronic hyperglycemia and is diagnosed as a combination of FPG, PPG, and hemoglobin A_{1C} ((Hb) A1C) (ADA, 2001; Monnier, 2015; Goyal & Jialal, 2022). An individual is diabetic if they have a FPG of greater than 125 mg/dL, an (Hb)A1C of 6.5% or higher, and/or a plasma glucose level of more than 200 mg/dL within two hours following an OGTT (Goyal & Jialal, 2022; Villegas-Valverde et al., 2018).

T2DM, while characterized by hyperglycemia, is not defined by it. This chronic hyperglycemia is secondary to impaired insulin secretion and insulin resistance (ADA, 2020).

The normal response of the body following the ingestion of carbohydrate is to release endogenous insulin from the pancreas which facilitates the transport of glucose into target cells, so that skeletal muscle and cardiac muscle can function properly along with other physiological functions. If more energy is consumed than is needed to complete these processes, the body will store it in adipocytes as triglycerides (Malone & Hansen, 2019). Insulin and glucose are necessary for the storage of fat, but insulin receptors become less sensitive over time in the presence of consistently elevated insulin (most prevalently at the skeletal muscle). Over time, there is a blunting of skeletal muscle insulin receptors to the point of compromising glucose and fatty acid transport that in turn elevates glucagon which increases hepatic glucose production in a negative feedback loop that is somewhat out of control (Malone & Hansen, 2019). This increase in hepatic glucose production results in fatty acid release (the body searching for an alternative fuel source as glucose is not able to be used correctly). Ultimately, the body is left in a state of constantly elevated glucose and insulin until the pancreas eventually loses the ability to produce insulin (Malone & Hansen, 2019). In the case of T1DM, there is autoimmune destruction of beta cells of the pancreas that prevents the body from producing meaningful quantities of insulin (ADA, 2020).

Risk Factors for Hyperglycemia and Diabetes

There is a slew of risk factors that increased risk of developing hyperglycemia as well as many potential secondary causes. Some major risk factors include having a history of gestational diabetes (Bashir et al., 2019), hyperlipidemia, hypertension, and family history of DM (Mouri & Badireddy, 2022). Secondary causes of hyperglycemia can include chronic pancreatitis, hemochromatosis, Cushing syndrome, and cystic fibrosis (Mouri & Badireddy, 2022). However,

the single largest risk factor for developing hyperglycemia is obesity, with somewhere from 30% to 50% of newly diagnosed cases of DM being secondary to obesity (Feng et al., 2021; Tanamas et al., 2016).

T1DM and T2DM share some of the same risk factors, but generally speaking, T1DM is typically diagnosed relatively early in life while T2DM is typically diagnosed relatively late in life. Naturally, it can be inferred that there is a different set of risk factors for each disease. T1DM cannot exclusively, but largely be attributed, to genetic risk factors. It is a disease characterized by beta cell autoimmunity and destruction of those beta cells which inhibits or stops the secretion of insulin from the pancreas (Pociot & Lermark, 2016). Initially, autoantibody biomarkers that target insulin or glutamic acid decarboxylase are typically present, and the larger the number of antibodies, the faster the progression to clinically measurable disease (Pociot & Lermark, 2016). The largest risk factor is genetic. It "mainly occur(s) in individuals with either *HLA-DR3-DQ2* or *HLA-DR4-DQ8* haplotypes, or both, but a trigger from the environment is generally needed" (Pociot & Lermark, 2016). Some of these environmental factors are prenatal and include high birthweight, low maternal intake of vegetables, and older maternal age (Rewers & Ludvigsson, 2016). Postnatal factors include early exposure to eggs and cow milk, cereals, frequent infection, and abnormal infant weight gain (Rewers & Ludvigsson, 2016). Puberty, steroids, psychological stress, and being overweight, among other factors, can encourage further disease progression up to clinically diagnosed T1DM (Rewers & Ludvigsson, 2016).

T2DM has a potentially better understood but seemingly more multi-factored set of risk factors. Some specific possible genetic risk factors include a potassium rectifying channel gene called KCNJ11, and transcription factor 2-like 2 called TCF7L2 (van Exel et al., 2002; Wu et al., 2014). More relevant to this dissertation are environmental risk factors that are at least partly

controllable. Some examples of these environmental risk factors are physical inactivity, alcohol consumption, and smoking (Cullmann et al., 2012; Hu et al., 2001; Manson et al., 2000). Obesity is the primary risk factor for developing T2DM, and the World Health Organization cites that roughly 65-80% of newly diagnosed T2DM patients are carrying excess body weight (WHO, 2011). Specifically related to the topic of this dissertation is the GI. Diet is a modifiable risk factor, and there is significant evidence that a high-GI diet coupled with low-fiber intake correlates to the risk of developing T2DM (Greenwood et al., 2013).

Hypoglycemia

Also concerning is the reverse of hyperglycemia–that is hypoglycemia. Hypoglycemia is typically broken into three separate levels based on severity (Agiostratidou et al., 2017). Level 1 hypoglycemia is defined as having a blood glucose concentration of < 70 mg/dL and >54 mg/dL. Level 2 is defined as being below 54 mg/dL, and this requires some immediate intervention to fix. Lastly, level 3 hypoglycemia is not necessarily tied to a specific value but is consistent with change in mental status and requires immediate intervention (Agiostratidou et al., 2017).

Typically, beyond T1DM patients, hypoglycemia manifests either as a side effect of insulin management therapies (Yale et al., 2018), or it is caused by exercise in exercise-induced hypoglycemia (Coyle et al., 1985). Events of severe hypoglycemia are most common in those patients with T1DM, and annual incidence ranges anywhere between roughly 3% and about 13.5% (Pettus et al., 2019). Within T2DM patients, those treated with insulin or sulfonylureas and meglitinides are at increased risk of severe hypoglycemia due to their effects on circulating glucose levels (Gangji et al., 2007). However, generally speaking, events of severe hypoglycemia are less common in T2DM patients when compared to T1DM patients (Nakhleh &

Shehadeh, 2021). Beyond these risk factors, there are also slightly less common risk factors for developing hypoglycemia such as renal failure, hepatic failure, significant weight loss, and hypothyroidism (IHSG, 2015).

Symptoms of hypoglycemia are not always easily diagnosed and can vary based on patient age and disease state and manifest as both neurological and autonomic symptoms (Nakhleh & Shehadeh, 2021). Some neurological symptoms are potentially vague and difficult to recognize and include restlessness, dizziness, headache, and confusion. These neurological symptoms do not typically manifest at plasma glucose concentrations above 54 mg/dL (Agiostratidou et al., 2017), and are not typically regulated or affected by counter-regulatory hormones failing or history of hypoglycemia (McAulay et al., 2001; Nakhleh & Shehadeh, 2021). Some autonomic symptoms include anxiety, tremor, diaphoresis, and paresthesia (Nakhleh & Shehadeh, 2021).

Burdens of Diabetes Mellitus and Dysglycemia

According to the Centers for Disease Control and Prevention (CDC), obesity is defined as having a body mass index (BMI) of 30 kg/m^2 or higher (CDC, 2022). Recent research conducted in the United States suggests somewhere around 40% of all adults, regardless of sex, are categorized as obese (Hales et al., 2017). Worryingly, from 2003 to 2014, the frequency and prevalence of a number of diseases such as obesity and DM increased significantly, and they continue to increase (Palmer & Toth, 2019). The frequency of obesity increased by approximately 20.4 million while the frequency of DM increased by 9 million (Palmer & Toth, 2019).

Clearly, obesity is a problem in the United States, but what does that mean for researchers, clinicians, and educators in practice? Obesity is in and of itself a disease, but possibly more worrying is that it often leads to and is strongly associated with one of the leading causes of death and morbidity in the United States, specifically T2DM (Guh et al., 2009). An individual is considered to be diabetic if they have a FPG of greater than 125 mg/dL, an (Hb)A1C of 6.5% or greater, or a 2-hr plasma glucose of greater than 200 mg/dL following an OGTT (ADA, 2015; Goyal & Jialal, 2022; Villegas-Valverde et al., 2018). Approximately 10.5% of the United States population, which is around 34 million people, have DM, and approximately 8.7 million people are undiagnosed according to the American Diabetes Association (ADA) (ADA, 2021). In 2021, DM was listed as the primary cause of death for 103,294 individuals and listed as either a primary cause of death or contributing cause of death for 378,075 individual death certificates (ADA, 2021).

The impact of DM on longevity is obvious, but it is not the only burden of disease on a population. Cost of a disease is both an individual and societal burden in terms of both monetary cost and the sheer amount of resources dedicated to treatment and management. In 2017, the estimated cost of treatment for diagnosed DM was $327 billion in the United States—which is further broken down by $237 billion in direct medical cost and $90 billion in loss of productivity (ADA, 2018). On a per-person basis, that averages out to be approximately $16,750 per year, which is around 2.3 times higher than the cost of medical expenditures per year for an individual that does not have DM (ADA, 2018). As a result of loss of life from premature death, there is an estimated loss (either via employment productivity or productivity from non-labor-force individuals) of approximately $19.9 billion in addition to the $37.5 billion lost due to inability to work related to diabetes (ADA, 2018). While this is the most up-to-date information, it is worth

noting the context of this data being from 2017, so six years on it is fair to infer that these numbers have grown significantly given that the incidence and prevalence of DM have indeed increased (CDC, 2022).

Hypoglycemic episodes also represent an important burden of disease in both T1DM and T2DM. In a 2020 systematic review and meta-analysis of hypoglycemia in both T1DM and T2DM that included studies from around the world, Alwafi and colleagues (2020) found a wide range of incidence of hypoglycemia. In the 39 included studies, involving 45,768,950 individuals and 2,462,810 individuals with DM, incidence rates of hypoglycemia ranged from 0.072 to 42,890 "episodes" per 1,000 person-years in general, from 14.5 to 42,890 per 1,000 episodes per 1,000 person-years for individuals with T1DM, and from 0.072 to 16,360 per 1,000 person-years for individuals with T2DM (Alwafi et al., 2020).

Brief Overview of Measurement Methods for Blood Glucose

It is natural to now review the different methods by which blood glucose is measured and some overall pros and cons of each method. Typically, blood glucose is monitored in one of three ways. Firstly, probably most commonly and typical of management of chronic disease, is the capillary blood glucose test, commonly referred to as the fingerstick (Mathew & Tadi, 2022). Secondly, and most commonly applied in a clinical setting, is a venous or plasma measurement that is acquired via venipuncture (Mathew & Tadi, 2022). Lastly is continuous monitoring utilizing a continuous glucose monitoring (CGM) device which measures interstitial fluid glucose at regular time points (Mathew & Tadi, 2022). In general, there are advantages and disadvantages to each method.

Capillary blood glucose testing is advantageous in that it takes typically very small samples of blood (on the order of ~0.5 microL), the testing site can be altered for each measurement, testing time is typically very short, and it is relatively painless (Mathew & Tadi, 2022). Disadvantages of this method include that test strips often have short shelf lives and are relatively costly, and they are affected potentially by temperature, humidity, quality of the blood sample, etc. Additionally, they are not always particularly accurate in patients with altered blood concentrations such as in patients with anemia, hypotension, or those that are critically ill (Mathew & Tadi, 2022). Lastly, these devices can be particularly inaccurate if not properly calibrated which can lead to significant issues (Acar et al., 2014; Ginsberg, 2009)

Venous blood that is collected via venipuncture is regarded as the most accurate method of assessment as it should be performed in a laboratory setting with established industry standards (Mathew & Tadi, 2022). The accuracy of this testing method is much less influenced by extraneous factors similar to those listed previously. Disadvantageously, venipuncture can by painful, cause localized tissue damage (Wei et al., 2017), and it requires higher levels of training and rigor to collect the sample and to measure glucose.

Lastly, CGM, or flash monitoring, tends to be particularly useful for patients with DM or require some sort of insulin therapy (Mathew & Tadi, 2022). This method requires the insertion of a monitor on the back of the arm or on the abdomen, which can be done at home, and it is monitored typically by a handheld device that can measure interstitial glucose as frequently as every few seconds. This can be particularly advantageous in patients who are at risk for hypoglycemia during sleep, for example (Mathew & Tadi, 2022). Disadvantageously, the CGM monitors interstitial fluid rather than actual blood, so there is a natural delay in measurement making real time data and monitoring for rapid glucose changes less than ideal (Mathew & Tadi,

2022). Lastly, these devices tend to have a particularly high cost, and they may be inaccessible for many patient populations (Hellmund et al., 2018).

As it relates to this dissertation and its proposed laboratory intervention, CGMs are unproven as tools for measuring blood glucose during exercise. In a review of CGMs and their accuracy during exercise in T1DM patient populations, Muñoz Fabra and colleagues reviewed a total of 54 studies dating back to 2011. To date, very few studies have actually been conducted using more modern sensors–the underlying assumption is that more modern sensors will provide better data during exercise protocols (Muñoz Fabra et al., 2021). Still, in this review and meta-analysis, the mean absolute relative difference scores, which is a measure of accuracy, were unimpressive. In short, the accuracy of CGMs were negatively impacted by exercise protocols, suggesting that they are not yet proven tools in measuring blood glucose during exercise (Muñoz Fabra et al., 2021).

Mechanisms and Regulation of Exercise-Induced Glucose Uptake

In order to understand better understand the potential implications of this dissertation's proposed laboratory intervention, it is necessary to have an understanding of the behavior of glucose regulation during exercise conditions. Under regular, resting conditions, glucose transporter-1 (GLUT1) is not exclusively, but largely responsible for glucose transport while glucose transporter-4 (GLUT4) is not active in the sarcolemma and t-tubules (Flores-Opazo et al., 2020; Ploug et al., 1998; Rodnick et al., 1992). During exercise, GLUT4 is the primary transporter of glucose into the muscle, and there is translocation of GLUT4 from within the cell to the sarcolemma and t-tubules which is a necessary step of facilitating glucose transport during exercise (Flores-Opazo et al., 2020). The mechanisms behind this are not entirely understood,

but there is a strong relationship between muscle contraction and GLUT4 translocation as well as the presence of insulin, but there is a distinction in their additive effects (Flores-Opazo et al., 2020; Lund et al., 1995) that can be observed through the exercise-induced translocation of GLUT4 in an insulin-resistant state (Flores-Opazo et al., 2020).

There are a number of pathways through which exercise increases the expression of GLUT4 transiently. Firstly, myocyte enhancer factor 2 (MEF2) and GLUT4 enhancer factor necessarily bind to the GLUT4 promoter which in turn increases GLUT4 expression (Flores-Opazo et al., 2020; Knight et al., 2003). Interestingly, histone acetylation of the nuclear respiratory factor 1 (NRF-1) binding sequence of the Mef2a promoter was also associated with an uptick in MEF2-NRF-1 binding to that region (Joseph et al., 2017). During acute exercise bouts, DNA binding of these two transcriptional factors occurs (McGee et al., 2006). Further GLUT4 expression is encouraged by the reduction of AMPK association as it is a histone deacetylase 5 (HDAC5) (McGee et al., 2008), and HDAC5 is a class of kinases that are partially responsible for transcriptional repression which would result in limited GLUT4 expression (Holmes et al., 2005).

In a similar vein, GLUT4 is not just expressed in skeletal muscle but also in adipose (fat) tissue as well (Flores-Opazo et al., 2020). Regular exercise is generally associated with increased or enhanced action of insulin in adipose tissue (Rodnick et al., 1987). It has been speculated that such enhanced insulin action may be mediated by changed GLUT4 expression (Flores-Opazo et al., 2020). In a study published in 2011, Hussey and a team that included Dr. Jeukendrup from the University of Birmingham in the United Kingdom, examined the effects of exercise training on adipose tissue and skeletal muscle GLUT4 expression in T2DM patients (Hussey et al., 2011). Their study included seven patients and seven controls that were sent through a four-week

exercise training program. Interestingly, adipose GLUT4 expression was increased by 36% in the T2DM patients after training while skeletal muscle expression was increased by 20% (Hussey et al., 2011). This result has potential implications as it relates to enhancing the action of insulin, but such changes are beta cell function dependent (Hussey et al., 2011), so the results may not be particularly applicable in T1DM patients. Potentially surprisingly, and in contrast, a separate study that included 20 healthy subjects that completed 10 days of exercise training did not find increased GLUT4 expression in adipose tissue while it was increased by >40% in skeletal muscle (Flores-Opazo et al., 2018).

Ordered Eating and Its Effects on Blood Glucose and Related Markers

At present, there are a wide variety of approaches used to manage chronic hyperglycemia and DM that typically combine pharmacological, dietary, and exercise interventions (ADA, 2020; Taylor et al., 2021). The primary aim of treatment, generally speaking, is to prevent the occurrence of cardiometabolic complications that are the primary causes of death and disability in T2DM (Taylor et al., 2021). In an ideal world, chronic hyperglycemia and DM would be treatable without pharmacological intervention–or at least minimal pharmacological intervention. Certainly, that has been the goal of some researchers, and the ordered eating concept provides a possible avenue to this end.

Initially proposed by Dr. Saeko Imai and Dr. Shizuo Kajiyama in 2010 at Osaka Prefecture University in Japan (Imai et al., 2010), eating foods within a meal in a standardized order to reduce PPG excursions is a fairly new idea. In an early study, the researchers reported the acute effect of a meal order (i.e., one day for each condition) that included vegetables before carbohydrates on PPG and PPI as well as chronic FPG in patients with T2DM (Imai et al., 2013).

The whole of the study included 19 patients with T2DM and 21 healthy controls. Interestingly, in the patients with T2DM, mean PPG peak excursions were reduced, incremental glucose peaks were reduced, and the amplitude of glycemic excursions were reduced from the 1- to 3-hour postprandial measuring points after eating vegetables first and carbohydrates last (Imai et al., 2013). Within the healthy controls, PPG at 1 hour was also significantly reduced along with mean area under the curve (AUC) for insulin over the 3-hour postprandial period, similar to the T2DM group. One possible explanation for these observations that was offered by the authors is that the high dietary fiber of vegetables slowed down the digestion and absorption of carbohydrate and thereby reduced insulin requirement (Imai et al., 2013; Wong & Jenkins, 2007). A second possible reason for the observed effects is that vegetable consumption could stimulate the secretion of incretin hormones which in turn reduces glycemic excursions (Imai et al., 2013; Ma et al., 2009), though neither of these explanations had concrete evidence, and it became clear that there is a need for further inquiry into this physiological phenomenon.

In a subsequent study, Imai and colleagues reviewed what effect eating vegetables before carbohydrates may have in a more ecological setting. The study included 15 outpatients who had well-managed T2DM. Included test meals consisted of white rice and vegetable salad with the order of eating being either carbohydrate (white rice) first or last (Imai et al., 2014). Plasma glucose and serum insulin were measured at 30, 60, and 120 minutes after eating. At both 30 and 60 minutes postprandial, there were significantly lower glucose and insulin levels in the carbohydrate-last condition. In a secondary, retrospective cohort study including 333 participants that were studied over a two-year period (included within the same manuscript), the group assessed hemoglobin A_{1C}. Imai and colleagues found that from baseline, there was no shift over 2.5 years in the control group, but in the education group, there was a drop from 8.6 ± 1.8% to

7.5 ± 1.7% that was significant at the $p < 0.001$ level (Imai et al., 2014). Education consisted of encouraging the participants to eat their carbohydrates last and describing the potential benefits of such an intervention. The evidence presented from these two studies gives credence to the idea that ordered eating can not only yield positive effects of acute PPG excursions, but it can also have a beneficial effect in a more ecologically valid, chronic condition (Imai et al., 2014). As it relates to glucose regulation, it is theoretically possible that consuming carbohydrate-heavy foods at the end of a meal could mitigate some of the steep rises in PPG that contribute to hyperglycemia and DM and their poor long-term outcomes.

A new research group under the direction Dr. Alpana Shukla entered the scene relating to ordered eating in 2015. Dr. Shukla is probably the most published author in this area of research as of today. During their 2015 study, Shukla and colleagues studied 11 participants that were split between 6 females and 5 males, and all participants were actively being treated with metformin for T2DM (Shukla et al., 2015). The study followed a within-subject crossover design. Average HbA_{1c} at baseline was 6.5 ± 0.7%. In this study, participants were fasted for 12 hours overnight and given an isocaloric meal containing ciabatta bread, orange juice, grilled chicken breast, lettuce, tomato, and steamed broccoli. The meals were consumed on two separate occasions one week apart. The first was consumed in a carbohydrate-first order (Ciabatta bread + orange juice) fashion, and the second meal was consumed in the reverse order. The team found that blood glucose was 28.6%, 37.0%, and 16.8% lower at minutes 30, 60, and 120 postprandial, respectively, in the carbohydrate-last condition. Similar results were found regarding AUC with values at each time point being 39.2%, 48.1%, and 48.5% lower. The takeaway here is clear: among people with T2DM, consuming carbohydrate-dense foods last in a meal appears to mitigate glucose peak excursions and AUC (Shukla et al., 2015).

Following this initial study, Shukla and colleagues released a series of papers on the topic, and each was a slight deviation from the preceding in terms of the total number of participants or disease state. In their 2019 publication, Shukla and colleagues included prediabetes patients while all prior studies included T2DM patients (Shukla et al., 2019). Overall results were largely the same as it relates to meal order and its impact on glucose. In each study, regardless of population, there was some impact on PPG by modifying meal order. Specifically, in the carbohydrate-last conditions, PPG peaks and AUC tended to be lower (Shukla et al., 2015, 2017, 2018, 2019).

Hitoshi Kuwata and his team from Japan are also a highly cited group in this area of inquiry. From their publication titled, "Meal sequence and glucose excursion, gastric emptying, and incretin secretion in type 2 diabetes: a randomized, controlled crossover, exploratory trial," published in *Diabetologia* in 2016, Kuwata has received roughly 100 citations to date according to Google Scholar. The participants consumed either boiled mackerel followed by steamed rice, grilled beef followed by steamed rice, or steamed rice followed by boiled mackerel (Kuwata et al., 2016). In many ways this study followed similar protocols to others in the field such as their inclusion of T2D patients and healthy controls and the general idea of carbohydrate placement in meal order affecting postprandial outcomes. Uniquely, however, patients enrolled in this study were diagnosed with T2DM but were not actively being treated with any sort of medication or noted lifestyle intervention which offers a potentially unique opportunity to observe the acute effects of a meal sequence intervention in this population. Additionally, they also included unique outcomes of interest such as gastric emptying rate, C-peptide, and 4-hour AUC incretin levels along with 4-hour blood glucose and insulin. Potentially unsurprisingly, gastric emptying of rice in the rice-first condition was fastest when compared to a rice-last condition. The 4-hour

AUC of glucose was lower in the T2DM patients when eating rice last, but it was the same as eating rice first in the controls. As it relates to insulin, in the rice-last conditions, 4-hour AUC was lower in the T2DM patients, and 4-hour C-peptide AUC was lower in both samples. Incretins (GLP-1 and GIP) were highest in the beef before rice condition in both samples (Kuwata et al., 2016).

Among the referenced literature, there is certainly some trend that is of note. That is: consuming a carbohydrate-dense food in a given meal and its position sequentially in that meal exerts a significant effect on PPG–more specifically, carbohydrate-dense foods last in a meal seems to lower PPG peaks and AUC. Yet, the current literature is incomplete at present, and the proposed intervention of this dissertation will address at least some of that gap. Major holes in the literature concerning meal sequence and its effect on postprandial measures include small sample sizes, an over-reliance on T2DM patients, and a complete absence of exercising populations. There is no mention of how such an intervention may affect glycemic responses and substrate utilization during an exercise bout, which is a natural area of inquiry given the already obvious effects of the intervention. While that is understandable given the exploratory nature of the area of inquiry, there is enough evidence that carbohydrate positioning in meal sequence does exert an effect on PPG to justify not only a systematic review of the literature to elucidate on these gaps, but also a laboratory-based intervention to include exercising populations.

Carbohydrate and its Relationship to Exercise

Carbohydrate and Glycemic Index (GI)

The effects of carbohydrate on exercise and its usefulness as an ergogenic aid are well documented across many sources of literature. One such source is the American College of

Sports Medicine (ACSM); in their position stand, the ACSM recommends, to varying degrees, carbohydrate consumption before, during, and after exercise (ACSM, 2016). To further contextualize the literature, it is important to understand exactly what carbohydrate is and what GI means. A carbohydrate is an organic molecule that is comprised of carbon, hydrogen, and oxygen and typically has two hydrogens for every one oxygen (Holesh et al., 2023). More specific to the purposes of this review, carbohydrate is one of three macronutrients consumed by humans–protein and fat are the other two. There is variability in their structure determined by the number of monosaccharides they are composed of. The simplest are monosaccharides which have the formula $C_6H_{12}O_6$ and include glucose, fructose, and galactose (Holesh et al., 2023). Other carbohydrates, in order of increasing complexity are disaccharides, oligosaccharides, and polysaccharides, which are all just comprised of increasing numbers of monosaccharides (Holesh et al., 2023). Further classification of carbohydrate is by type. Simple carbohydrates are those that are monosaccharides or disaccharides while complex carbohydrates are those which are made of three or more sugars and are oligosaccharides and polysaccharides (Holesh et al., 2023). Starches are specific complex carbohydrates that are produced by plants and include potatoes and wheat while fiber are non-digestible complex carbohydrates that are utilized by intestinal bacteria (Holesh et al., 2023). Digestion of carbohydrate begins with salivary amylase in the mouth and eventually ends in a monosaccharide that can be absorbed directly into the bloodstream and used as energy in the form of glucose or stored as glucagon (Holesh et al., 2023). Fiber is the exception as it is non-digestible but provides indirect health benefits within the intestines (Holesh et al., 2023).

The glycemic index, or GI, is a simple means by which carbohydrate-containing foods and their effects on blood glucose can be quantified. This value is calculated by dividing the

AUC of blood glucose concentration (usually 2 hours) after ingestion of a test food with 50 g of carbohydrate by an equal amount of carbohydrate as glucose or from white bread (Jenkins et al., 1981). For the purposes of this review, it is important to note the three categories that carbohydrates fall into on this scale. Low-GI foods are rated as 55 or less, medium-GI foods are rated as 56-69, and high-GI foods are rated from 70-100. Examples of each are peas, brown rice, and white potatoes, respectively (Holesh et al., 2023). Ultimately, a well-balanced diet will likely include all of these, but these higher GI foods, when consumed in larger quantities, do tend to contribute to heart disease, obesity, and other morbidities (Holesh et al., 2023).

Pre-Exercise Meals, Blood Glucose Changes, and Rebound Hypoglycemia

During a 2002 study, Jentjens and Jeukendrup studied the effects of pre-exercise ingestion of carbohydrate on metabolism, and they found that some athletes may experience rebound hypoglycemia. The purpose was to further explore the idea of rebound hypoglycemia, which is hypoglycemia that presents early in exercise as a result of the combined effects of increased glucose uptake and increased circulating insulin following a pre-exercise meal (Jentjens & Jeukendrup, 2002). Twenty trained athletes performed a cycle ergometer exercise bout and consumed a 500 mL beverage containing 75 g of glucose 45 minutes prior to the onset of exercise. Exercise was a 20-minute bout at ~75% of VO_2max followed by a time trial. Participants were divided into a hypo or non-hypo group based on plasma glucose nadir that they reached during submaximal exercise (>/< 3.5 mmol/L). Ultimately, the authors found that plasma glucose, at its lowest, was significantly lower ($p < .01$) in the hypo group compared to the non-hypo group. In the hypo group, plasma glucose nadir was 2.7 ± 0.1 mmol/L whereas in the non-hypo group, plasma glucose nadir was 4.1 ± 0.2 mmol/L (Jentjens & Jeukendrup, 2002).

A review by Jeukendrup and Killer (2011) summarized the literature on pre-exercise carbohydrate feeding and rebound hypoglycemia and came to several conclusions. Namely, for most athletes there is little, if any, negative effect(s) on performance related to consuming carbohydrate prior to exercise. Still, individuals who are prone to developing reactive hypoglycemia should consider avoiding carbohydrate in the 90 minutes prior to exercise or consume low-GI carbohydrates (Jeukendrup & Killer, 2011).

Concerned with the potential risk in diabetic patient populations as it relates to exercise-associated hypoglycemia, a small team in Brazil conducted a systematic review of pre-exercise meals in DM patients. Unfortunately, only two studies were included in the review and included a total of 17 subjects (Faria et al., 2018). They found that a low-GI meal tends to lead to more stable blood glucose values and lower risk of exercise-induced hypoglycemia (Faria et al., 2018). Given the nature of their review and considering that it only included two studies, it is clear that more research is needed in these populations, and there is no clear indication for clinical decision making relating to glycemic content of pre-exercise meals (Faria et al., 2018).

In a study including exclusively well-managed T2DM patients (5 were Metformin-managed, and 5 were not taking any pharmaceuticals), Ferland and colleagues administered a high-GI meal, a low-GI meal, a high-fat/low-carbohydrate meal, and a low-calorie meal to each participant in a crossover trial where they served as their own controls (Ferland et al., 2009). Two hours following their meal, participants engaged in a 60-minute submaximal aerobic exercise bout on a cycle ergometer at 60% of their VO_{2max}. Outcomes of interest included glucose, insulin, free fatty acid, catecholamine, glucagon, and cortisol responses. Unsurprisingly, blood glucose levels decreased in all conditions after which exercise took place, and this effect was strongest in conditions that had higher starting blood glucose. The low-GI meal produced

the lowest fall in blood glucose (2.9 1.7 mmol/L), and this may be analogous to a carbohydrate-last condition.

Pre-exercise Meals and Substrate Utilization in Healthy Populations

An investigation by Sun et al. (2012) included 10 apparently healthy, young males who completed 60 minutes of brisk walking after eating a standardized breakfast. The 3 meals were a low-GI meal, a low-GI meal that included fructose, and a high-GI meal without fructose (Sun et al., 2012). It follows naturally that blood glucose was elevated in the high-GI condition, and it was elevated in the low-GI condition that included fructose. However, of most interest here is substrate utilization during exercise. Based on the pre-exercise meal, there was a significant preference for substrates during exercise. In the low-GI meal, there was a preference for fat utilization and lower carbohydrate oxidation during exercise relative to the other two conditions. The conclusion here is that there is a potentially significant effect of the GI (and possibly fructose content) of a pre-exercise meal on substrate utilization during moderate-intensity exercise.

Such an observation is not entirely novel. One of the first studies performed in this area was published by Thomas and colleagues in 1991 in the *International Journal of Sports Medicine*. In this study, eight well-trained cyclists were tested to exhaustion one hour after consuming a test meal that was either high-GI or low-GI (Thomas et al., 1991). Unsurprisingly, plasma glucose was higher in the 30-60 min postprandial period in the high-GI meal condition compared to the low-GI meal. What may be surprising is that endurance, measured by time to exhaustion, was 20 minutes longer following the low-GI pre-exercise meal compared to the high-GI meal. There is now significant evidence that this is due to what the authors expected at the

time–increased fat oxidation and lower hyperglycemia and hyperinsulinemia following a low-GI meal (Thomas et al., 1991).

A series of studies have also been conducted within healthy populations that are of a similar design to the proposed intervention in this dissertation. One such study was conducted by Wu and colleagues in 2003. In this study, nine participants were sent through three separate trials where the pre-exercise meals were either a high-GI meal, a low-GI meal, or a fasting condition (Wu et al., 2003). Three hours following meal consumption, participants were instructed to run for 60 minutes at 65% VO_{2max}. The authors found that fat oxidation was increased in the fasted state compared to both meals and fat oxidation was increased in the low-GI meal condition when compared with the high-GI meal condition. This is potentially analogous to a carbohydrate-first (high-GI meal) and a carbohydrate-last (low-GI meal) condition. There is potential to increase exercise tolerance and time to exhaustion with such an intervention or target specific substrates for either performance or health benefits.

Pre-Exercise Meals and Substrate Utilization in Sedentary and Unhealthy Populations

Dr. Emma Stevenson and her team at the University of Nottingham in the United Kingdom conducted a somewhat unique study that included eight healthy, sedentary female participants with sedentary being defined as "not taking part in any structured exercise and scoring ≤ 1.2 on the International Physical Activity Questionnaire" (Craig et al., 2003; Stevenson et al., 2009). They were all free from any sort of disease, either chronic or acute and were non-dieting, non-smokers. Participants were fed a standardized meal each evening prior to their experiment that were either comprised of high-GI foods or low-GI foods, and foods were matched for proteins, fats, and carbohydrates. Each participant then came in the following

morning (overnight fast) and was fed a second standardized meal after which they waited three hours prior to beginning the exercise protocol. During this waiting period, expired air samples and glucose measurements were taken at regular intervals. The exercise protocol consisted of a 60-minute walk on a treadmill that was at 50% of the calculated VO_{2max} of each individual. Following exercise, participants were given a standard meal, after which they were monitored for 2 hours further while continuing to measure glucose and expired air samples at regular intervals. Peak glucose excursions and total postprandial AUC of glucose for the three-hour period were both higher in the high-GI meal condition compared to the low-GI meal condition. Of particular interest in this case, though, is not necessarily glucose directly. Rather, substrate utilization in these trials is keenly interesting. Following the low-GI meal, the amount of fat oxidized during the exercise bout was higher than in the high-GI meal condition while the total amount of carbohydrate oxidized was higher in the high-GI meal condition. While this may not come as a major surprise, it does demonstrate a potential acute health benefit of such an intervention in a sedentary population. Lastly, of note, following the standard post-exercise lunch, perception of fullness was higher in the low-GI pre-exercise meal when compared to the high-GI meal condition (Stevenson et al., 2009). Such a result is encouraging. Not only does a low-GI pre-exercise meal increase fat oxidation, it also may encourage a feeling of fullness in overweight individuals which may lead to individuals consuming fewer calories overall.

A 2012 study that was conducted by Dr. König and his team in Freiburg, Germany examined postprandial substrate utilization following isomaltulose (a low-GI sugar) ingestion in 20 overweight males that were affected by metabolic syndrome. Participants were fed either a breakfast of a 250-mL drink and 140 g of cookies that contained a total of 50 g of isomaltulose or 50 g of glucose and sucrose (König et al., 2012). Following a two-hour break after breakfast,

participants exercised at a moderate intensity for 30 minutes on a treadmill. After exercise, they consumed a 250-mL drink that was 10% isomaltulose along with mini pizzas and an apple. This second meal had either 25 g of isomaltulose or 25 g of glucose syrup and sucrose. Plasma glucose was lower in the first hour following breakfast and had lower AUC for the pre-exercise period in the isomaltulose condition. Blood glucose drop after exercise was much slower in the same condition and significantly higher at three hours after meal consumption. Insulin was lower at every time point in the isomaltulose condition. Of note, high-GI foods being replaced, even partially, by isomaltulose resulted in higher utilization of fat during exercise and lower glycemic and insulinemic responses. These results are seemingly in agreement with the data presented by Dr. Stevenson's team and suggest that low-GI pre-exercise meals may result in higher fat oxidation when compared with a high-GI pre-exercise meal (König et al., 2012; Stevenson et al., 2009).

In a different study that included girls aged 11-13 years, Zakrzewski and colleagues examined the effects of mixed breakfasts with varying GI on substrate metabolism. There was a total of eight overweight girls and twelve non-overweight girls included in the study (Zakrzewski et al., 2012). Each participant consumed isoenergetic meals with either a high GI (73) or a low GI (44). At 120 minutes following meal consumption, the girls completed a 30-minute walk at 50% of their VO_2 peak. The authors found that peak glucose was higher in the high-GI condition in the overweight girls but not in the non-overweight girls while AUC was 13% higher in the overweight girls and 4% higher in the non-overweight girls in the same respective meal conditions. Plasma insulin mirrored glucose and was both higher at its peak and had a larger area AUC in the overweight group compared to the non-overweight group. Interestingly, there was no difference in either group relating to fat oxidation during exercise or at rest regardless of meal,

though this may have been partly due to the small sample size (Zakrzewski et al., 2012). The main conclusion here is that there may be a reduced ability to control the metabolic load of a higher-GI meal in overweight populations when compared to a non-overweight population.

In conclusion, to this point, no study has examined the possible effect of ordered eating on the glycemic and metabolic responses to an acute exercise bout. There is clearly significant evidence that substrate utilization is significantly altered in both healthy and unhealthy populations by altering the GI of a pre-exercise meal. As such, there is a need for a study that combines both an ordered eating protocol and a subsequent exercise bout to examine if the same responses can be reproduced in the absence of altering the GI of foods eaten. Additionally, such a study may provide some evidence for the potential of altering substrate utilization during exercise to either be advantageous for health or performance.

Chapter Summary

Blood glucose and its intrinsic management are certainly essential to overall health. Consistent aberrations in these control systems and in overall blood glucose can lead to severe negative health outcomes such as hypertension, DM, heart disease, and cancer, just to name a few (Monnier & Colette, 2015). Beyond potential disease risk, glucose is necessary in its fundamental form for the proper functioning of virtually all tissues and organ systems in the human body (Cryer & Gerich, 1985). Because dysglycemia is caused by such a complex array of risk factors, it is difficult to pin a direct, traceable pathophysiology profile on disease progression. Possible contributing factors to dysglycemia are GLUT4 translocation inhibition, beta cell failure, and obesity (ADA, 2020; Khan & Pessin, 2002; Röder et al., 2016).

A relatively novel method of potentially treating dysglycemia is ordered eating–that is, changing the order of food consumption within a meal, with the most carbohydrate-dense portion consumed either first or last. While Imai and colleagues initially began this line of inquiry in 2010 (Imai et al., 2010), there has been relatively little subsequent research on the topic since then. Most notably spearheading the lion's share of that research has been Dr. Alpana Shukla. Dr. Shukla and her team have found, most notably, that consuming carbohydrates last in a meal order tends to lower PPG both in terms of AUC and peak excursions (Shukla et al., 2015, 2017, 2018, 2019). Other research teams have found similar results (Kuwata et al., 2016). Still, among these studies, there has been inconsistency in methodology and in participant profile, both in demographics and in health status. As such, there remains a significant amount of gap to fill in this literature.

Potentially more well researched is the comparison of pre-exercise meals and their potential effects on substrate utilization based on GI. Importantly, there is some evidence that a high-GI pre-exercise meal tends to lead to shorter time to exhaustion–possibly owing to higher rates of fat oxidation in a low-GI pre-exercise meal, which may serve to spare glycogen (Thomas et al., 1991; Wu et al., 2003). Beyond this, a low-GI pre-exercise meal may also lead to lower chance of rebound hypoglycemia (Faria et al., 2018) which has potential implications for those with a history of hypoglycemia or T1DM–specifically that they should avoid carbohydrate consumption within 90 minutes of exercise onset (Jeukendrup & Killer, 2011). These pre-exercise meals and their effects on substrate utilization are understudied in sedentary populations and obese populations. In these groups, it seems that low-GI pre-exercise meals tend to lead to higher fat oxidation rates and may increase feelings of fullness which could lead to consuming fewer overall calories (Stevenson et al., 2009).

To this point, there remains a gap in the literature that the proposed studies fill. Specifically, the systematic review comprehensively addresses what established literature does exist on the topic of ordered eating. The proposed laboratory study further elucidates on what effects ordered eating has in an acute setting, and it explores how a carbohydrate-last condition (akin to a low GI pre-exercise meal) impacts PPG, substrate utilization, and perceptual responses during exercise.

CHAPTER III

METHODS

The purpose of this dissertation was to determine the role of ordered eating as it relates to a number of relevant postprandial measures. This was done in two separate studies. The first was a systematic review of the current state of the literature as it relates to ordered eating and its effects on a variety of postprandial measures to include glucose, insulin, C-peptide, satiety, ghrelin, and others. The second study was a randomized, crossover experimental study that sought to examine the effects of meal order on PPG, substrate utilization, and relevant perceptual responses during an acute exercise bout.

Study 1 Methods

Included Studies in Review

The procedures for this unregistered systematic review were developed, in part, by consulting the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines (Higgins et al., 2011; Page et al., 2021). A formal protocol document was not created prior to conducting the review process. Initially, three databases were searched: PubMed, Web of Science, and the Cochrane CENTRAL Library. Search terms were structured as follows: (random* OR "clinical trial" OR experiment* OR control* OR crossover OR "within subjects" OR "between subjects" OR counterbalance*) AND ("food order" OR “food sequence” OR "food pattern" OR "meal order" OR "meal sequence" OR "meal pattern") AND (postprandial OR glucose OR glycemia OR glycemic OR insulin OR triglyceride OR GLP-1 OR ghrelin OR cholecystokinin OR "peptide YY" OR "neuropeptide Y").

Targeted reports were those that presented original research based on an experimental design (either randomized or nonrandomized). Master's theses, dissertations, and conference abstracts were not eligible for inclusion. To be included in the final review, a report had to be an acute experiment that compared the glycemic effects of altering food order within meals. Further, meals had to be isocaloric and consistent in composition between conditions or groups. There also could not be ≥30 minutes between the administration of two consecutive components of a meal. Studies were not excluded based on the specific characteristics of participants, including disease state. Outcome measures could include blood or plasma glucose, insulin, C-peptide, and other glycemia-related measures, as well as measures of lipemia, gut hormones, perceptual responses such as hunger and satiety, and feeding behavior at a subsequent meal. Other variables extracted from reports included study design, sample size, participant characteristics (age, gender/sex, race/ethnicity, anthropometrics, diagnosis of diabetes or prediabetes, hemoglobin A_{1c}), and meal/feeding details (specific foods, macronutrient composition, energy content).

Two reviewers screened each record independently. Each title was examined, and if the title was potentially eligible, the abstract was examined; if the article appeared eligible based on the abstract, the full text was read. After reading the full text, if the article was eligible, the references were examined following the same protocol. After this screening process, the reviewers compared finalized lists of reports and resolved any discrepancies.

Two other search strategies were used to identify potentially relevant records. First, the reference lists of pertinent review articles that the authors came across during the database search were examined. Second, one researcher who was listed as a first author on multiple papers

identified from initial searching was emailed and asked if they have knowledge of any reports that appear to be missing from the list of records from searching databases and reference lists.

The original plan was to evaluate risk of bias for each report using the Cochrane Risk of Bias tool for parallel group randomized trials (Ding et al., 2015), with an adapted tool being used for crossover trials (Murad et al., 2017). The certainty of evidence was evaluated using a modified version of the GRADE approach that is appropriate in the absence of a pooled effect estimate (Faber et al., 2018). The initial GRADE assessment was done by one author (PW) and then checked and edited by the other (BF).

Participants and Trial Features

Prior to data analysis, data was organized and extracted and into tables. Of those studies included in the review, participant characteristics were included in some capacity, and participant characteristics were noted in as much detail as given.

Data Analysis

Given the nature of systematic reviews, there is not a standardized statistical approach to analysis of data per say. As such, analysis took the form of results by outcome variable. For each outcome variable, the studies that included that variable were discussed and the relevant outcomes for that specific study. Outcome variables included in the results were: 1) glucose; 2) insulin and C-peptide; 3) incretins, gut hormones, and gut function; and 4) perceptual responses to feeding behavior.

Study 2 Methods

General Design

Similar to experimental studies that have been conducted previously in the area of ordered eating, our laboratory-based project included a standardized meal that each participant consumed with the foods eaten in a prescribed order. In one condition, the carbohydrate-dense food was consumed first (rice first (RF)), and in the opposite condition, the carbohydrate-dense food was consumed last (rice last (RL)). Dissimilar to previous research, and unique to this project, following consumption of the prescribed meal, participants completed an acute exercise bout during which their respiratory gaseous exchange was measured as well as their exertion and blood glucose, among other outcomes. The laboratory-based intervention followed a randomized crossover design. Blinding either the participants or the research team in this trial was not practical.

Participants went through three separate laboratory visits at the Old Dominion University Human Performance Laboratory (HPL). Participants also had the option of completing the first visit virtually, to include only the consent process and reviewing instructions for the subsequent visits. Visit two and visit three followed mirrored protocols that included a pre-exercise meal, but the order by which specific components of the meal were consumed was randomized. In a similar fashion to existing literature (Thomas et al., 1994), an exercise bout occurred 60 minutes after meal completion. In addition to the standardized meal and exercise bout, during visit two, participants had their body composition assessed in addition to relevant anthropometrics. Visits two and three began prior to 12 p.m. to help accommodate for fasting and standardization.

The protocol for this project was submitted to the Old Dominion University Institutional Review Board for approval prior to beginning. Additionally, the trial was pre-registered prior to participant enrollment using clinicaltrials.gov—pre-registration ID number: NCT06242015.

Recruitment and Participants

Initial recruitment took the form of speaking to classrooms of students and sending the study flyer to local gyms, sports clubs, etc. Word of mouth within the investigators' networks was also used, and participants who enrolled in the study were asked to share the flyer with individuals they knew who might be interested in participating. Individuals contacting the HPL about testing services were also told about the study.

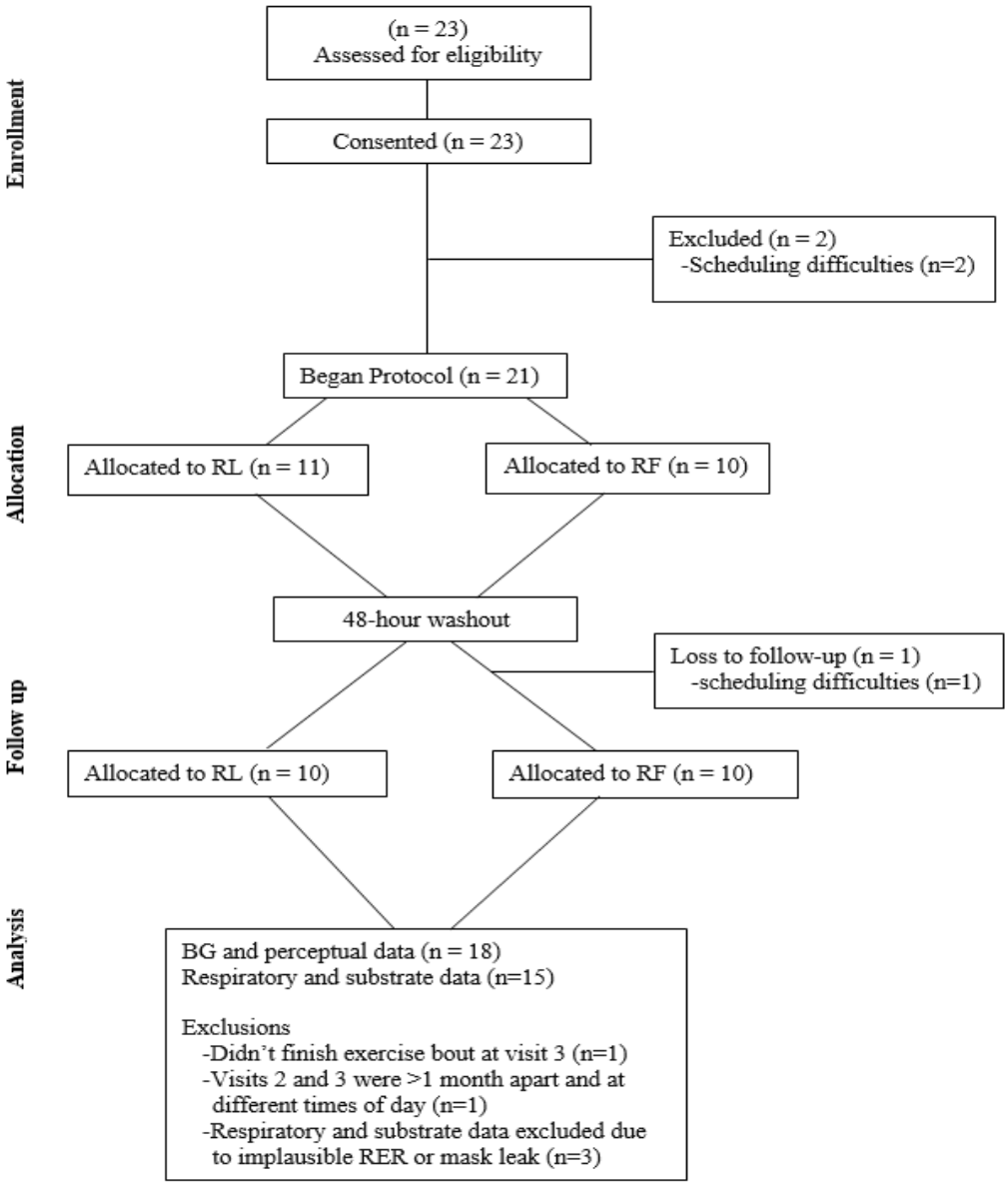
Inclusionary criteria were: 1) physically active, defined by the American College of Sports Medicine (ACSM) as participating in scheduled exercise at least three days per week for 30 minutes each time over the last three months, 2) ability to perform moderate-to-high intensity running for at least 30 minutes, 3) free from any allergy or other condition that would prohibit the consumption of poultry, rice, or broccoli, 4) no injury or disease (cardiovascular disease, diabetes, pulmonary disease except controlled asthma) precluding physical exercise, 5) not currently pregnant, 6) 18-60 years of age, and 7) no implanted electrical devices such as a pacemaker.

A total of 23 individuals were screened for eligibility, with all 23 consenting and enrolling in the study (**Figure 1**). Five participants did not complete the protocol as designed. Among those, three participants did not complete all visits, due to personal scheduling conflicts that did not allow for timely collection of data. Two participants attended all three visits but were excluded from the analysis: one individual was unable to complete the exercise portion of visit

three due to acute leg discomfort, and one completed visits two and three >1 month apart and their third visit was not started in the morning. Eighteen individuals completed the study and were included in the analysis for blood glucose and perceptual variables. Three participants' respiratory exchange and substrate use data were omitted from the analysis, so a smaller number (n = 15) had complete data for those outcomes. In one of those cases, there were implausible RER values (i.e. > 1.1) during the RF condition. In the remaining two cases, VO_2 values between conditions were significantly different (>15%), which indicated evidence of mask leaks.

No formal a priori power analysis was conducted to determine an adequate sample size. Previous research employing a crossover design to examine the impact of ordered eating on postprandial resting measures have utilized samples of less than 20 in most cases (Kuwata et al., 2016; Shukla et al., 2015, 2017; 2018; 2019; 2020).

Figure 1 Enrollment and Follow-up.



Visit Two

Participants were instructed to avoid prolonged (i.e., >60 min) or vigorous exercise for at least 24 hours prior to coming into the HPL. If they chose to exercise in the 48-hour window leading up to visit two, they were asked to replicate that exercise in terms of duration, time, intensity, modality, and volume in the leadup to visit three. Physical activity was tracked by

having participants wear an Actigraph GT3X-BT (Actigraph, Pensacola, FL) at the waist for the two days prior to the visit. Additionally, they arrived at the HPL fasted for at least eight hours to include avoidance of all food, energy-containing beverages, caffeine, other stimulants, and recreational drugs. They were allowed to consume water during this period. In addition, participants were asked to drink 20 ounces of water at home, on the morning of the visit, before coming to the laboratory, which helped to ensure adequate hydration status upon arrival. Lastly, they were asked to keep a food record for two days prior to the visit and asked to duplicate their food and beverage intake as best they could prior to visit three.

Upon arrival, urine specific gravity (USG) was measured using a urine refractometer (PAL-10S, Atago, Japan) in order to assess hydration status. Initial body anthropometrics were measured and included height, mass, and body composition. Body composition was assessed using the InBody 770 (InBody Co., LTD; Seoul, South Korea), and assessed data included body mass and body fat percentage.

The participant was then familiarized with specific perceptual scales and provided baseline ratings. The scales included rate of perceived exertion (RPE) measured by the original Borg Scale (Borg, 1982) and a Feeling Scale (FS) that ranked how they were feeling from "-5" (very bad) to "+5" (very good) (Hardy & Rejeski, 1989). Seven GI symptoms were rated on a 0-10 scale where 0 means "no discomfort," 5 means "moderate discomfort," and 10 means "unbearable discomfort" (Wilson, 2017). Symptoms included nausea, regurgitation/reflux, bloating, abdominal cramps, side stitch, gas/flatulence, and urge to defecate. Participants were provided with definitions of each symptom. In addition, hunger, satiety, fullness, and appetite were measured using a similar 0-10 Likert scale (0=none, 10=extremely) after participants were

provided definitions of each phenomenon. Likert scales have demonstrated good agreement with visual analog scales when rating these types of perceptual sensations (Holliday et al., 2021).

Heart rate (HR) was monitored using a Polar H10 heart rate monitor (Polar Electro, Inc.; Bethpage, NY). After allowing the participant to rest in a seated position for five minutes, HR was recorded three times over the course of one minute (baseline, 30 seconds, and 60 seconds).

Next, baseline resting gas exchange measurements were collected using a TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT). The metabolic cart's flow and gas sensors were calibrated with a 3-liter syringe and calibration gas (O_2, 16.0%; CO_2, 4.0%) prior to baseline measurements being taken. A slight adjustment to the calibration procedures was made after the first four participants completed the study. Specifically, additional gas sensor calibrations were completed after meal consumption and within 5 minutes of the start of exercise. This change was made to reduce any small potential impacts of drift in the CO_2 sensor over time. After applying a silicone rubber facemask connected to a two-way non-rebreathing value, 10 minutes of volume of oxygen consumption (VO_2) and volume of carbon dioxide production (VCO_2) were collected with the participant seated in a chair. Frayn's equations (carbohydrate = $4.55VCO_2 – 3.21VO_2 – 2.87n$ and fat = $1.67VO_2 – 1.67VCO_2 – 1.92n$) were used to calculate estimated rates (g/min) of carbohydrate and fat utilization at rest (Frayn, 1983), with protein oxidation assumed to be negligible. Respiratory exchange ratio (RER) was also calculated.

Thereafter, fasting blood glucose was measured using the FreeStyle Lite Blood Glucose Monitor System (Abbott Diabetes Care Inc., Alameda, CA, USA) due to its relatively high accuracy when assessing glucose compared to the YSI 2300 Stat Plus, a reference method (Tack et al., 2012).

A website (https://www.sealedenvelope.com/simple-randomiser/v1/lists) was used to generate a randomization list, which was used to assign a particular meal order to each participant (of the two available meal orders). The participant either ate chicken (100 g, Tyson Grilled and Ready Chicken Breast Strips) and broccoli (150 g, Harris Teeter Broccoli Florets) together followed by eating rice (150 g, microwavable Ben's Original Jasmine Rice), or the reversed order. Masses of chicken and broccoli were measured out in a frozen state before cooking, while the rice was measured after cooking. The masses of food used were similar to protocols laid out in previous studies (Imai et al., 2014; Shukla et al., 2017). The participant was asked to consume all food within 15 minutes to include 7.5 minutes per phase. If they did not consume all of the food in the allotted time, the remaining balance was measured on a food scale (i500 Balance, My Weigh, Pheonix, AZ), and those adjusted masses were provided to the participant during visit three. Participants were allowed to drink water ad libitum during the meal, and water consumed was measured. During the third visit, participants were required to consume the same amount of water.

After the meal was ingested, a 5-10-minute period was allowed for the participant to use the restroom (if needed) and to refit the facemask before gas exchange was collected again. Resting gas exchange was collected and analyzed for a 45-minute period, beginning at 10 minutes postprandial (PP) until 55 minutes PP. Participants remained seated throughout this period. Blood glucose was assessed at 30 minutes PP, at 59 minutes PP (exercise began at 60 minutes PP), immediately after the conclusion of exercise (approximately 90 minutes PP), and at the conclusion of the visit (120 minutes PP). Perceptual data were collected at specified intervals (see **Figure 2**).

Exercise began 60 minutes after the meal was finished and was carried out on H/P Cosmos T170 DE SPORT MED Treadmill (Nussdorf-Traunstein, Germany). Duration of exercise was set at 30 minutes. Considering that this is the first known study of its kind that incorporated ordered eating and comparing substrate utilization under those manipulated conditions, it was deemed justifiable to use a relatively short exercise duration, especially since it is a duration that most recreationally active people can complete without becoming too tired. Exercise intensity was determined as a percentage of estimated maximum HR. We used the Tanaka estimation (HR_{max} = 208-0.7 x age; Tanaka et al., 2001) as opposed to the Fox estimation, as the Fox estimation tends to underestimate maximum HR in men whereas both seem to equally overestimate in women (Nikolaidis et al., 2018). Exercise was conducted at 70% HR_{max}. Treadmill speed started at 5 mph for the first minute, and adjustments were every 1-2 minutes thereafter as necessary to find the correct speed to reach the target HR. Once the target heart was achieved (within ±4 bpm), the speed was maintained for the remainder of the protocol. The same speed adjustments and final speed were utilized during visit three. Respiratory exchange data were collected for the duration of the exercise bout. Equations from Jeukendrup and Wallis (2005) were used to estimate rates of carbohydrate and fat oxidation during exercise.

Fat oxidation: $(1.695 \times VO_2) - (1.701 \times VCO_2)$

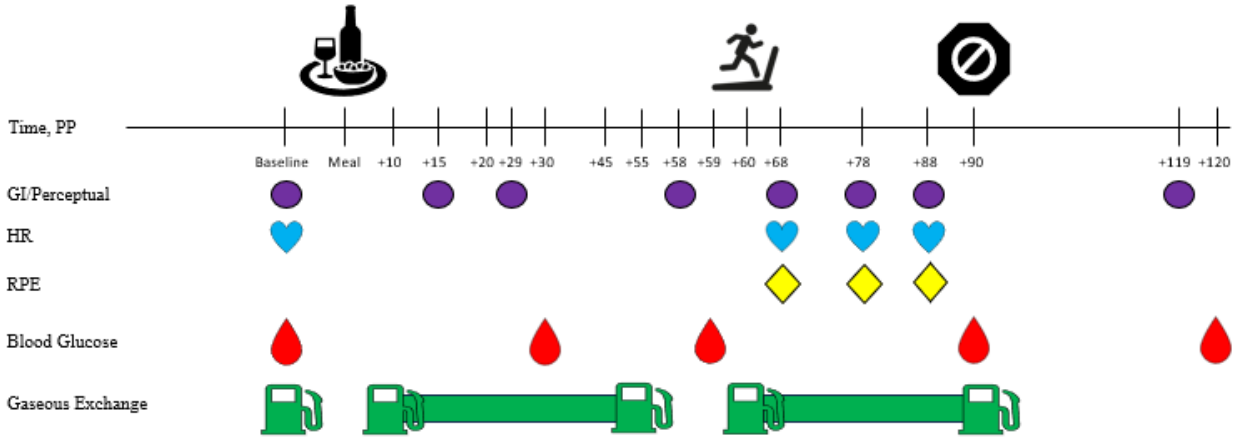
Carbohydrate oxidation: $(4.21 \times VCO_2) - (2.962 \times VO_2)$

Perceptual data were collected again at 8, 18, and 28 minutes after the onset of exercise (i.e., 68, 78, and 88 minutes PP). Over one-minute periods from 9-10, 19-20, and 29-30 minutes during exercise, HR was collected at 30-second intervals and averaged. Blood glucose measurement was also repeated at the conclusion of exercise.

Following the exercise bout, participants rested for 30 minutes. During this time, respiratory exchange measurements were not recorded, but perceptual and blood glucose measurements were taken 29 and 30 minutes after the conclusion of exercise (119 and 120 minutes PP), respectively. Participants were also offered water, and the amount consumed was measured and duplicated in visit three.

A summary of the study protocol and measurements is shown in **Figure 2.**

Figure 2. Order of Events for Laboratory Visits 2 and 3.



**Time bars are not drawn to scale*

Visit Three

Visit three, as well as the pre-visit procedures leading up to visit three, followed essentially the same protocol as visit two with the major exception of the changed meal order. Whatever the meal order was in visit three (either RF or RL), it was reversed in visit three. Participants were given the same quantities of food and water they consumed during visit two and were asked to consume the entire amount of each. If all the provided food was not consumed, the remaining balance was measured.

There was a minimum of 48 hours between visits two and three for all participants. Although menstrual cycle status and oral contraceptive use have been reported to modestly impact substrate use in some individual studies, a recent meta-analysis of 25 studies (483 participants) found no significant effect of menstrual cycle phase (standardize mean difference = 0.05, 95% confidence interval = -0.15 to 0.25) (D'Souza et al., 2023). The authors of the article ultimately concluded that there is likely no need to control for menstrual cycle phase so long as other important factors (exercise duration and intensity, nutritional status, etc.) are controlled for. A motivation for avoiding menstrual cycle control, beyond the marginal effect it may have, is to avoid inherent disenfranchisement of female participants by including a higher barrier to participation. In a 2014 analysis, authors conducted a search to include three years of studies published in the following three journals: *British Journal of Sports Medicine, Medicine and Science in Sports and Exercise,* and *American Journal of Sports Medicine* (Costello, et al., 2014). Within the 1,382 articles included, 6,076,580 participants were totaled. Of those, only 2,366,968–roughly 39%–were female (Costello, et al., 2014). Further, the authors found that females were under-represented across all of the three included journals (Costello et al., 2014). As such, it is important to be aware of such biases and attempt to correct them where applicable.

Data Processing

Blood glucose change values were calculated as peak-nadir, PP peak-nadir, and delta exercise change from pre-to-post exercise. Peak-nadir was calculated as the maximum measured blood glucose value minus the minimum measured blood glucose value and included baseline while PP peak-nadir was the same but did not include baseline values. Delta exercise change was

calculated as the blood glucose value measured at 59 minutes (just prior to exercise onset) minus blood glucose measured at 90 minutes (at exercise cessation).

Respiratory exchange data were exported from the Parvo software using 30-second averages. Because the data were collected for a relatively long duration at each visit (~90 minutes total), they needed to be averaged into smaller blocks of time. Five-minute blocks of time were used, including the following: the last 5 minutes of the 10-min baseline period; nine blocks during PP rest (10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, and 50-55 minutes); and six blocks during exercise (0-5, 5-10, 10-15, 15-20, 20-25, and 25-30 minutes).

Actigraph accelerometer data were downloaded directly from the accelerometer devices and processed with ActiLife version 6.13.5 (Actigraph, Pensacola, FL). Wear time was validated through the software using the algorithm of Choi and colleagues (Choi et al., 2011). Scoring for different intensities of activity was performed using the methods described by Troiano et al. (2008) and utilized the following counts-per-minute thresholds (light, 100-2019; moderate, 2020-5998; vigorous, ≥5999)

Area under the curve (AUC) values for hunger, appetite, satiety, and fullness were calculated with Microsoft Excel using the trapezoid method (Matthews et al., 1990). Essentially, the segments between timepoints were considered different trapezoids on a graph, and the area of each trapezoid was calculated and summed to determine AUC.

Because gastrointestinal symptoms were highly right skewed with many 0 values, all symptom values at each time point were summed and added together to reach a total sum score for each variable.

Dietary information from food logs was quantified, including total energy consumption, carbohydrate content, fat content, protein content, and fiber content. Although participants were

asked to complete the logs for two days before each visit in order to enhance matching of intake between visits, only intake from the day before each visit was analyzed. Each value was estimated based on individual participant reporting in their provided food logs. If specific brand information was either not provided by the participant or unavailable directly from the manufacturer website, these values were estimated using a separate online tool (Cronomter (https://cronometer.com)) or taken from distributor websites such as Walmart.

There was a small amount of missing perceptual data due three participants unintentionally failing to write down some values on the provided recording sheet. Two participants did not report information for any of the perceptual variables at the final timepoint (minute 119 PP) for one condition, and one participant did not report FS values at three timepoints (15, 29, and 45 minutes PP) for one condition. In the cases of missing data at minute 119, the last observation was carried forward to fill in the missing data. In the case of the missing feeling scale data, linear interpolation was used to fill in the missing data.

Before any statistical analyses were undertaken, values for perceptual, blood glucose, metabolic/substrate, and Actigraph data were checked via a double entry method. Specifically, two of the authors (BKF and PBW) independently transferred all the data to Microsoft Excel. Differences were noted by examining average condition values for each variable, and any differences between the two authors' entries were corrected by re-confirming with the original data.

Statistical Analysis

The analysis of the outcome data was conducted as follows using SPSS version 29 (IBM, Armonk, NY, USA). Visual inspection of histograms and Q-Q plots was used to determine

normality of data distribution. Normally distributed data are presented as means and standard deviations, with skewed data presented as median (25^{th}-75^{th} percentile).

A two-way ANOVA with time and condition as within-subject factors was conducted to compare the RL and RF conditions as it relates to PPG, FS, and RPE. Two-way ANOVAs for gas exchange (i.e., VO_2) and substrate oxidation were broken up into two separate analyses, one that included baseline and PP resting data, with the other including exercise data separately. This allowed us to evaluate whether there were any exercise-specific effects separate from the resting PP period. In the event there were any time effects with no interaction effect, pairwise comparisons with a Bonferroni correction were applied. With significant interaction effects, between-condition effects at each timepoint were explored using paired t-tests with Bonferroni adjustments for multiple comparisons.

Paired t-tests were also used for comparing blood glucose change/delta values, AUCs for hunger, appetite, satiety, and fullness, and Actigraph data. Some variables did have a non-normal distribution, so square-root and natural-log transformations were used to achieve normal distribution. Hunger and appetite AUC values were transformed via natural log. Because some participants answered "0" for hunger and appetite throughout the visit, a trivial value of 1 was added to each AUC in order to calculate natural log. Moderate-to-vigorous physical activity (MVPA) was modestly right-skewed, and square-root transformation was best-suited for making the data normal. Gastrointestinal-symptom sum scores were compared using a Wilcoxon signed-rank test. A value of $<.05$ was considered significant.

CHAPTER IV

ORDERED EATING AND ITS EFFECTS ON VARIOUS POSTPRANDIAL HEALTH MARKERS: A SYSTEMATIC REVIEW

Introduction

According to the Centers for Disease Control and Prevention, approximately 40% of adult men and women are obese in the United States, typically defined as a body mass index (BMI) of 30 or higher (Hales et al., 2017). To say that there is an obesity epidemic in the United States is appropriate, and this concerning trend holds true in many other countries in the Americas, Europe, Australia, and the Middle East (Blüher, 2019). Obesity is associated with numerous long-term health outcomes, including type 2 diabetes (T2D), cardiovascular disease, hypertension, and multiple cancers (Fava et al., 2019). The association between obesity and T2D is particularly strong, with anywhere from about 30% to 50% of diabetes cases being attributable to obesity (Feng et al., 2021; Tanamas et al., 2016). As such, there are ongoing efforts at every level of policy making to attempt to curb what is now commonly termed the 'obesity epidemic.'

Healthcare providers commonly assess blood glucose as a measure of the effects that obesity and other T2D risk factors have on cardio-metabolic health. Specifically, fasting and postprandial glucose (PPG) are the clinical diagnostic tools for diabetes (ADA, 2001), and although both are important targets for management in diabetes, PPG is usually the more important contributor to overall hyperglycemia early on in the disease course and among patients who have slightly elevated hemoglobin A_{1c} (Monnier & Colette, 2015). Further, elevated PPG and postprandial insulin (PPI) are risk factors in developing a number of chronic diseases to include T2D and cardiovascular disease (Monnier & Colette, 2015), though there remains a lack

of high-level evidence that specifically targeting PPG improves clinical outcomes (Monnier & Colette, 2015).

Over the years there have been many different attempted interventions, from lifestyle changes to pharmacological interventions, to mitigate increases in PPG. One relatively recent intervention is the idea of consuming foods within a meal in a particular sequence (e.g., vegetables followed by protein-rich foods followed by carbohydrate-rich foods). From a physiological perspective, consuming carbohydrate-rich foods at the end of a meal, as opposed to the beginning, could theoretically attenuate sharp rises in PPG and PPI. Among other mechanisms, this could be due to delays in gastric emptying and absorption of glucose (Monnier & Colette, 2015).

Some studies, such as those done by Shukla and colleagues, have shown some promise for ordered eating as an approach to tackling the effects of some foods on PPG (IDF, 2017; Ma et al., 2009; Shukla et al., 2017). That said, to the knowledge of the authors, there has been no attempt to consolidate this information into a systematic review. Thus, the goal of this paper was to systematically examine the literature and consolidate the available evidence of the effects of ordered eating on postprandial glycemic measurements including glucose and insulin. A secondary goal was to examine the effects of eating order on a variety of other potentially relevant outcomes, including postprandial lipemia, hunger-related hormones, perceptual (e.g., hunger, satiety) responses, and feeding behavior. A formal meta-analysis was not carried out due to notable variation in the types of foods used between studies, as well as because of variability in participant characteristics and the methods (area under the curve [AUC], peak levels, time to peak, etc.) that were employed for quantifying PPG and PPI.

Methods

The procedures for this unregistered systematic review were developed, in part, by consulting the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines (Moher et al., 2009; Page et al., 2021). A formal protocol document was not created prior to conducting the review process. Initially, after cursory searching, we developed a specific set of search terms and chose three databases to search: PubMed, Web of Science, and the Cochrane CENTRAL Library. Search terms were structured as follows: (random* OR "clinical trial" OR experiment* OR control* OR crossover OR "within subjects" OR "between subjects" OR counterbalance*) AND ("food order" OR “food sequence” OR "food pattern" OR "meal order" OR "meal sequence" OR "meal pattern") AND (postprandial OR glucose OR glycemia OR glycemic OR insulin OR triglyceride OR GLP-1 OR ghrelin OR cholecystokinin OR "peptide YY" OR "neuropeptide Y").

Targeted reports were those that presented original research based on an experimental design (either randomized or nonrandomized). Master’s theses, dissertations, and conference abstracts were not eligible for inclusion. To be included in the final review, a report had to be an acute experiment that compared the glycemic effects of altering food order within meals. Further, meals had to be isocaloric and consistent in composition between conditions or groups. There also could not be ≥ 30 minutes between the administration of two consecutive components of a meal. Studies were not excluded based on the specific characteristics of participants, including disease state. Outcome measures could include blood or plasma glucose, insulin, C-peptide, and other glycemia-related measures, as well as measures of lipemia, gut hormones, perceptual responses such as hunger and satiety, and feeding behavior at a subsequent meal. Other variables extracted from reports included study design, sample size, participant

characteristics (age, gender/sex, race/ethnicity, anthropometrics, diagnosis of diabetes or prediabetes, hemoglobin A_{1c}), and meal/feeding details (specific foods, macronutrient composition, energy content).

The search of each database was conducted on February 15th, 2022. Two reviewers screened each record independently. Each title was examined, and if the title was potentially eligible, the abstract was examined; if the article appeared eligible based on the abstract, the full text was read. After reading the full text, if the article was eligible, the references were also examined following the same protocol. After this screening process, the reviewers compared finalized lists of reports and resolved any discrepancies.

Two other search strategies were used to identify potentially relevant records. First, the reference lists of pertinent review articles that the authors came across during the database search were examined. Second, one researcher who was listed as a first author on multiple papers identified from initial searching was emailed and asked if they had knowledge of any reports that appeared to be missing from the list of records from searching databases and reference lists.

The original plan was to evaluate risk of bias for each report using the Cochrane Risk of Bias tool for parallel group randomized trials (Higgins et al., 2011), with an adapted tool being used for crossover trials (Ding et al., 2015). Ultimately, all included trials used a crossover design, so only the tool from Ding et al. (2015) was used. The certainty of evidence was evaluated using a modified version of the GRADE approach that is appropriate in the absence of a pooled effect estimate (Murad et al., 2017). The initial GRADE assessment was done by one author (PW) and then checked and edited by the other (BF).

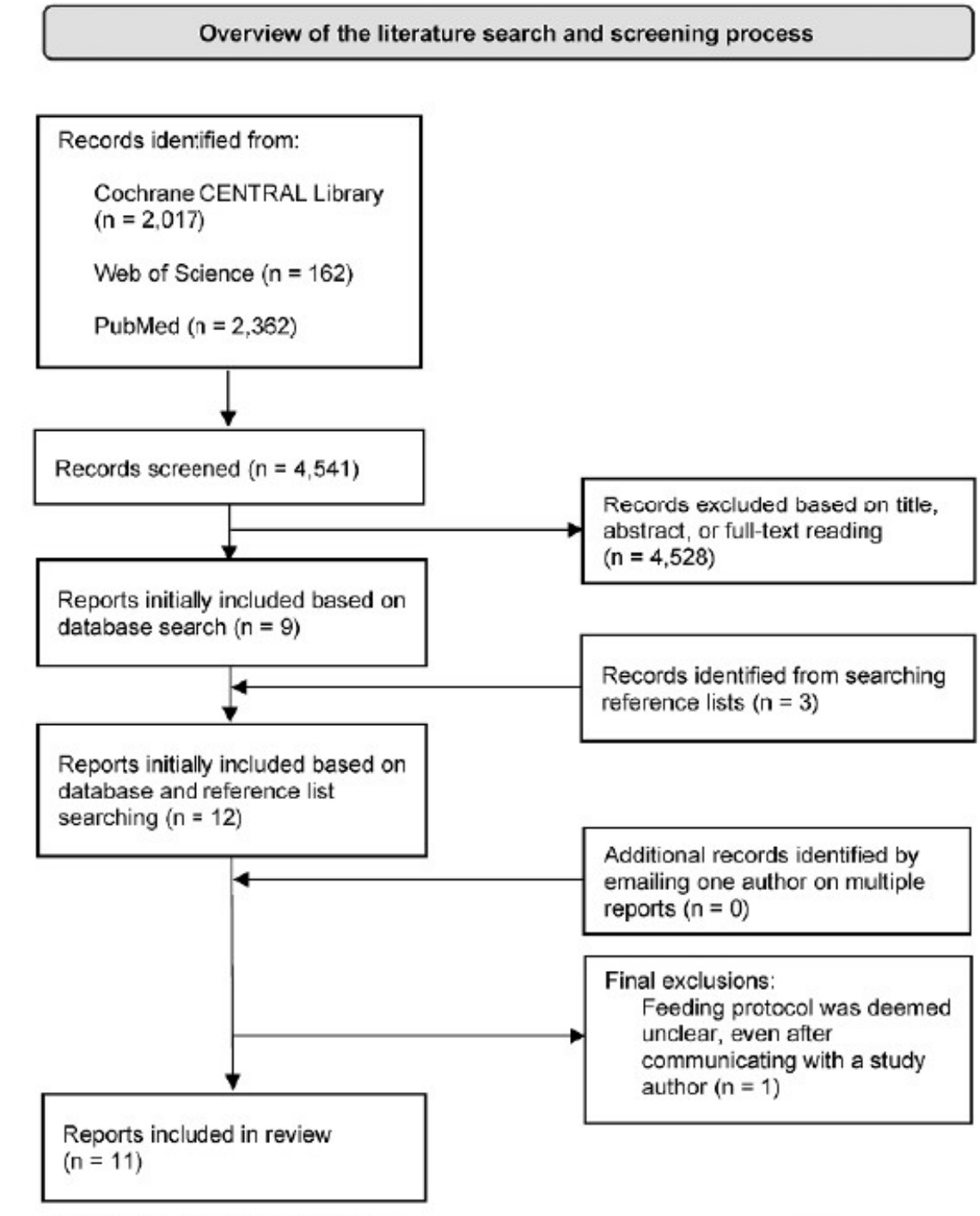
Results

Search Results

An overview of the search and screening process is presented in **Figure 3.** Total records screened from the database searching included 2,017 from the Cochrane CENTRAL Library, 162 from Web of Science, and 2,362 from PubMed. After screening, a total of nine reports were considered likely eligible for extraction (Faber et al., 2018; Kuwata et al., 2016; Lee et al., 2021; Nagoro et al., 2019; Shukla et al., 2015, 2017, 2018, 2019; Sun et al., 2020). Three additional records were identified from reference lists (Imai et al., 2010, 2013; Nishino et al., 2018), raising the total to 12 reports. No other records were identified by emailing a researcher (Shukla) who was a first author of multiple reports. Finally, one article that appeared to be eligible initially was subsequently excluded due to a lack of clarity around the feeding protocol (Nagoro et al., 2019), which remained unresolved after emailing with one of the report's authors.

Ten of 11 identified reports were in English (Shukla et al., 2015, 2017, 2018, 2019; Faber et al., 2018; Imai et al., 2013; Kuwata et al., 2016; Lee et al., 2021; Nishino et al., 2018; Sun et al., 2020). The other report was written primarily in Japanese (Imai et al., 2010), but it was still included in this review because it presented the meal content and composition in English, and a limited version of the methods and results was re-reported in English in a review article published several years later (Imai et al., 2014). Therefore, data from both articles was extracted and considered as a single report. Of note, one included report (Shukla et al., 2018) presented data from a previously published experiment (Shukla et al., 2017), but the data presented (ghrelin and perceptions of hunger and fullness) were not published in the earlier report.

Figure 3. Systematic Review Screening Process



Trial Features and Participant Characteristics

Extracted data was organized using Microsoft Tables. Initial extraction was performed by one author (BF) and edited and checked by the second (PW). Out of the final 11 reports included in this review, nine reported results from randomized crossover trials. The remaining two included data from studies that used a non-randomized crossover design (Nishino et al., 2018; Shukla et al., 2015).

Not every report listed specific racial/ethnic characteristics of participants. That said, roughly half of reports either explicitly stated that participants had some type of East or Southeast Asian (Japanese, Chinese, Malaysian Indian, etc.) background (Imai et al., 2013; Lee et al., 2021; Sun et al., 2020) or implied so by referring to the study taking place in an East Asian country (Imai et al., 2010; Kuwata et al., 2018; Nishino et al., 2018).

Most of the administered meals were moderate in energy content (range: 340-628 kcal), with carbohydrate making up the largest share of energy provision in all cases (**Table 1**). The specific foods administered to participants varied substantially, but the most common sources of carbohydrate were rice (six reports), bread (six reports), and orange juice (four reports). Ten reports fed meat or fish, while nine provided vegetables in varying quantities.

The results of the Cochrane Risk of Bias evaluation are presented in **Figure 4**. Overall, most reports were rated as either having low or unclear risk of bias for most items. Lack of randomization and allocation concealment were a source of bias for two reports (Imai et al., 2010; Shukla et al., 2015), while potential selective reporting or retrospective trial registration was a source of bias for three reports (Kuwata et al., 2016; Shukla et al., 2019; Sun et al., 2020).

Figure 4. Overview of Cochrane Risk of Bias

Author	Appropriate Crossover Design	Randomized Treatment Order	Carry-Over Effect	Unbiased Data	Allocation Concealment	Blinding	Incomplete Outcome Data	Selective Outcome Reporting	Other Bias
Faber et al. (2018)	+	+	+	+	?	+	+	+	+
Imai et al. (2013)	?	?	?	+	?	+	?	?	?
Imai et al. (2010) + Imai et al. (2014)	?	?	?	+	?	+	?	?	?
Kuwata et al. (2016)	?	?	?	+	?	+	?	-	+
Lee et al. (2021)	+	+	+	+	+	+	+	?	+
Nishino et al. (2018)	+	-	+	+	-	+	?	?	+
Shukla et al. (2015)	+	-	+	+	-	+	?	?	?
Shukla et al. (2017)	+	+	+	+	?	+	+	?	+
Shukla et al. (2018)	+	+	+	+	?	+	+	?	-
Shukla et al. (2019)	+	+	+	+	?	+	+	-	+
Sun et al. (2020)	+	?	+	+	?	+	+	-	+

Results by Outcome

For the remainder of the results section, outcomes are reported using the following four categories: 1) glucose; 2) insulin and C-peptide; 3) incretins, gut hormones, and gut function; 4) perceptual responses and feeding behavior. An overview of the statistically significant findings for all outcomes is presented in **Table 1**.

Table 1. Systematic Review Results by Outcome

Article	Participants	Design	Meals	Included Foods	Order of Foods	Main Relevant Outcomes§	Notable Significant (p≤0.05) Findings
Faber et al. [16]	20 (5 M, 15 F) youth T1D patients (age: 12.1 ± 2.2 y; median HbA1c: 7.4%)	Randomized crossover trial	▪ 398 kcal ▪ 54 g C + 22 g P + 9 g fat	1. Cheese (22 g) 2. Turkey breast (30 g) 3. Brown bread (2 slices) 4. Strawberry jam (15 g) 5. Orange juice (150 mL)	▪ T1 = 1 + 2 (15-min break) 3 + 4 + 5 ▪ T2 = 1 + 2 + 3 + 4 + 5	3-h PP blood glucose (peak, time to peak, mean, AUC, hypoglycemic events, proportion of time >10 and >12 mmol/L)	▪ T1 had lower mean glucose levels than T2 (10.2 vs. 9.3 mmol/L) ▪ T1 spent less time >10 mmol/L (38.6% vs. 56.5%) and >12 mmol/L (20.8% vs. 34.5%) than T2
Imai et al.[22]+ Imai et al.[25]	15 (7 M, 8 F) T2D patients (age: 61.7 ± 11.6 y; BMI: 24.7 ± 4.3; kg/m²; HbA1c: 6.4 ± 0.6%)	Randomized crossover trial	▪ 340 kcal ▪ 61.8 g C (1.9 g fiber) + 4.8 g P + 7.7 g fat‡	1. White rice (150 g) cooked with salt (0.3 g) 2. Salad with tomato (30 g), cabbage (60 g), olive oil (7 g), rice vinegar (7 g), soy sauce (0.3 g), mustard (0.5 g), salt (0.3 g), and maltose (1.3 g)	▪ T1 = 2 then 1 ▪ T2 = 1 then 2	2-h PP plasma glucose 2-h PP serum insulin	▪ At 30-min PP, T1 had lower plasma glucose than T2 (~10 vs 13 mmol/L)¥ ▪ At 30- and 60-min PP, T1 showed approximately 25-35% lower serum insulin than T2¥
Imai et al. [23]†	19 (6 M, 13 F) T2D patients (age: 65.5 ± 9.4 y; BMI 22.5 ± 3.1 kg/m²; HbA1c: 7.2 ± 1.0%) 21 (2 M, 19 F) individuals with normal glucose tolerance (age: 29.8 y; BMI: 20.8 ± 3.0 kg/m²; HbA1c: 5.4 ± 0.6%)	Randomized crossover trial	▪ 30 kcal/kg of mass spread for 3 meals over 24 h ▪ 58% C + 17% P + 25% fat	1. Rice/bread (unspecified amount) 2. Meat/fish (unspecified amount) 3. Vegetables including tomato, spinach, broccoli, and radish (500 g)	▪ T1 = 3 then 2 then 1 ▪ T2 = 1 then 2 then 3	Continuous glucose monitoring to assess mean plasma glucose, standard deviation of glucose, mean and largest amplitude of glucose excursions, 1- and 2-h PP glucose at each meal, and 3-h incremental AUC after all 3 meals	▪ T1 led to lower 1-h glucose than T2 in normal tolerance group at all 3 meals, as well lower amplitudes of excursions and standard deviations of glucose ▪ T1 led to lower 1-h glucose than T2 in T2D group after breakfast and at 2 h after lunch and dinner, as well as lower amplitudes of excursions and standard deviations of glucose ▪ T1 led to lower 3-h AUC than T2 after all three meals combined in T2D group (334 vs. 546 mmol/L) and normal tolerance group (132 vs. 191 mmol/L)
Kuwata et al. [17]¶	12 (9 M, 3 F) unmanaged T2D patients (age 59.7 ± 9.7 y; BMI: 25.3 ± 4.1 kg/m²; HbA1c: 6.6 ± 0.5%) 10 male healthy controls (age: 38.4 ± 4.9 y; BMI: 22.7 ± 2.4 kg/m²; HbA1c: 5.4 ± 0.3%)	Randomized crossover trial	▪ 460 kcal ▪ 53 g C + 19-20 g P + 18 g fat	1. Boiled mackerel (100 g, 220 kcal) 2. Grilled beef (79 g, 220 kcal) 3. Steam rice (150 g, 240 kcal)	▪ T1 = 1 (15-min break) 3 ▪ T2 = 2 (15-min break) 3 ▪ T3 = 3 (15-min break) 1	4-h AUC PP blood glucose 4-h AUC PP insulin and C-peptide Gastric emptying (time required for half of ^{13}C-labelled acetate to leave stomach 4-h AUC incretin levels (GLP-1, GIP)	▪ T1 and T2 led to lower 4-h glucose AUC than T3 in T2D patients, but not controls ▪ T1 and T2 led to lower 4-h insulin AUC than T3 in T2D patients, and lower 4-h C-peptide AUC in both samples ▪ Gastric emptying of rice was faster in T3 than the other two conditions ▪ T1 led to higher 4-h GLP-1 AUC values than T3 in T2D patients ▪ T2 tended to lead to the highest 4-h GIP AUC values
Lee et al. [18]	14 (6 M, 8 F) non-diabetic normal weight individuals (age: 21.9 ± 2.1 y; BMI: 21.0 ± 1.1 kg/m²) 17 non-diabetic overweight or obese individuals (age: 25.5 ± 5.2 y; BMI: 28.9 ± 4.2 kg/m²)	Randomized crossover trial	▪ 431 kcal ▪ 56 g C + 19 g P + 15 g fat	1. Jasmine white rice (150 g) 2. Chicken breast (76 g) with green capsicum (40 g), red onion (10 g), corn oil (5 g), ginger root (3 g), garlic clove (3 g), turmeric (2 g), and curry paste (10 g)	▪ T1 = 1 (10-min break) 2 ▪ T2 = half of 1 + 2 (10-min break) half of 1 + 2 ▪ T3 = 2 (10-min break) 1	PP peak glucose and 2-h incremental AUC	▪ 1.8 mmol/L lower glucose peak with T3 vs. T1 in normal weight group ▪ 1 mmol/L lower glucose peak with T3 vs. T1 in overweight/obese group ▪ 2-h AUC lower in T3 than T1 in normal weight group (122 vs. 272 mmol/L x min) and overweight/obese group (114 vs. 193 mmol/L x min) ▪ 2-h AUC lower in T2 than T1 in normal weight group (206 vs. 272 mmol/L x min)
Nishino et al. [24]	8 (4 M, 4 F) healthy individuals (age: 20 ± 1.2 y; BMI: 20.3 ± 1.1 kg/m²; HbA1c: 5.2 ± 0.2%)	Non-randomized crossover	▪ 544 kcal ▪ 78.8 g C (6.0 g fiber) + 21.9 g P + 15.1 g fat	1. Boiled rice (150 g), braised pumpkin (45 g), and orange (75 g) 2. Grilled pork (60 g) 3. Cucumber with sesame and vinegar (50 g); salad with lettuce (30 g), broccoli (50 g), tomatoes (20 g), olive oil (5 g) and rice vinegar (5 g)	▪ T1 = 1 then 3 then 2 ▪ T2 = 3 then 1 then 2 ▪ T3 = 3 then 2 then 1	2-h PP plasma glucose at multiple timepoints and 2-h AUC 2-h PP serum insulin at multiple timepoints and 2-h AUC	▪ T3 led to lower PP glucose after 30 min, 45 min, and 60 min than T1 ▪ T3 led to lower PP insulin after 30 min than T1 ▪ T3 had lower 2-h AUC for insulin than T1 (6,310 vs. 9,890 μU•min/mL) ▪ 2-h AUC for glucose was not statistically different between conditions but tended to be lowest with T3 (975 mg•min/dL), followed by T2 (1,230 mg•min/dL), then by T1 (1,878 mg•min/dL)

Shukla et al. [20]	11 (5 M, 6 F) metformin-treated T2D patients (age: 54 ± 9 y; BMI: 32.9 ± 5 kg/m²; HbA1c: 6.5 ± 0.7%)	Non-randomized crossover	▪ 628 kcal ▪ 68 g C + 55 g P + 16 g fat	1. Ciabatta bread 2. Orange juice 3. Skinless grilled chicken breast 4. Lettuce and tomato salad with low-fat Italian dressing 5. Steamed broccoli with butter	▪ T1 = 1 + 2 (15-min break) 3 + 4 + 5 ▪ T2 = 3 + 4 + 5 (15-min break) 1 + 2	2-h PP blood glucose at multiple timepoints and 2-h AUC 2-h PP serum insulin at multiple timepoints and 2-h AUC	▪ From 30-120 min, glucose was 17-37% lower for T2 than T1 ▪ Serum insulin was 40-50% lower from 60-120 min in T2 than T1 ▪ 2-h glucose AUC was 74% lower with T2 than T1 ▪ 2-h insulin AUC was 49% lower with T2 than T1
Shukla et al. [8]	16 (7 M, 9 F) metformin-treated T2D patients (age: 57.7 ± 7.6 y; BMI: 32.8 ± 3.3 kg/m²; HbA1c: 6.5 ± 0.7%)	Randomized crossover trial	▪ 574 kcal ▪ 64.5 g C + 55.3 g P + 9.6 g fat	1. Ciabatta bread (90 g) 2. Orange juice (120 g) 3. Skinless grilled chicken breast (150 g) 4. Lettuce (45 g), tomato (50 g), cucumber (75 g), and low-fat Italian dressing (15 g)	▪ T1 = 1 + 2 (10-min break) 3 + 4 ▪ T2 = 3 + 4 (10-min break) + 1 + 2 ▪ T3 = half of 1 + 2 + 3 + 4 (10-min break) half of 1 + 2 + 3 + 4	PP blood glucose every 30 min over 3 h and 3-h incremental AUC PP plasma insulin every 30 min over 3 h and 3-h incremental AUC PP plasma GLP-1 every 30 min over 3 h and 3-h incremental AUC	▪ T2 showed lower glucose 3-h AUC than T1 and T3 (173 vs. 372 and 310 mmol/L•min) ▪ T2 had a lower insulin 3-h AUC than T1 (51,074 vs. 67,851 pmol/L•min) ▪ T2 had higher 3-h AUC GLP-1 than T1 (1,057 vs. 764 pmol/L•min) ▪ T3 tended to have 3-h glucose, insulin, and GLP-1 AUCs that were between T1 and T2 ▪ From 30-90 min, T2 had lower glucose and insulin than T1; but, T2 had higher glucose and insulin than T1 at 180 min
Shukla et al. [9]	Same as above	Randomized crossover trial	Same as above	Same as above	Same as above	PP ghrelin % change (from baseline) every 30 min over 3 h and 3-h decremental AUC PP hunger and fullness perceptions every 30 min over 3 h	▪ At 180 min, ghrelin levels remained 11.5% below baseline levels in T2 while they were 4.1% above baseline in T1
Shukla et al. [10]	15 (4 M, 11 F) prediabetes patients (age: 52.4 ± 3.4 y; BMI: 34.2 ± 1.1 kg/m²; HbA1c: 6.0 ± 0.06%)	Randomized crossover trial	▪ 578 kcal ▪ 58.3 g C + 41.0 g P + 20.0 g fat	1. Ciabatta bread (90 g) 2. Skinless grilled chicken breast (100 g) 3. Lettuce (35 g), red bell pepper (110 g), tomato (110 g), cabbage (30 g), olive oil (15 g), balsamic vinegar (8 g), and 0.1 g each of thyme, oregano, and garlic powder	▪ T1 = 1 (10-min break) 2 + 3 ▪ T2 = 2 + 3 (10-min break) 1 ▪ T3 = 3 (10-min break) 1 + 2	PP blood glucose every 30 min over 3 h and 3-h incremental AUC PP plasma insulin every 30 min over 3 h and 3-h incremental AUC	▪ From 30-60 min, T1 showed higher glucose and insulin than T2 and T3 ▪ Glucose was lower for T1 than T2 and T3 from 120-180 min ▪ At 180 min, T1 had lower insulin than T2 ▪ T2 and T3 had 39% and 23% lower 3-h glucose AUCs than T1, respectively ▪ T3 had 44% lower 3-h insulin AUC than T1
Sun et al. [21]	16 (13 M, 3 F) healthy individuals (age: 25.8 ± 4.8 y; BMI: 22.0 ± 2.0 kg/m²)	Randomized crossover trial	▪ 364 kcal ▪ 50 g available C (1.3 g fiber) + 28.1 g P + 3.3 g fat	1. Rice (63 g raw) 2. Skinless chicken breast (100 g) 3. Boiled leafy (xiao bai cai) vegetables (180 g)	▪ T1 = 3 then 1 + 2 ▪ T2 = 2 then 1 + 3 ▪ T3 = 3 then 2 then 1 ▪ T4 = 1 + 2 + 3 ▪ T5 = 1 then 2 + 3	PP blood glucose over 3 h incremental AUCs PP serum insulin over 3 h and incremental AUCs PP plasma incretin (GLP-1, GIP, active ghrelin) over 3 h and incremental AUCs PP hunger, fullness, and desire to eat incremental AUCs Ad libitum lunch several hours after meals	▪ T5 had higher 1-h glucose AUC (135 mmol/L•min) than all other conditions, which ranged from 46-99 mmol/L•min ▪ T4 had higher 1-h glucose AUC (99 mmol/L•min) than T2 (70 mmol/L•min) and T3 (46 mmol/L•min) ▪ T5 showed 31-46% higher 3-h glucose AUC than other conditions, though only the differences with T2 and T4 were significant ▪ T3 had lower 1-h insulin AUC compared to T2, T4, and T5; 3-h insulin AUCs were not different between groups ▪ T4 had lower 2-h GLP-1 AUC than T1, T2, and T3, but only the difference with T3 remained significant for 3-h AUC ▪ T5 had higher 1-h GIP AUC than T1, T3, and T4, but there were no differences in 3-h AUC for GIP

Abbreviations/Acronyms: AUC, area under the curve; BMI, body mass index; C, carbohydrate; F, female; g, gram; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; M, male; P, protein; PP, postprandial; T1D, type 1 diabetes; T2D, type 2 diabetes; y, years old; +, consumed together.
†Additional details of foods eaten and timing of ingestion were taken from Imai et al. [25] review article. ‡The original 2010 article was written primarily in Japanese, but the nutritional information for the meals was presented in English in Table 1 of the 2010 paper. §Occasionally, additional methods of reporting outcomes were present in some studies but not reported here due redundancy and space limitations in the table. ¥Mean glucose levels and % differences in insulin were estimated from figures 1a and 1b in the Imai et al. [22]. ¶There were results from a rice-before-beef condition presented in online supplementary material for this report, but it appeared to be from a separate study sample, and few details on the methods used were available.

Glucose. The most common outcome studied was PPG, which was analyzed in 10 of 11 reports. The lone paper to not include glucose is the aforementioned report (Shukla et al., 2018) that presented ghrelin and perceptual data, which were not included in the original publication of the same experiment (Shukla et al., 2017).

All experiments that assessed PPG found that, in conditions where carbohydrate-rich food was eaten last or in the last portion of the meal, PPG was significantly lower as a function of AUC, peak glucose, and/or glucose at certain timepoints post-feeding. Notably, the majority of studies that evaluated an AUC-type measure of PPG showed significant treatment differences (Imai et al. 2013; Kuwata et al. 2016; Lee et al. 2021; Shukla et al. 2015; Shukla et al. 2017; Shukla et al. 2019; Sun et al., 2020). Likewise, most studies that assessed peak glucose concentrations, time-to-peak glucose concentrations, or incremental peak glucose found significant treatment effects (Imai et al., 2013; Lee et al., 2021; Sukla et al., 2017; Shukla et al., 2019; Sun et al., 2020), though one study did not (Faber et al., 2018).

The lower PPG associated with eating carbohydrate-rich foods last was relatively consistent regardless of the health status of participants. Three studies included "at-risk" samples (T2D, overweight/obesity) alongside healthy controls (Imai et al., 2013; Kuwata et al., 2018; Lee et al., 2021), facilitating more direct comparisons. While two of these studies found significantly lower PPG in both healthy and at-risk samples after eating carbohydrate-rich foods last, Kuwata et al. (2016) only found statistically significant effects for glucose AUC among those with T2D. While the pattern appeared similar in controls, it was less pronounced and statistically insignificant.

Eating carbohydrate-rich foods at the end of a meal consistently lowered PPG, but the magnitude of benefit varied depending on what it was compared against. Generally, reductions in PPG were largest when contrasted against conditions where carbohydrate-rich foods were eaten first, whereas the effects were somewhat attenuated when compared against eating all foods mixed together (Lee et al., 2021; Shukla et al., 2017; Sun et al., 2020). Isolating the differences between eating vegetables and protein-rich foods prior to carbohydrate is problematic from the

available studies, given the lack of consistency in which those foods were ordered in the studies that included both.

Something to consider in all studies, as it relates to postprandial measures, is that the time of measurement was generally capped at 2-3 hours after meals, with the longest going out to 4-h postprandial (Kuwata et al., 2016). In Shukla et al. (2017), eating carbohydrate-rich foods last led to lower PPG from 30-90 min post-ingestion as compared to eating carbohydrate-rich foods first, but by 180 min, PPG was actually lower with the carbohydrate-first pattern. This same pattern (i.e., eating carbohydrate-rich foods first vs. last leads to higher PPG over 30-90 post-ingestion, but lower PPG later on) was also seen among T2D patients, but not controls, in Kuwata et al. (2018). The lack of measurements beyond four hours postprandial somewhat limits conclusions that can be made regarding PPG responses.

Two studies were relatively unique in terms of either population sampled, or the number of feedings applied per condition. Imai et al. (2013) examined PPG responses for three consecutive meals (breakfast, lunch, and dinner) over a day, rather than after a single meal. In both participants with T2D and normal glucose tolerance, eating rice and bread at the beginning of each meal, as compared to the end, led to higher 3-h incremental AUC glucose when averaged across all three meals. However, in the normal tolerance group, there was no effect of eating order on 3-h incremental AUC at breakfast specifically. In another somewhat unique study, because it exclusively included youth type 1 diabetes (T1D) patients, Faber and colleagues found that eating carbohydrate-rich foods last led to lower mean glucose levels and less time spent with glucose at an elevated level, as compared to eating all foods at the same time (Faber et al., 2018). Insulin was administered based on each T1D patient's own insulin-to-carbohydrate ratio, with either multiple dosing of insulin injection therapy or continuous subcutaneous insulin infusion.

Insulin and C-Peptide. Secondary to PPG, PPI was measured most often, and it was included in seven of 11 reports. The pattern for insulin followed a similar one to PPG, in that it tended to be lower in conditions that fed carbohydrate-rich foods last. In fact, all seven reports observed at least one PPI outcome that was lower after eating carbohydrate-rich foods at the end of a meal. In the earliest study that included PPI, eating white rice after a vegetable salad lowered serum insulin by approximately 25-35% after 30-60 min (but not at 120 min) versus eating rice before vegetables (Imai et al., 2010, 2014). Similarly, Shukla and colleagues found in their 2017 experiment that PPI had a lower 3-h AUC in the carbohydrate-last condition compared to a carbohydrate-first condition (Shukla et al., 2017). A similar pattern can be seen in a later study by the same group (Shukla et al., 2019). In terms of AUC measures, which better reflect cumulative insulin load, at least five studies found treatment effects over 2-4 h postprandially (Kuwata et al. 2016; Nishino et al., 2018; Shukla et al., 2015, 2017, 2019).

A pair of studies found lower insulin levels at 3-h postprandial when carbohydrate-rich foods were consumed at the beginning of a meal, as compared to at the end of a meal (Shukla et al., 2017, 2019). Insulin levels rose sharply and peaked at 60-min postprandial when carbohydrate-rich foods were eaten first. In contrast, when carbohydrate was consumed at the end of a meal, insulin rose more gradually and, after peaking between 60-120 min postprandial, remained at that level until 180-min postprandial. The overall incremental AUC was lower in both studies in the carbohydrate-last conditions. Overall, this suggests a spike-then-sharp-drop pattern in insulin when carbohydrate is consumed before other macronutrients, and a more gradual rise and plateauing in insulin when vegetables and/or meat is eaten before carbohydrates.

In what was a unique approach – in that it included a group with untreated T2D and that the postprandial period extended out to 4 hours – one study found that eating fish before rice (as

compared to eating rice before fish) led to lower PPI total AUC for the T2D group along with lower PPG (Kuwata et al., 2016). While it was not statistically significant, insulin AUC among controls was also seemingly lower in the fish-first condition. Additionally, C-peptide was measured, and AUC values were significantly higher with eating rice before fish than fish before rice, in both T2D patients and controls. This further supports the notion that insulin was elevated with the rice-first condition among controls, even if not statistically significantly (Kuwata et al., 2016).

Incretins, Gut Hormones, and Gut Function. Four reports included data on gut incretins and related hormones, including GLP-1, GIP, and ghrelin (Kuwata et al., 2016; Shukla et al., 2017, 2018, Sun et al., 2020), and one reported on gut function, as quantified by gastric emptying time (Kuwata et al., 2016). GLP-1 and GIP are the two primary peptides secreted from L and K cells in the intestines and have many effects, including affecting the paracrine vagus nerve pathway, blood-brain signaling, inhibiting postprandial acid secretion, and decreasing overall gut motility (MacDonald et al., 2002; Seino et al., 2010). Primarily, GLP-1 and GIP target beta cells on the pancreas to encourage insulin secretion (Seino et al., 2010). Ghrelin is a hormone originating in the stomach that targets type 1a growth hormone secretagogue receptor (GHSR1a), which increases appetite (Sun et al., 2020).

Kuwata et al. (2016) found that 4-h AUC incretin levels (GLP-1 and GIP) tended to be higher in a fish-followed-by-rice condition relative to a rice-followed-by-fish condition, although the difference was only statistically significant for GLP-1 in patients with T2D. Eating beef before rice consistently led to higher AUC levels for GLP-1 and GIP than eating rice before fish

in both T2D patients and controls. However, it is difficult to interpret this result, as a full set of methods and results was not available for a rice-before-beef condition.

A second study that observed postprandial changes in GLP-1 specifically found that 3-h incremental AUC was higher in the carbohydrate-last condition than the carbohydrate-first condition (Shukla et al., 2017). The investigators also measured postprandial ghrelin and reported it in a later paper (Shukla et al., 2018); they found that ghrelin levels had returned to baseline at 180-min postprandial in the carbohydrate-first condition, while they were still below baseline in the carbohydrate-last condition.

The most recent study to measure postprandial incretins (GLP-1, GIP) and ghrelin found that 2-h GLP-1 AUC was significantly higher in the three conditions in which rice was eaten at the end of a meal (either alone or with chicken or vegetables), as compared to when all foods were eaten as a mixed meal (Sun et al., 2020). However, the 3-h GLP-1 AUC was only significantly different between two conditions: vegetable-then-chicken-then-rice was higher than the mixed meal. For GIP, AUC over the first hour was significantly higher in the rice-first condition than three other conditions (vegetable-then-chicken-then-rice; mixed-meal; vegetables-then-meat-plus-rice), but no significant differences were found between any conditions for 3-h GIP AUC (Sun et al., 2020). There were no treatment effects observed for active ghrelin levels.

Lastly, gastric emptying time (as quantified by the time required for half of ^{13}C-labelled acetate to leave the stomach) was lower when rice was eaten before fish, relative to eating fish before rice (as well as beef before rice) (Kuwata et al., 2016). In other words, gastric emptying of rice was fastest in the rice-first condition.

Perceptual Responses and Feeding Behavior. Two studies measured perceptual responses to different meal orders (Shukla et al., 2018; Sun et al., 2020). In one of the studies, participants rated hunger and satiety on a visual analog scale, and there were no significant differences between participants based on meal order (Shukla et al., 2018). A second study that included perceptions of fullness, hunger, desire to eat, and prospective consumption (on a visual analog scale) found no significant differences between meal orders (Sun et al., 2020). Of note, this study also used a buffet-style, ad libitum lunch to evaluate whether meal order impacted subsequent food consumption, though there was a lack of specific information on the foods available at the lunch and the exact amount of time between the experimental test meals and the buffet lunch (Sun et al., 2020). There were no significant differences between conditions in food consumption at the buffet lunch.

Certainty of Evidence

A summary of the GRADE assessment findings, organized by various outcomes, is presented in **Table 2**. In brief, the evidence that PPG and PPI were lowered by eating carbohydrate-rich foods last was rated as moderate. Evidence for GLP-1 was rated as low, while evidence for GIP, ghrelin, and eating-related perceptions was very low.

Table 2. Grade Assessments

Outcome	Effect	Number of Participants (Trials)	Certainty of Evidence
PPG	10 of 10 trials found that, in conditions where carbohydrate-rich foods were eaten last or in the last portion of the meal, PPG was lower as a function of AUC, peak glucose, and/or glucose at some timepoints (particularly within 90 minutes of feeding)	194 (10)	⨁⨁⨁◯†
PPI	7 of 7 trials observed at least one PPI outcome that was lower after eating carbohydrate-rich foods at the end of a meal.	103 (7)	⨁⨁⨁◯†
GLP-1	GLP-1, as measured by AUC postprandially, tended to be higher with carbohydrate-last eating conditions in 3 of 3 trials.	54 (3)	⨁⨁◯◯†‡
GIP	Inconsistent effects were observed for time-course changes.	38 (2)	⨁◯◯◯†‡¥
Ghrelin	1 trial showed no effect of food order, while 1 showed that eating carbohydrate-rich foods last led to a greater suppression after 3 h.	32 (2)	⨁◯◯◯†‡¥
Eating-related perceptions (hunger, fullness/satiety, etc.)	No effects of food order were observed.	32 (2)	⨁◯◯◯†‡¥

⨁⨁⨁⨁ = high certainty; ⨁⨁⨁◯ = moderate certainty; ⨁⨁◯◯ = low certainty; ⨁◯◯◯ = very low certainty. †Downgraded one level due to imprecision. ‡Downgraded one level due to methodological limitations. ¥Downgraded one level due to inconsistency. AUC, area under the curve; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; PPG, postprandial glucose; PPI, postprandial insulin.

Discussion

There are a few key takeaways from this systematic review. Primarily, consuming carbohydrates last in meal order seems to consistently have a positive effect (lower value) on PPG and PPI, especially when contrasted against beginning a meal with carbohydrate-rich foods. Among studies that evaluated AUC glucose and insulin concentrations specifically, most found that eating carbohydrate toward the end of a meal improved glycemic regulation. Given the use of glucose AUC in the clinical management of diabetes (Sakaguchi et al., 2016), the results of our review suggest that shifting from a carbohydrate-first to a carbohydrate-last eating pattern

can be utilized to help manage T2D and perhaps other cardiometabolic diseases, though this is speculative and warrants confirmation from longer term trials (e.g., >12 weeks) of food-order-based interventions.

Secondly, significant relationships between food order, postprandial hormone secretion, and satiety are largely absent or inconsistent, and the evidence base for these outcomes is largely very low in quality. One possible exception is that a carbohydrate-last eating pattern may lead to higher AUC GLP-1 levels, which could be one mechanism by which food order modulates the PPG response (Edwards et al., 1999). However, no clear effects on hunger, fullness, desire to eat, and subsequent feeding behavior were found, though it is important to note that most of the 11 reports did not include those outcomes. So, while meal sequence appears to be a potentially useful tool for managing aberrations in glucose metabolism, there is poor evidence that it is a means to control hunger at this point.

Notably, the focus of this review was on acute feeding studies. However, a small number of trials have attempted to apply ordered eating principles using longer exposure timeframes. Tricò et al. (2016), for example, included 50-75-year-old T2D patients treated with metformin and/or sitagliptin. In the experimental group, participants were instructed to consume their meals with a carbohydrate-last emphasis while the control group was told to consume similar meals but without instruction regarding meal order. All dietary plans were meant to induce a 200-kcal daily deficit and implemented over the course of eight weeks. It can be inferred that participants followed the energy prescriptions reasonably well as there were not significant weight differences between groups at baseline or at the eight week follow up at the end of the intervention (MacDonald et al., 2002). As compared to baseline, participants in the experimental group had significantly lower hemoglobin A1c, fasting plasma glucose, and PPG excursions after

eight weeks, while the control group did not experience statistically significant changes in these outcomes (MacDonald et al., 2002). However, the statistical analyses that incorporated between-group comparisons were less impressive, only finding time x group interactions for post-lunch glucose excursions and for the coefficient of variation of glucose concentrations. This trial, to some degree, validates the carbohydrate-last method of meal consumption as a means of managing T2D chronically, but the small sample size (n=20) and lack of between-group differences for most outcomes limits the strength of evidence.

Another study, which was originally reported in Japanese (Seino et al., 2010) but later covered in an English-language review paper (Imai et al., 2014) included 333 individuals with T2D who received either education to consume carbohydrate-rich foods after vegetables and meat/protein or general education about lifestyle and diabetes risk. After 2.5 years, hemoglobin A1c declined more in the food order education group (-1.1% vs. -0.1%), and HDL was also higher. Further, overall energy consumption, including from carbohydrate, decreased over the same time period in the food order education group, while vegetable intake also increased (Imai et al., 2014). Consequently, it is somewhat challenging to say what specific role food order played (vs. energy and macronutrient changes) in the observed changes. Still, when combined with the results of the present systematic review of acute studies, we can infer there are likely some long-term benefits of a carbohydrate-last meal order to long-term glycemic control in T2D.

A contextual factor that is important to consider is that measures tended to stop at either 2-h or 3-h postprandial, with the exception of one that went out as far as 4-h postprandial (Kuwata et al., 2016). Experiments providing protein and/or fat pre-loads before carbohydrate have shown delays in both gastric emptying and absorption of glucose, which, in some cases, can result in a later peak in PPG and prolong the elevation (Sakaguchi et al., 2016). However, these

effects likely depend on several factors, including the quantity and type of protein and fat consumed. For example, pre-loading with refined/processed proteins like whey and soy does not seem to significantly delay the glucose peak (Edwards et al., 1999; Ma et al., 2009), whereas meat and oil do seem to have some delaying effects (Kuwata et al., 2016; Sakaguchi et al., 2016). For protein, solid food sources like meat could delay the glucose peak through effects on gastric emptying, as prior research has shown that larger food particles empty slower than smaller particles (Imai et al., 2010; Tricò et al., 2016). Ultimately, the fact that most experiments used a 2-3-h postprandial period may bias the AUC data to being higher in conditions where carbohydrate-rich foods were eaten first. It is possible, albeit speculative, that the differences in AUC may be less pronounced if longer postprandial measurements are used.

Although the findings of this review support that idea that eating carbohydrates last may positively impact glycemic measures, there were no clear effects on perceptual responses among the minority of studies that measured those outcomes. One potential explanation for this is simply the subjective nature of those phenomenon. Hunger and satiety are subjective experiences, so one person's perception of hunger may be vastly different from another's. A second possible explanation is simply owing to small sample sizes; both studies that measured perceptual responses had 16 participants (Shukla et al., 2018; Sun et al., 2020). Although moderate-to-large effects on postprandial hunger can be detected with small, paired samples (<20 participants), ensuring there is sufficient statistical power to detect small effects generally requires more than 20 participants (Gentilcore et al., 2006).

Limitations

Studying the health impacts of the order of what we eat, rather than just what we eat, is relatively young, and the earliest included study in this review, which was published in 2010, reflects that (Imai et al., 2010). Consequently, the number of available reports to review was modest. Why an intervention of this nature has not caught on more prevalently globally is worthy of speculation. T2D and chronic disease are significant health concerns worldwide in general and the United States more specifically. It is possible that this sort of intervention has not gained significant traction, in part, due to the lack of understood mechanistic theory behind it. The lack of chronic trials on this topic is particularly noteworthy, because it limits what can be concluded about the effectiveness of manipulating food order on clinical management of diabetes and related disorders. Long-term interventions are somewhat cost-prohibitive to run and require significant amounts of time to produce meaningful results, which may also explain the paucity of data.

Even though the studies included in this review largely used robust designs, there are shortcomings across the board that need to be taken into consideration. In general, the total number of participants in each study was not high (eight of 11 used a total sample of less than 20), and the outcome measurements were not completely consistent, which is not surprising given there is no established protocol for this line of inquiry. Further, in cases where control groups were implemented and compared to a diseased or at-risk population (T2D, overnight/obese), controls did not accurately match the opposite group. For example, in one study, 10 healthy males with an average age of 38.4 years were used to control for 9 males and 3 females with an average age of 59.7 years (Kuwata et al., 2016). In general, control participants

tended to be younger and have lower BMIs among studies that included at-risk and control groups.

Additional to the issues with sample size and control discrepancies is the fact that most of these studies included east Asian populations, and as such, are representative of those largely homogenous populations. That is not an issue in itself, but it does call into question the generalizability of these findings to other racial and ethnic groups. Of note, dysglycemia and other metabolic aberrations tend to occur at lower BMI values and younger ages in east Asian populations (Kashima et al., 2016), which may indeed indicate to some degree why such an intervention could be perceived as more valuable in those populations.

Another limitation of the included studies has to do with the types and quantities of foods that were used. The most frequent sources of carbohydrate (white rice, bread, and orange juice) are relatively low in fiber. The rapid rise in PPG that can occur when these foods are eaten first may not completely reflect the responses to other carbohydrate-rich foods that are higher in fiber, like legumes and berries. In addition, the meals were generally moderate in caloric content (<700 kcal), and it remains less clear whether the observed responses should be expected with large meals.

Beyond the limitations of the identified studies, the systemic review itself has limitations. Only acute studies were examined, meaning inferences to long-term outcomes are somewhat hampered. In addition, given that there is no standard phrasing or terminology used to describe the concept of food order, it is possible that relevant experiments were missed in the search process. However, strategies (reference list searching and communicating with an experienced researcher in the field) were used to minimize this possibility.

Conclusions

Despite the aforementioned limitations, the included studies used relatively robust protocols and showed consistent results with respect to PPG and PPI. Therefore, moderate certainty evidence exists that changing the order of eating can significantly impact measures of glycemic regulation that are indicative of overall health status. More specifically, a carbohydrate-last approach to eating tends to appreciably lower PPG and PPI, especially when compared to eating carbohydrate-rich foods at the beginning of a meal. Multiple studies also showed that GLP-1 AUC was higher when carbohydrate was eaten at the end of a meal. In addition, one trial showed a reduction in ghrelin with a carbohydrate-last eating pattern as compared to a carbohydrate-first pattern. However, these responses in incretins and gut hormones come from an evidence base that is low-to-very-low quality, indicating a clear need for additional research. Likewise, the evidence related to perceptions of hunger, appetite, satiety, etc. was of very low quality, and no benefit of food order was observed. Thus, although switching from eating carbohydrates from first to last might improve glycemic measures over the long term, it remains unclear whether this type of dietary change, on its own, would facilitate weight loss.

In the future, researchers should consider sampling larger and more heterogenous populations when possible. Further, there should be a focus on extending the postprandial measurement to at least four hours. Still, with what we can infer from the presented studies and literature at large, practitioners could consider advising patients, specifically those with prediabetes or T2D, to consume carbohydrates last in their meals in order to lower blood sugar elevations and spikes.

CHAPTER V

THE EFFECT OF ORDERED EATING ON BLOOD GLUCOSE, PERCEPTUAL RESPONSES, AND SUBSTRATE UTILIZATION WITH A STEADY-STATE EXERCISE BOUT

Introduction

The concept of ordered eating and its likely effects on postprandial glucose (PPG) are not necessarily well studied, but there does seem to be a common consensus that the position of a carbohydrate-dense food in a meal sequence significantly affects PPG. More specifically, carbohydrate-dense foods placed last in meal sequence tends lower peak PPG (Faber et al., 2018; Imai et al., 2013; 2014; Shukla et al, 2015; 2017; 2018; 2019), which may have beneficial long-term effects as it relates to chronic diseases such as heart disease, type 2 diabetes mellitus (T2DM), and other diseases. As a specific example of its potential as an intervention, Imai and colleagues performed a randomized controlled trial in 101 patients with T2DM and assigned them to either a vegetable-before-carbohydrate intervention (n = 69) or exchange-based meal plan (n = 32). Participants were followed for 24 months (Imai et al., 2010). Hemoglobin A1c (HbA1c) improved in both groups, but HbA1c levels were significantly lower in the vegetable-before-carbohydrate group compared to the exchange-based meal plan group at 6-, 9-, 12-, and 24-months follow-up.

Despite the growing body of research on ordered eating, some specific shortfalls of the existing literature are apparent, including a systematic sampling bias towards Asian participants, type 1 diabetes mellitus (T1DM), and T2DM patients. Beyond that, there is minimal inclusion of incurred perceptual responses. Of note, during a 2017 study that included 16 participants (9 F, 7

M) that had T2DM, Shukla and colleagues did include some perceptual data and found no significant differences in responses relating to hunger and fullness but did find some marginal differences in ghrelin levels based on meal sequence (Shukla et al., 2017). Potentially more importantly, there is no existing literature surrounding the effects of ordered eating on physiological or perceptual responses to exercise.

As it relates to substrate utilization during exercise, manipulating the glycemic index (GI) of feedings prior to exercise has been an active area of research for several decades (Ormsbee et al., 2014). In one example crossover study that included 10 trained cyclists completing three exercise-to-exhaustion trials, researchers at San Jose State University compared a high-GI pre-exercise meal to a low-GI pre-exercise meal and a control condition (DeMarco et al., 1999). The research team found that in the low-GI pre-exercise meal condition, respiratory exchange ratio (RER) was significantly lower out to 120 minutes when measured at 20-minute intervals. This indicates that out to 120 minutes, fat utilization was significantly higher following the low-GI pre-exercise meal. In line with these findings, a review by Ormsbee et al. (2014) reported that multiple studies have found increased fat oxidation and lower reliance on carbohydrate with lower-GI meals, which could theoretically translate to sparing of muscle glycogen stores.

The purpose of this study, therefore, was multi-faceted. Initially, we, in part, set out to determine how much of an effect ordered eating would have on a healthy individual's PPG. We hypothesized that there would be a significantly lower peak PPG in the carbohydrate-last (referred to as rice last (RL) going forward) sequence compared to a carbohydrate-first (rice first (RF)) meal sequence. Further, we hypothesized that there would be a larger delta change in peak-nadir glucose in the RF sequence. More novelly, this study sought to explore what effects ordered eating may have on carbohydrate and fat utilization at rest and during exercise. We

hypothesized that carbohydrate utilization would be higher, and fat oxidation would be lower, at rest and during exercise in the RF condition. Lastly, we set out to determine if there was some effect of meal sequence on perceptual responses. In this regard, we hypothesized that the RF condition would result in higher perceptions of satiety based on data showing that there is an association between higher insulin levels and levels of satiety in healthy weight populations (Flint et al., 2007).

Methods

General Design

Similar to experimental studies that have been conducted previously in the area of ordered eating, our laboratory-based project included a standardized meal that each participant consumed with the foods eaten in a prescribed order. In one condition, the carbohydrate-dense food was consumed first (RF), and in the opposite condition, the carbohydrate-dense food was consumed last (RL). Dissimilar to previous research, and unique to this project, following consumption of the prescribed meal, participants completed an acute exercise bout during which their respiratory gaseous exchange was measured as well as their exertion and blood glucose, among other outcomes. The laboratory-based intervention followed a randomized crossover design. Blinding either the participants or the research team in this trial was not practical.

Participants went through three separate laboratory visits at the Old Dominion University Human Performance Laboratory (HPL). Participants also had the option of completing the first visit virtually, to include only the consent process and reviewing instructions for the subsequent visits. Visit two and visit three followed mirrored protocols that included a pre-exercise meal, but the order by which specific components of the meal were consumed was randomized. In a

similar fashion to existing literature (Thomas et al., 1994), an exercise bout occurred 60 minutes after meal completion. In addition to the standardized meal and exercise bout, during visit two, participants had their body composition assessed in addition to relevant anthropometrics. Visits two and three began prior to 12 p.m. to help accommodate for fasting and standardization.

The protocol for this project was submitted to the Old Dominion University Institutional Review Board for approval prior to beginning. Additionally, the trial was pre-registered prior to participant enrollment using clinicaltrials.gov—pre-registration ID number: NCT06242015.

Recruitment and Participants

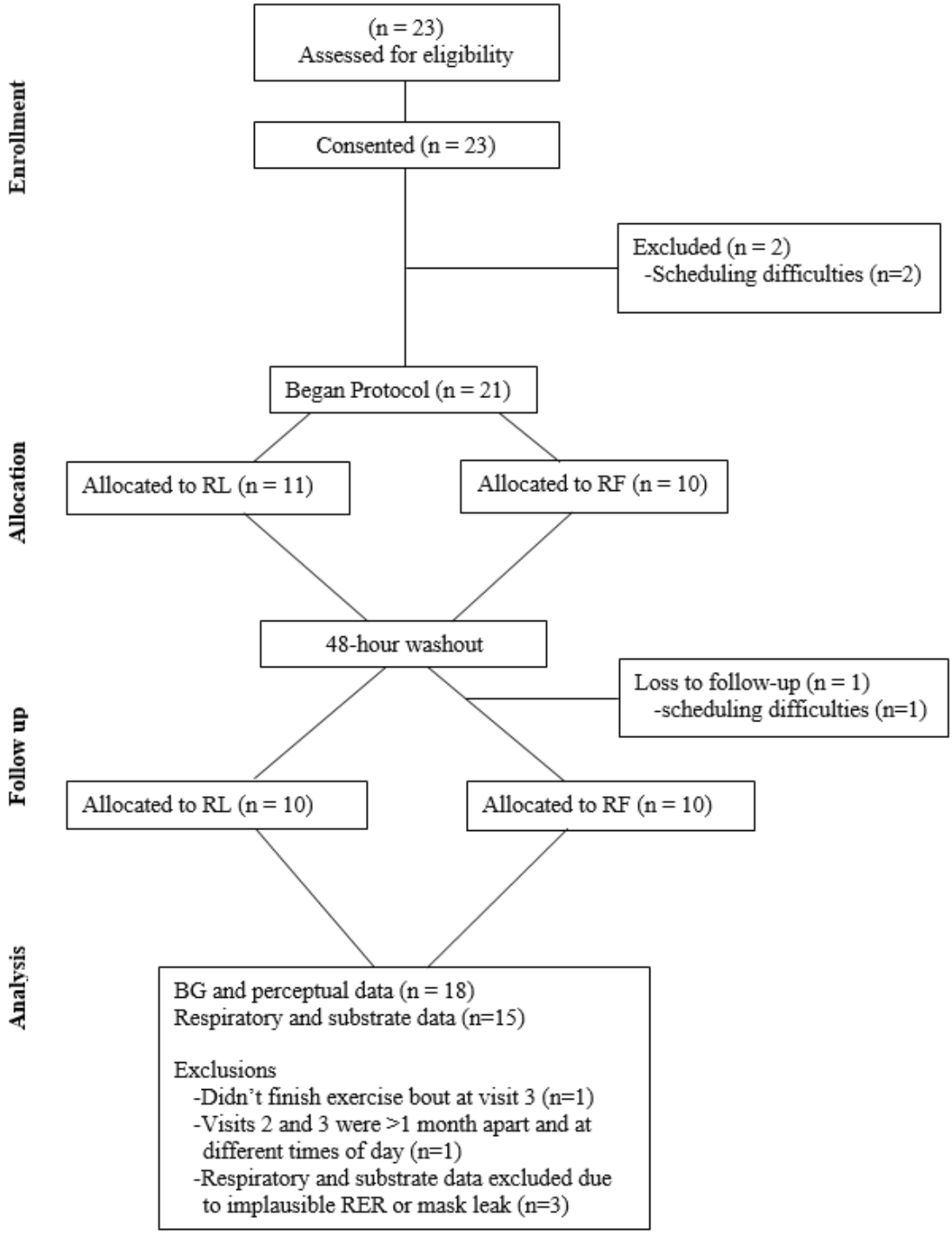
Initial recruitment took the form of speaking to classrooms of students and sending the study flyer to local gyms, sports clubs, etc. Word of mouth within the investigators' networks was also used, and participants who enrolled in the study were asked to share the flyer with individuals they knew who might be interested in participating. Individuals contacting the HPL about testing services were also told about the study.

Inclusionary criteria were: 1) physically active, defined by the American College of Sports Medicine (ACSM) as participating in scheduled exercise at least three days per week for 30 minutes each time over the last three months, 2) ability to perform moderate-to-high intensity running for at least 30 minutes, 3) free from any allergy or other condition that would prohibit the consumption of poultry, rice, or broccoli, 4) no injury or disease (cardiovascular disease, diabetes, pulmonary disease except controlled asthma) precluding physical exercise, 5) not currently pregnant, 6) 18-60 years of age, and 7) no implanted electrical devices such as a pacemaker.

A total of 23 individuals were screened for eligibility, with all 23 consenting and enrolling in the study (**Figure 1**). Five participants did not complete the protocol as designed. Among those, three participants did not complete all visits, due to personal scheduling conflicts that did not allow for timely collection of data. Two participants attended all three visits but were excluded from the analysis: one individual was unable to complete the exercise portion of visit three due to acute leg discomfort, and one completed visits two and three >1 month apart and their third visit was not started in the morning. Eighteen individuals completed the study and were included in the analysis for blood glucose and perceptual variables. Three participants' respiratory exchange and substrate use data were omitted from the analysis, so a smaller number (n = 15) had complete data for those outcomes. In one of those cases, there were implausible RER values (i.e. > 1.1) during the RF condition. In the remaining two cases, VO_2 values between conditions were significantly different (>15%), which indicated evidence of mask leaks.

No formal a priori power analysis was conducted to determine an adequate sample size. Previous research employing a crossover design to examine the impact of ordered eating on postprandial resting measures have utilized samples of less than 20 in most cases (Kuwata et al., 2016; Shukla et al., 2015, 2017; 2018; 2019; 2020).

Figure 5. Enrollment and Follow-up.



Visit Two

Participants were instructed to avoid prolonged (i.e., >60 min) or vigorous exercise for at least 24 hours prior to coming into the HPL. If they chose to exercise in the 48-hour window leading up to visit two, they were asked to replicate that exercise in terms of duration, time, intensity, modality, and volume in the leadup to visit three. Physical activity was tracked by having participants wear an Actigraph GT3X-BT (Actigraph, Pensacola, FL) at the waist for the two days prior to the visit. Additionally, they arrived at the HPL fasted for at least eight hours to include avoidance of all food, energy-containing beverages, caffeine, other stimulants, and recreational drugs. They were allowed to consume water during this period. In addition, participants were asked to drink 20 ounces of water at home, on the morning of the visit, before coming to the laboratory, which helped to ensure adequate hydration status upon arrival. Lastly, they were asked to keep a food record for two days prior to the visit and asked to duplicate their food and beverage intake as best they could prior to visit three.

Upon arrival, urine specific gravity (USG) was measured using a urine refractometer (PAL-10S, Atago, Japan) to assess hydration status. Initial body anthropometrics were measured and included height, mass, and body composition. Body composition was assessed using the InBody 770 (InBody Co., LTD; Seoul, South Korea), and assessed data included body mass and body fat percentage.

The participant was then familiarized with specific perceptual scales and provided baseline ratings. The scales included rate of perceived exertion (RPE) measured by the original Borg Scale (Borg, 1982) and a Feeling Scale (FS) that ranked how they were feeling from “-5” (very bad) to “+5” (very good) (Hardy & Rejeski, 1989). Seven GI symptoms were rated on a 0-10 scale where 0 means “no discomfort,” 5 means “moderate discomfort,” and 10 means

"unbearable discomfort" (Wilson, 2017). Symptoms included nausea, regurgitation/reflux, bloating, abdominal cramps, side stitch, gas/flatulence, and urge to defecate. Participants were provided with definitions of each symptom. In addition, hunger, satiety, fullness, and appetite were measured using a similar 0-10 Likert scale (0=none, 10=extremely) after participants were provided definitions of each phenomenon. Likert scales have demonstrated good agreement with visual analog scales when rating these types of perceptual sensations (Holliday et al., 2021).

Heart rate (HR) was monitored using a Polar H10 heart rate monitor (Polar Electro, Inc.; Bethpage, NY). After allowing the participant to rest in a seated position for five minutes, HR was recorded three times over the course of one minute (baseline, 30 seconds, and 60 seconds).

Next, baseline resting gas exchange measurements were collected using a TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT). The metabolic cart's flow and gas sensors were calibrated with a 3-liter syringe and calibration gas (O_2, 16.0%; CO_2, 4.0%) prior to baseline measurements being taken. A slight adjustment to the calibration procedures was made after the first four participants completed the study. Specifically, additional gas sensor calibrations were completed after meal consumption and within 5 minutes of the start of exercise. This change was made to reduce any small potential impacts of drift in the CO_2 sensor over time. After applying a silicone rubber facemask connected to a two-way non-rebreathing value, 10 minutes of volume of oxygen consumption (VO_2) and volume of carbon dioxide production (VCO_2) were collected with the participant seated in a chair. Frayn's equations (carbohydrate = $4.55VCO_2 – 3.21VO_2 – 2.87n$ and fat = $1.67VO_2 – 1.67VCO_2 – 1.92n$) were used to calculate estimated rates (g/min) of carbohydrate and fat utilization at rest (Frayn, 1983), with protein oxidation assumed to be negligible. Respiratory exchange ratio (RER) was also calculated.

Thereafter, fasting blood glucose was measured using the FreeStyle Lite Blood Glucose Monitor System (Abbott Diabetes Care Inc., Alameda, CA, USA) due to its relatively high accuracy when assessing glucose compared to the YSI 2300 Stat Plus, a reference method (Tack et al., 2012).

A website (https://www.sealedenvelope.com/simple-randomiser/v1/lists) was used to generate a randomization list, which was used to assign a particular meal order to each participant (of the two available meal orders). The participant either ate chicken (100 g, Tyson Grilled and Ready Chicken Breast Strips) and broccoli (150 g, Harris Teeter Broccoli Florets) together followed by eating rice (150 g, microwavable Ben's Original Jasmine Rice), or the reversed order. Masses of chicken and broccoli were measured out in a frozen state before cooking, while the rice was measured after cooking. The masses of food used were similar to protocols laid out in previous studies (Imai et al., 2014; Shukla et al., 2017). The participant was asked to consume all food within 15 minutes to include 7.5 minutes per phase. If they did not consume all of the food in the allotted time, the remaining balance was measured on a food scale (i500 Balance, My Weigh, Pheonix, AZ), and those adjusted masses were provided to the participant during visit three. Participants were allowed to drink water ad libitum during the meal, and water consumed was measured. During the third visit, participants were required to consume the same amount of water.

After the meal was ingested, a 5-10-minute period was allowed for the participant to use the restroom (if needed) and to refit the facemask before gas exchange was collected again. Resting gas exchange was collected and analyzed for a 45-minute period, beginning at 10 minutes postprandial (PP) until 55 minutes PP. Participants remained seated throughout this period. Blood glucose was assessed at 30 minutes PP, at 59 minutes PP (exercise began at 60

minutes PP), immediately after the conclusion of exercise (approximately 90 minutes PP), and at the conclusion of the visit (120 minutes PP). Perceptual data were collected at specified intervals (see **Figure 6**).

Exercise began 60 minutes after the meal was finished and was carried out on an H/P Cosmos T170 DE SPORT MED Treadmill (Nussdorf-Traunstein, Germany). Duration of exercise was set at 30 minutes. Considering that this is the first known study of its kind that incorporated ordered eating and comparing substrate utilization under those manipulated conditions, it was deemed justifiable to use a relatively short exercise duration, especially since it is a duration that most recreationally active people can complete without becoming too fatigued. Exercise intensity was determined as a percentage of estimated maximum HR. We used the Tanaka estimation (HR_{max} = 208-0.7 x age; Tanaka et al., 2001) as opposed to the Fox estimation, as the Fox estimation tends to underestimate maximum HR in men whereas both seem to equally overestimate in women (Nikolaidis et al., 2018). Exercise was conducted at 70% HR_{max}. Treadmill speed started at 5 mph for the first minute, and adjustments were made every 1-2 minutes thereafter as necessary to find the correct speed to reach the target HR. Once the target heart was achieved (within ±4 bpm), the speed was maintained for the remainder of the protocol. The same speed adjustments and final speed were utilized during visit three. Respiratory exchange data were collected for the duration of the exercise bout. Equations from Jeukendrup and Wallis (2005) were used to estimate rates of carbohydrate and fat oxidation during exercise.

Fat oxidation: $(1.695 \times VO_2) - (1.701 \times VCO_2)$

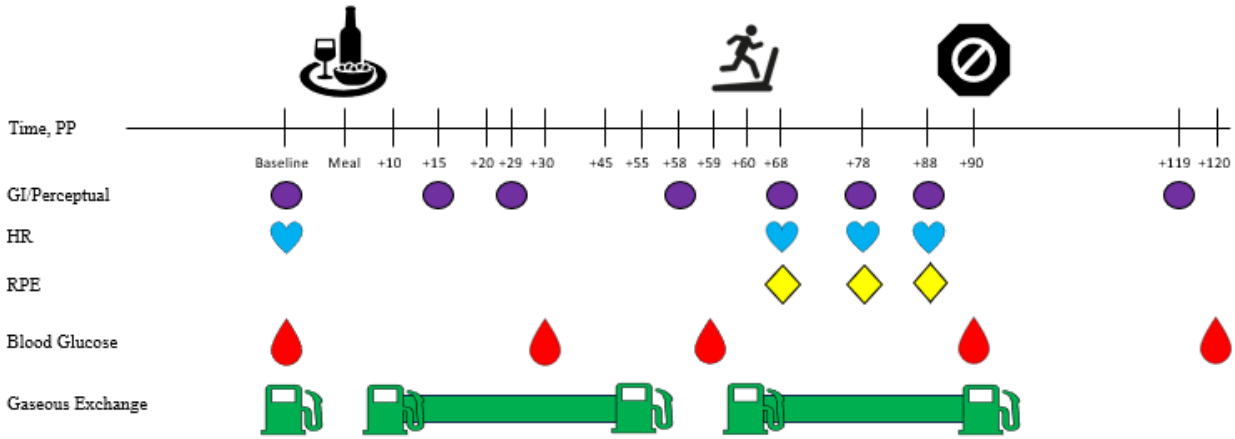
Carbohydrate oxidation: $(4.21 \times VCO_2) - (2.962 \times VO_2)$

Perceptual data were collected again at 8, 18, and 28 minutes after the onset of exercise (i.e., 68, 78, and 88 minutes PP). Over one-minute periods from 9-10, 19-20, and 29-30 minutes during exercise, HR was collected at 30-second intervals and averaged. Blood glucose measurement was also repeated at the conclusion of exercise.

Following the exercise bout, participants rested for 30 minutes. During this time, respiratory exchange measurements were not recorded, but perceptual and blood glucose measurements were taken 29 and 30 minutes after the conclusion of exercise (119 and 120 minutes PP), respectively. Participants were also offered water, and the amount consumed was measured and duplicated in visit three.

A summary of the study protocol and measurements is shown in **Figure 6.**

Figure 6. Order of Events for Laboratory Visits 2 and 3.



**Time bars are not drawn to scale*

Visit Three

Visit three, as well as the pre-visit procedures leading up to visit three, followed essentially the same protocol as visit two with the major exception of the changed meal order. Whatever the meal order was in visit two (either RF or RL), it was reversed in visit three.

Participants were given the same quantities of food and water they consumed during visit two and were asked to consume the entire amount of each. If all the provided food was not consumed, the remaining balance was measured.

There was a minimum of 48 hours between visits two and three for all participants. Although menstrual cycle status and oral contraceptive use have been reported to modestly impact substrate use in some individual studies, a recent meta-analysis of 25 studies (483 participants) found no significant effect of menstrual cycle phase (standardize mean difference = 0.05, 95% confidence interval = -0.15 to 0.25) (D'Souza et al., 2023). The authors of the article ultimately concluded that there is likely no need to control for menstrual cycle phase so long as other important factors (exercise duration and intensity, nutritional status, etc.) are controlled for. A motivation for avoiding menstrual cycle control, beyond the marginal effect it may have, is to avoid inherent disenfranchisement of female participants by including a higher barrier to participation. In a 2014 analysis, authors conducted a search to include three years of studies published in the following three journals: *British Journal of Sports Medicine, Medicine and Science in Sports and Exercise,* and *American Journal of Sports Medicine* (Costello, et al., 2014). Within the 1,382 articles included, 6,076,580 participants were totaled. Of those, only 2,366,968–roughly 39%–were female (Costello, et al., 2014). Further, the authors found that females were under-represented across all three included journals (Costello et al., 2014). As such, it is important to be aware of such biases and attempt to correct them where applicable.

Data Processing

Blood glucose change values were calculated as peak-nadir, PP peak-nadir, and delta exercise change from pre-to-post exercise. Peak-nadir was calculated as the maximum measured

blood glucose value minus the minimum measured blood glucose value and included baseline while PP peak-nadir was the same but did not include baseline values. Delta exercise change was calculated as the blood glucose value measured at 59 minutes (just prior to exercise onset) minus blood glucose measured at 90 minutes (at exercise cessation).

Respiratory exchange data were exported from the Parvo software using 30-second averages. Because the data were collected for a relatively long duration at each visit (~90 minutes total), they needed to be averaged into smaller blocks of time. Five-minute blocks of time were used, including the following: the last 5 minutes of the 10-min baseline period; nine blocks during PP rest (10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, and 50-55 minutes); and six blocks during exercise (0-5, 5-10, 10-15, 15-20, 20-25, and 25-30 minutes).

Actigraph accelerometer data were downloaded directly from the accelerometer devices and processed with ActiLife version 6.13.5 (Actigraph, Pensacola, FL). Wear time was validated through the software using the algorithm of Choi and colleagues (Choi et al., 2011). Scoring for different intensities of activity was performed using the methods described by Troiano et al. (2008) and utilized the following counts-per-minute thresholds (light, 100-2019; moderate, 2020-5998; vigorous, ≥5999)

Area under the curve (AUC) values for hunger, appetite, satiety, and fullness were calculated with Microsoft Excel using the trapezoid method (Matthews et al., 1990). Essentially, the segments between timepoints were considered different trapezoids on a graph, and the area of each trapezoid was calculated and summed to determine AUC.

Because gastrointestinal symptoms were highly right skewed with many 0 values, all symptom values at each time point were summed and added together to reach a total sum score for each variable.

Dietary information from food logs was quantified, including total energy consumption, carbohydrate content, fat content, protein content, and fiber content. Although participants were asked to complete the logs for two days before each visit in order to enhance matching of intake between visits, only intake from the day before each visit was analyzed. Each value was estimated based on individual participant reporting in their provided food logs. If specific brand information was either not provided by the participant or unavailable directly from the manufacturer website, these values were estimated using a separate online tool (Cronomter (https://cronometer.com)) or taken from distributor websites such as Walmart.

There was a small amount of missing perceptual data due three participants unintentionally failing to write down some values on the provided recording sheet. Two participants did not report information for any of the perceptual variables at the final timepoint (minute 119 PP) for one condition, and one participant did not report FS values at three timepoints (15-, 29-, and 45-minutes PP) for one condition. In the cases of missing data at minute 119, the last observation was carried forward to fill in the missing data. In the case of the missing feeling scale data, linear interpolation was used to fill in the missing data.

Before any statistical analyses were undertaken, values for perceptual, blood glucose, metabolic/substrate, and Actigraph data were checked via a double entry method. Specifically, two of the authors (BKF and PBW) independently transferred all the data to Microsoft Excel. Differences were noted by examining average condition values for each variable, and any differences between the two authors' entries were corrected by re-confirming with the original data.

Statistical Analysis

The analysis of the outcome data was conducted as follows using SPSS version 29 (IBM, Armonk, NY, USA). Visual inspection of histograms and Q-Q plots was used to determine normality of data distribution. Normally distributed data are presented as means and standard deviations, with skewed data presented as median (25^{th}-75^{th} percentile).

A two-way ANOVA with time and condition as within-subject factors was conducted to compare the RL and RF conditions as it relates to PPG, FS, and RPE. Two-way ANOVAs for gas exchange (i.e., VO_2) and substrate oxidation were broken up into two separate analyses, one that included baseline and PP resting data, with the other including exercise data separately. This allowed us to evaluate whether there were any exercise-specific effects separate from the resting PP period. In the event there were any time effects with no interaction effect, pairwise comparisons with a Bonferroni correction were applied. With significant interaction effects, between-condition effects at each timepoint were explored using paired t-tests with Bonferroni adjustments for multiple comparisons.

Paired t-tests were also used for comparing blood glucose change/delta values, AUCs for hunger, appetite, satiety, and fullness, and Actigraph data. Some variables did have a non-normal distribution, so square-root and natural-log transformations were used to achieve normal distribution. Hunger and appetite AUC values were transformed via natural log. Because some participants answered "0" for hunger and appetite throughout the visit, a trivial value of 1 was added to each AUC in order to calculate natural log. Moderate-to-vigorous physical activity (MVPA) was modestly right-skewed, and square-root transformation was best-suited for making the data normal. Gastrointestinal-symptom sum scores were compared using a Wilcoxon signed-rank test. A value of $<.05$ was considered significant.

Results

Sample Descriptives

Total number in the sample was n = 18 (8 male, 10 female). Average age was 37.1 ± 10.3 years with the oldest participant at 56 years old and the youngest at 21. Height (cm), mass (kg), and percent body fat (%) were M = 170.3, SD = 10.7; M = 73.0, SD = 15.9; and M = 22.6, SD = 7.2 respectively. Interestingly the sample did include a diverse group; 10 white, 2 black, 1 Hispanic/Latino, 2 Asian, and 3 mixed race/ethnicity (2 white-Hispanic, and 1 white-black).

With respect to the two meal phases/components, median (25^{th}-75^{th} percentile) amounts of broccoli and chicken consumed were 250 (250-250) g and 250 (244-250) g for the RL and RF conditions, respectively. Rice amounts consumed were 150 (150-150) g in both conditions.

USG was measured as 1.013 ± 0.008 prior to RL and 1.012 ± 0.007 prior to RF. Water consumed during the meal phase of the RL condition was 247 ± 131 g on average in both conditions. Post exercise, water consumed was measured as 297 ± 85 g in both conditions.

Amounts of physical activity were similar between conditions two days prior to the visits. On the day prior to the visits, MVPA (square-root transformed) was significantly higher (t = 3.8, p = 0.001) in the RL condition. Physical activity data are presented in **Table 3**, along with results of the t-tests in **Table 4**. MVPA total differences were quite small—on the order of 15 minutes.

Table 3. Actigraph Activity Levels

Activity Level	Condition	Days Before Visit	*N*	*M*	*SD*
Sedentary	RL	2	16	467.8	223.7
Sedentary	RF	2	16	462.8	175.2
Sedentary	RL	1	18	493.5	271.6
Sedentary	RF	1	18	537.3	281.8
Light	RL	2	16	213.6	93.5
Light	RF	2	16	238.5	101.9
Light	RL	1	18	235.4	120.6
Light	RF	1	18	235.4	109.9
SQRT MVPA	RL	2	18	5.6	3.3
SQRT MVPA	RF	2	18	5.8	3.4
SQRT MVPA	RL	1	18	6.5	2.5
SQRT MVPA	RF	1	18	4.5	3.1

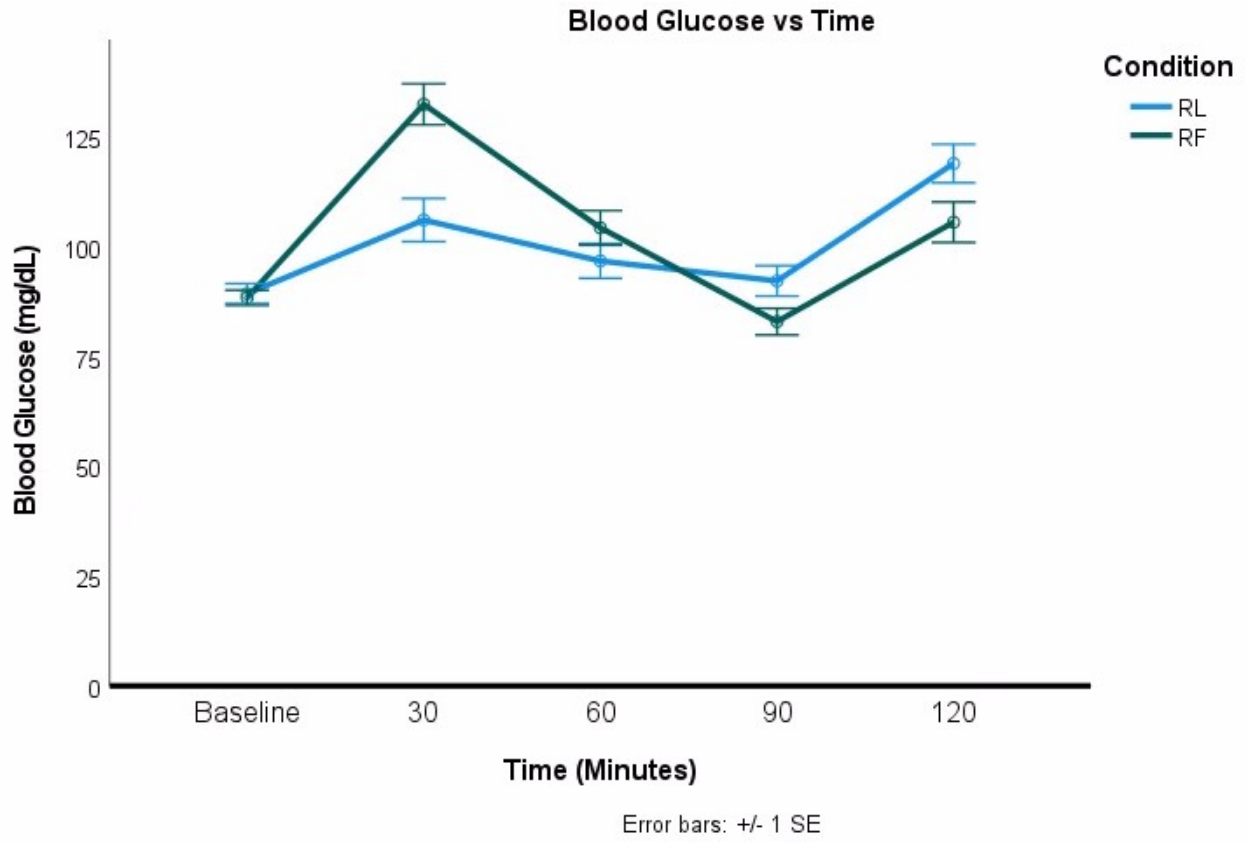
Table 4. Actigraph T-Tests

Activity Level	Days Before Visit	*N*	*M*	*SD*	*t*	*p*
Sedentary	2	16	5.0	192	0.10	0.3
Sedentary	1	18	-43.8	237.0	-0.79	.443
Light	2	16	-24.9	93.1	-1.07	.302
Light	1	18	0.1	100.2	0.00	.998
SQRT MVPA	2	18	-0.16	3.1	-0.22	.832
SQRT MVPA	1	18	1.9	2.1	3.80	.001

Blood Glucose

The primary outcome of interest was blood glucose—specifically, PPG. There were no significant differences between baseline glucose values. A two-way repeated measures ANOVA was performed to compare the effect of meal sequence on blood glucose. There was not a significant effect of condition on blood glucose F (1, 17) = 1.20, p = .288. There was, however, a significant effect of condition * time on blood glucose F (4, 68) = 14.39, p < .001, as well as a time effect F (4, 68) = 27.10, p < .001. Blood glucose was significantly lower in the RL condition when compared to the RF condition at minute 30 (see **Figure 7**).

Figure 7. Blood Glucose vs Time (Based on Condition)



Paired samples t-tests were conducted to determine the effect of meal sequence on peak-nadir glucose, PP peak-nadir glucose and delta exercise change. When baseline was included, peak-nadir glucose was not significantly larger in the RF condition, (M = 54.9, SD = 21.0), t = -2.04, p = .057, compared to the RL condition (M = 43.6, SD = 10.5). When only considering the PP period, peak-nadir glucose in the RF condition was not statistically significantly higher (M = 52.3, SD = 22.6), t = -1.7, p = .103, than the RL condition (M = 40.7, SD = 13.0). Delta exercise change was significantly higher in the RF condition (M = 21.4, SD = 22.1), t = -2.3, p = .035, than in the RL condition (M = 4.6, SD = 23.5).

Respiratory Exchange and Substrate Data

Absolute VO_2, during both the PP rest and exercise periods, did not show any condition or condition * time effects, indicating that exercise workload was well matched between conditions. During rest, condition effect and condition * time effects were F (1, 14) = 0.29, p = .600 and F (4, 68) = 0.66, p = .748 respectively. During exercise, the effect of condition on VO_2 was F (1, 14) = 0.001, p = .971, and the condition * time effect was F (4, 68) = 1.60, p = .223. However, VO_2 did significantly increase over time during both the resting PP and exercise periods; F (9, 126) = 9.8, p < .001 and F (5, 70) = 16.6, p < .001 respectively. The VO_2 data are visualized in **Figure 8** and **Figure 9**.

Figure 8. VO_2 in PP, Resting Phase

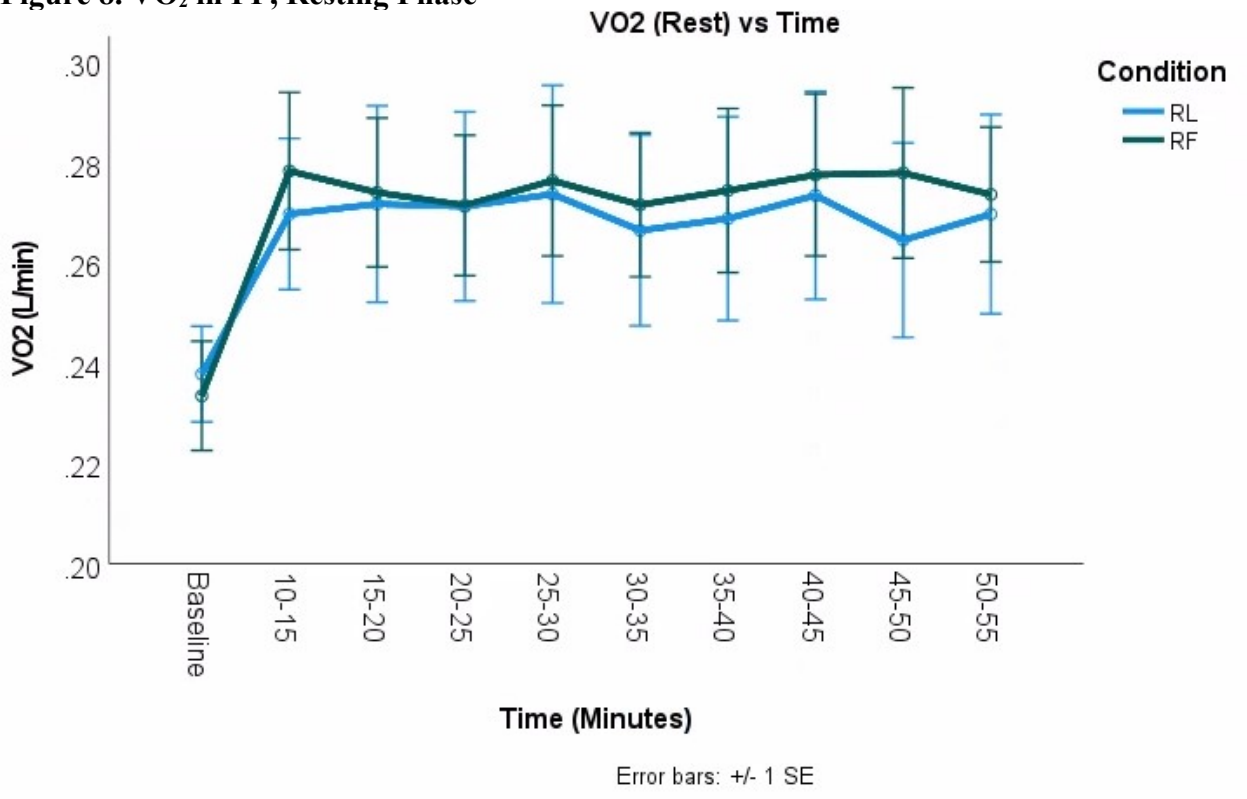
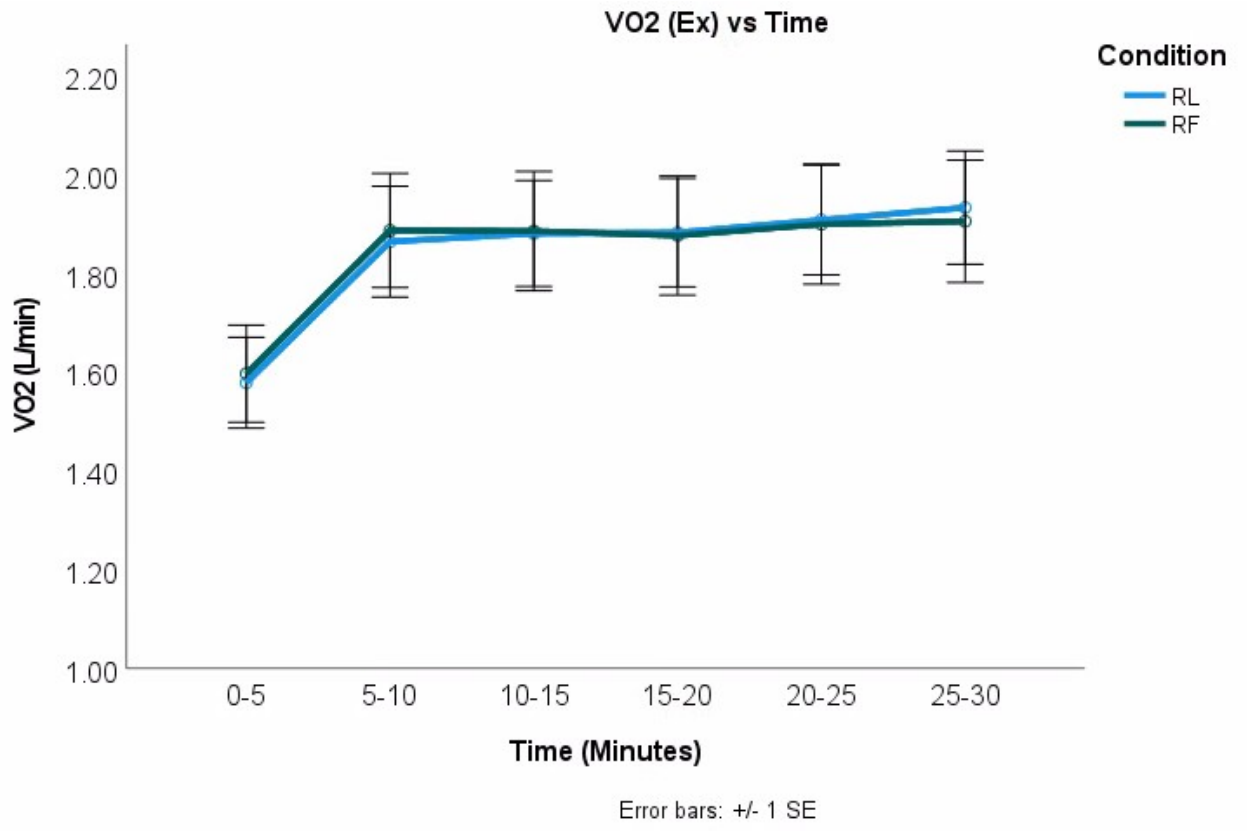


Figure 9. VO_2 During Exercise



There was a significant effect of condition on carbohydrate oxidation during the PP rest period, F ((1, 14) = 5.48, p = .034), a significant time effect, F (9, 126) = 17.11, p = < .001, as well as a significant condition * time interaction, F ((9, 126) = 3.37, p = .024). See **Figure 10** for a visual representation of the data. During the exercise protocol, there was a significant effect of condition on carbohydrate oxidation, F (1, 14) = 4.97, p = .043, as well as a significant time effect, F (5, 70) = 17.88, p < .001. There was no significant condition * time interaction F (5, 70) = 1.11, p = .364. Of note, there was no statistically significant difference in carbohydrate oxidation for any particular time period during exercise (see **Figure 11**).

Figure 10. Carbohydrate (CHO) Oxidation at Rest

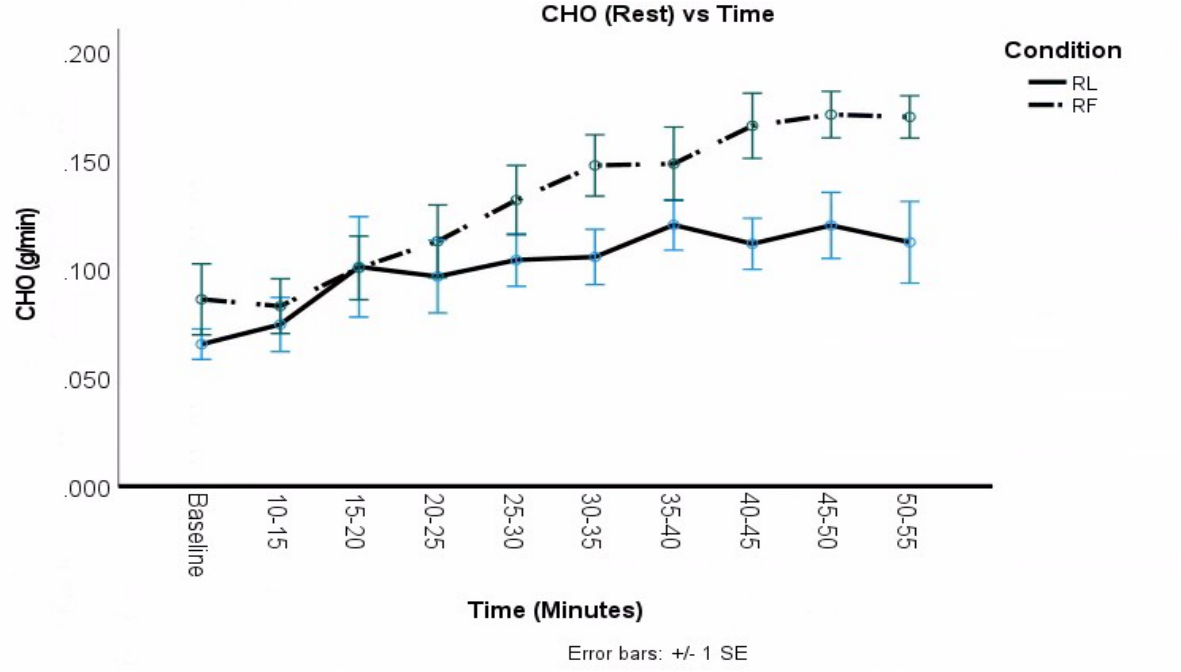
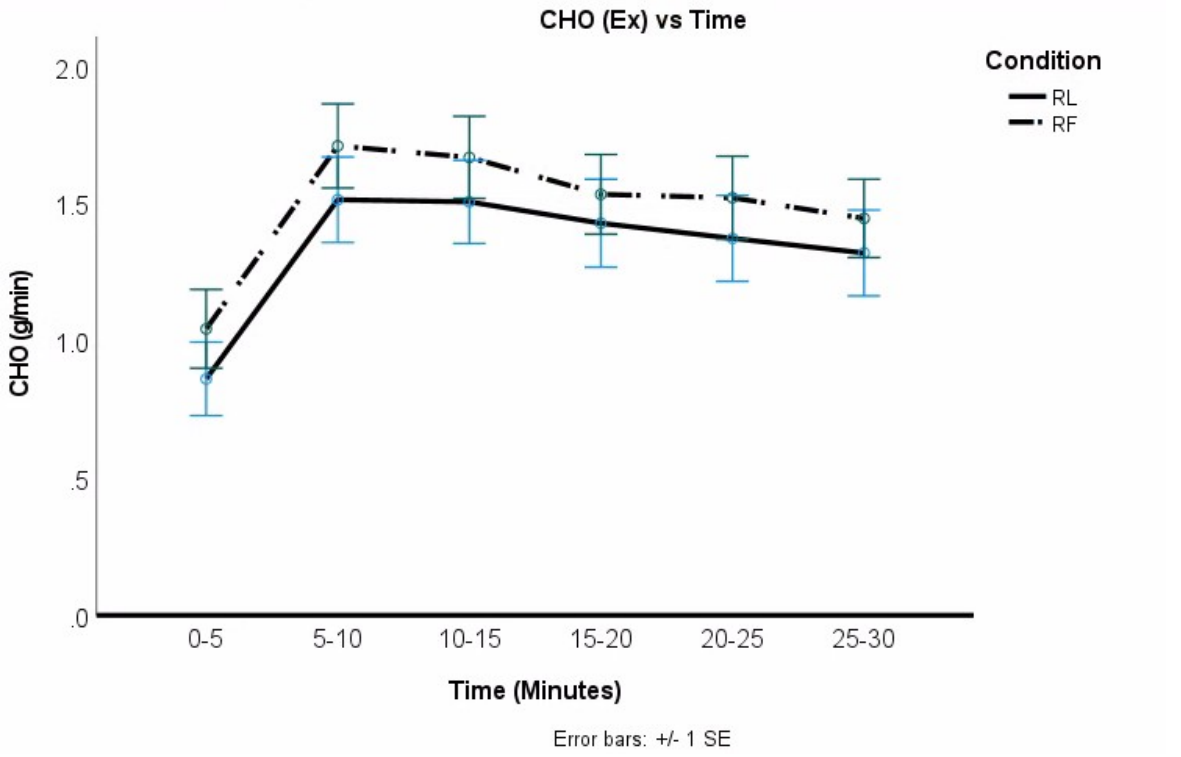


Figure 11. Carbohydrate (CHO) Oxidation During Exercise



With respect to fat oxidation, there was a significant effect of condition at rest, F (1, 14) = 5.67, p = .032, a significant effect of time, F (9, 126) = 10.49, p < .001, and a significant condition * time effect, F (9, 126) = 2.76, p = .038. Fat oxidation during exercise did not show a significant effect of condition, F (1, 14) = 3.99, p = .066 or a significant condition * time effect, F (5, 70) = 0.33, p = .801. There was, however, a significant effect of time, F (5, 70) = 13.06, p < .001. Fat oxidation is visualized in **Figure 12** and **Figure 13**.

Figure 12. Fat Oxidation at Rest

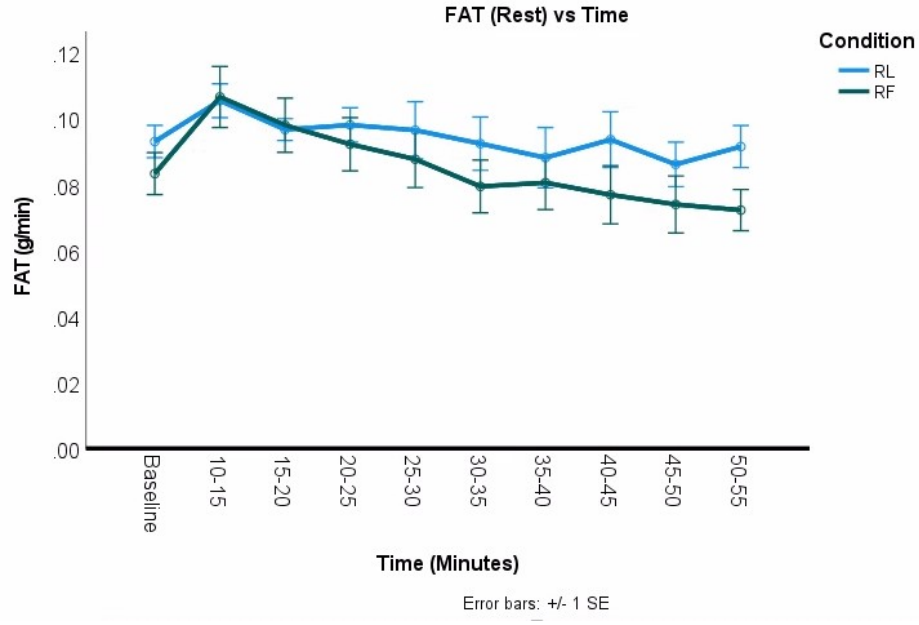
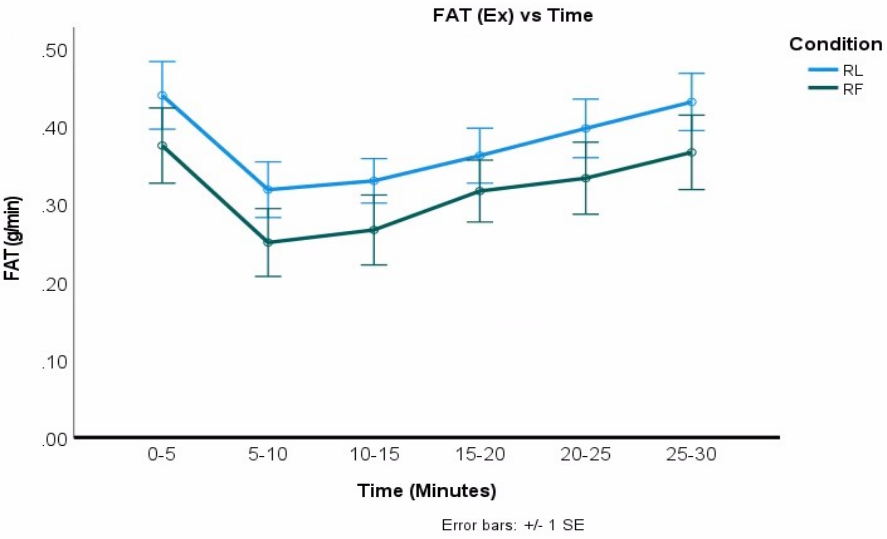


Figure 13. Fat Oxidation During Exercise



RER showed a significant effect of condition at rest, F (1, 14) = 8.21, p = .012, time; F (9, 126) = 18.75, p < .001; and condition * time, F (9, 126) = 3.79, p < .001. There were also significant effects of condition, F (1, 14) = 5.88, p = .029 and time, F (5, 70) = 17.14, p < .001 on RER during exercise. However, there was not a significant condition * time effect, F (5, 70) = 0.58, p = .713. A visualization is included in **Figure 14** and **Figure 15**.

Figure 14. RER at Rest.

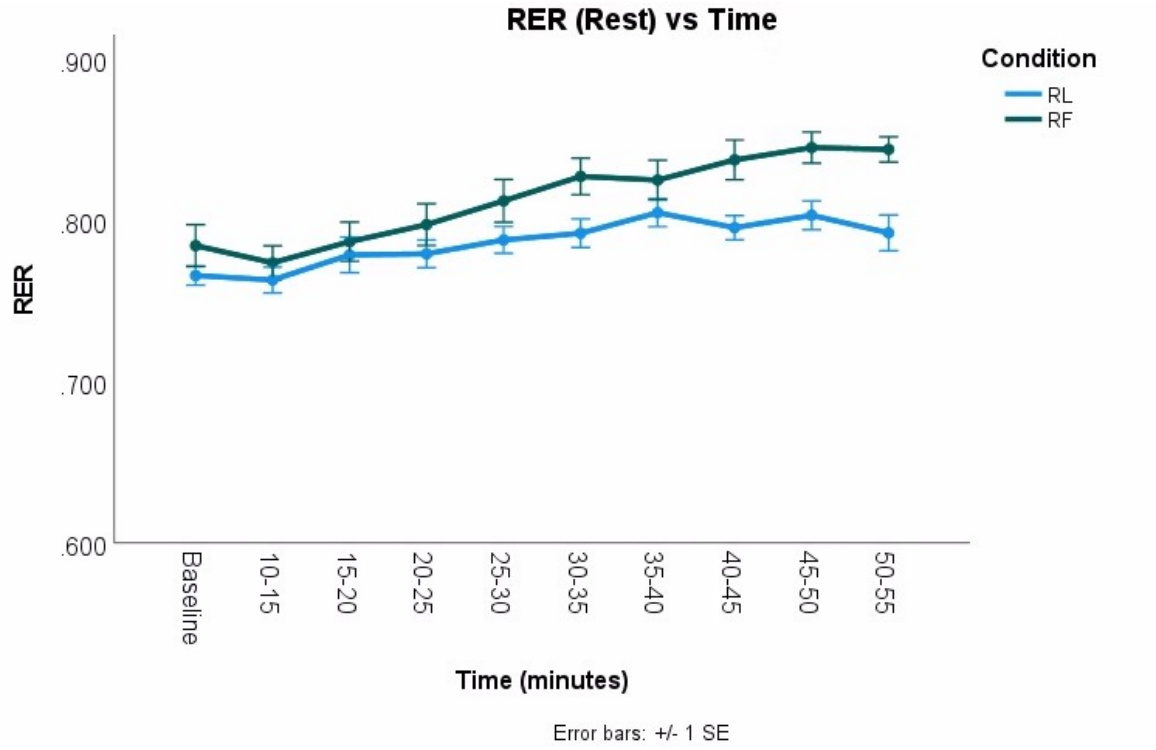
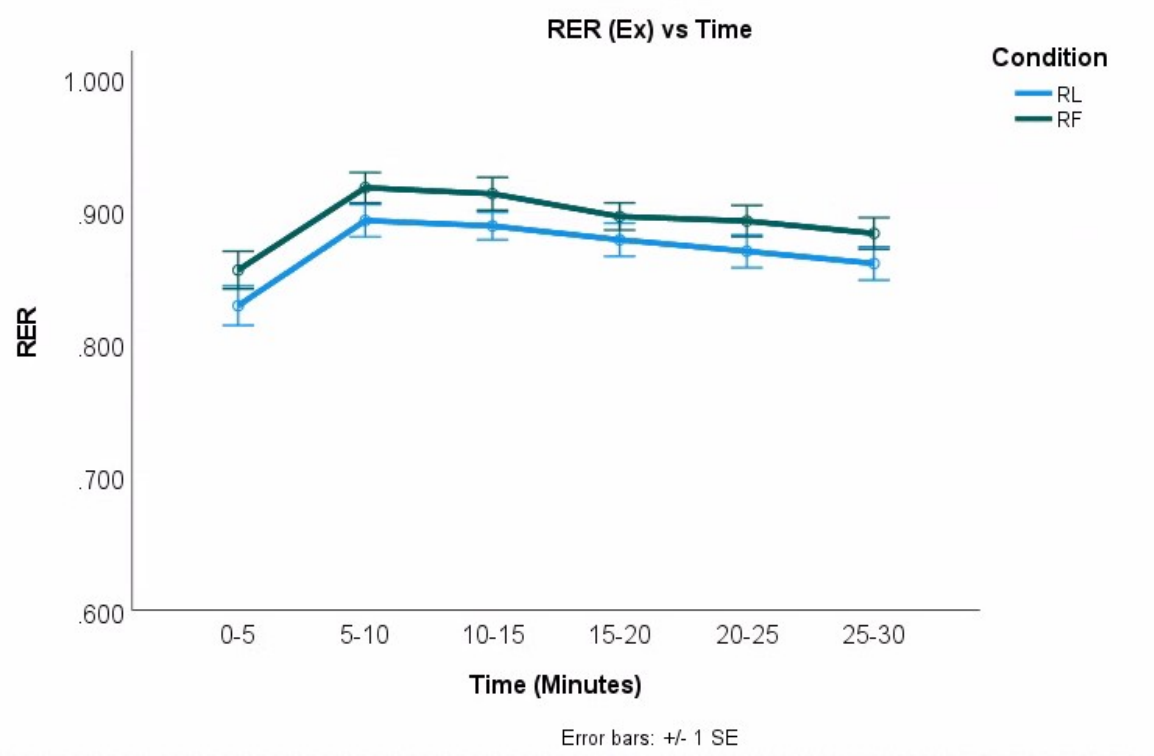


Figure 15. RER During Exercise



Perceptual Data

Regarding the AUC data, participants experienced significantly higher levels of satiety, t = -2.27, p = .037, in the RF condition (M = 487.5, SD = 306.8) compared to the RL condition (M = 366.5, SD = 278.9). There was not a significant difference in levels of fullness based on condition, t = -1.43, p = .171. Hunger and appetite also did not vary significantly based on condition. A paired samples t-test was conducted for hunger, t = -1.55, p = .140, while the same test for appetite resulted in, t = -1.34, p = .198.

FS showed no significant effect of condition, F (1, 17) = 1.87, p = .189, but it did show a significant effect of time; F (8, 136) = 2.91, p = .037. There was no significant condition * time effect, F (8, 136) = 0.77, p = .551. A visualization of these results are shown in **Figure 16**. RPE did not show any significant changes based on condition, time, or condition * time; F (1, 17) =

1.11, $p = .308$; $F\ (2, 34) = 4.20$, $p = .052$; and $F\ (2, 34) = 0.34$, $p = .633$, respectively. Results are visualized in **Figure 17**.

Figure 16. FS by Condition

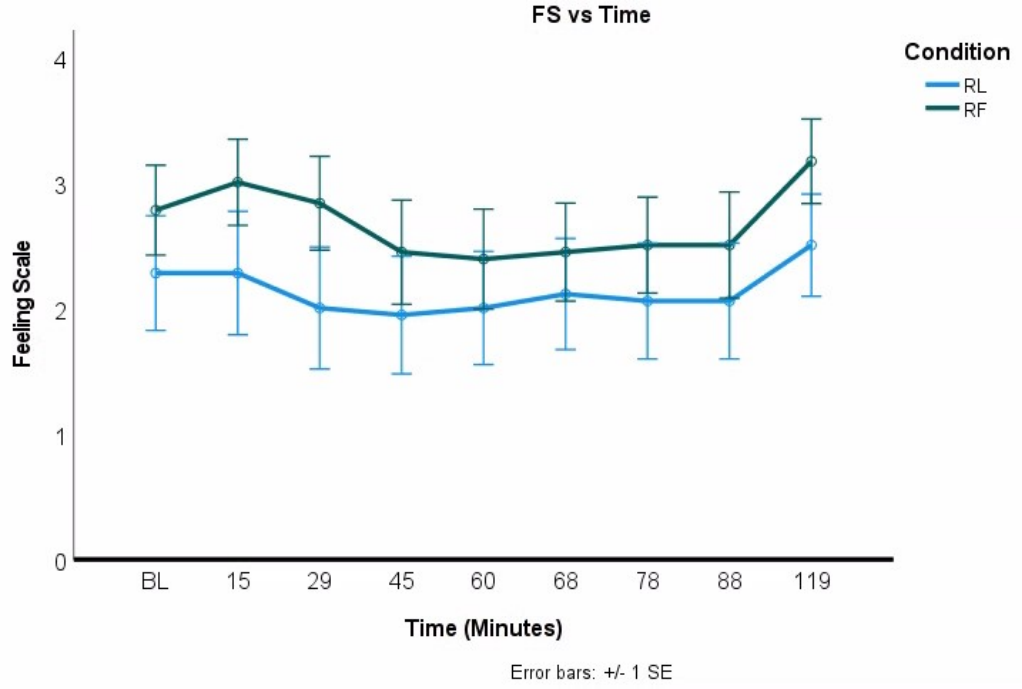
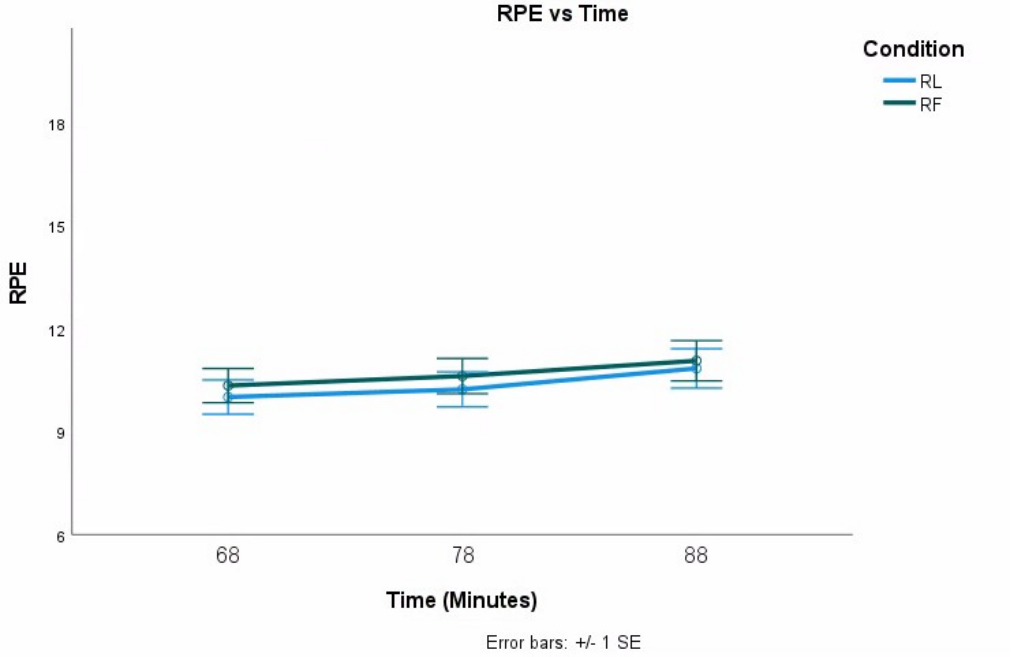


Figure 17. RPE by Condition



Discussion

Ours is not the first study to measure and notice significant effects of meal sequence or order of eating on PPG (Faber et al., 2018; Imai et al., 2010; Shukla et al., 2015, 2017, 2018, 2019; Sun et al., 2020). Nor is it the first study to examine the effects of meal sequence on PP perceptions of satiety (Shukla et al., 2018). However, this study is novel in that we measured respiratory gases and calculated CHO oxidation both at rest and during exercise and included an ostensibly healthy population as the intervention group. As it relates to blood glucose, we were successfully able to replicate findings of previous studies (Faber et al., 2018; Imai et al., 2010; Kuwata et al., 2016; Lee et al., 2021; Nishino et al., 2018; Shukla et al., 2015, 2017, 2018, 2019) that blood glucose generally has a higher peak and a larger peak-nadir difference in a carbohydrate-first meal sequence when compared to a carbohydrate-last meal sequence. Novelly, this study is the first to show that meal sequence does indeed have a significant effect on, not only blood glucose, but substrate oxidation both at rest and during steady state exercise. We also showed that individuals are likely to have higher feelings of satiety in a carbohydrate-last meal sequence when compared to a carbohydrate-first meal sequence.

Although not directly shown in the present study, a theoretical benefit of a carbohydrate-last meal with respect to exercise and glycemia is the prevention of exercise-associated hypoglycemia. In this study, there was a lower exercise-associated delta change in blood glucose following a RL meal sequence. In a well-illustrated example of this point, a group of 6,761 individuals wearing CGMs were made to self-report pre-exercise food ingestion in order to detect exercise-associated hypoglycemia events. The authors found that ingesting food 30-90 minutes prior to exercise can significantly impact the likelihood of this event occurring (Zignoli et al., 2023). In a more concentrated study involving highly trained cyclists, researchers found

that bioavailability of carbohydrate near the end of long exercise bouts was inversely correlated with glycemic index content of pre-exercise feedings (Thomas et al., 1994). Reducing potential incidence of exercise-associated hypoglycemia could have significant advantages for specific populations such as those with T1DM or T2DM, and while we did not observe any cases of severe hypoglycemia (i.e., <50 mg/dL), future studies consider examining the impact of meal order on the incidence of exercise-associated hypoglycemia in clinical populations.

Having higher carbohydrate oxidation during exercise may improve performance in certain activities such as cycling (DeMarco et al., 1999), and carbohydrate rich pre-exercise meals have shown to improve performance (Burdon et al., 2017). Given that our study included a non-exhaustive steady-state exercise protocol, we can only speculate that the RF condition *may* have improved short-term exercise performance. Still, other studies have shown that low-GI pre-exercise foods may improve endurance performance compared to high-GI by lowering carbohydrate oxidation rate and allowing for better performance later in competition (Donaldson et al., 2010; Kaviani et al., 2020). The effect that pre-exercise feeding has on performance will likely be highly individualized and influenced by acute factors such as exercise intensity, duration, and further ingestion of carbohydrate during exercise. At a minimum, though, the present study provides a solid basis for examining the effects of ordered eating on substrate use and performance in various athletic populations.

Potentially more impactful is that substrate utilization at rest was significantly mediated by meal sequence in our study, which may have implications for encouraging fat burning in certain populations. Specifically, consuming a carbohydrate-dense food at the end of a meal sequence may encourage fat burning even at rest. In healthy individuals, substrate utilization (i.e. what we are burning) can change quickly in response to stimuli like eating and is described as

'flexible' (Astrup, 2011). Still, certain groups such as those who have a history of obesity, often do not have this 'flexibility' in their substrate switching due to specific genetic factors or environmental factors (Astrup, 2011). For these reasons, it is possible that manipulating bioavailability of substrate through ordered eating may affect utilization without requiring metabolic flexibility. From a clinical perspective, it is possible that lowering peak excursions in the manner observed, over a number of days, weeks, and years may reduce incidence rates of chronic diseases such as CVD and T2DM.

Lastly, self-reported feelings of satiety were indeed significantly different based on meal sequence. Consuming a carbohydrate-dense food at the end of a meal sequence may leave individuals feeling satisfied for shorter periods when compared to the reverse order due to the association between insulin and immediate appetite control in healthy populations (Flint et al., 2007). Interestingly, in overweight and obese populations, this association may be blunted or affected due to insulin resistance such that satiety is not related to insulin in these populations (Flint et al., 2007).

While the results of this particular intervention are certainly interesting and novel, there were a number of limitations in the study design. The methods used to calculate carbohydrate and fat use during exercise are indeed well documented and valid (Jeukendrup & Wallis, 2005), but they are still not direct measurements. Beyond that, while 23 total individuals were consented, complete data was available for only 18 participants for blood glucose and perceptual outcomes and 15 participants for substrate use, which limits the power of the study for detecting small effects. It should be noted that, in the screening process, participants were asked if they were free from any sort of metabolic disorder symptoms, but no further inquiry was performed, so there is a possibility that individual(s) may have had undiagnosed, BG altering diseases.

Additionally, we did not have the capability to measure blood fatty acids, triglycerides, and most glaringly, insulin, which could have added significant value to these results. Lastly, it is important to recognize that while we did include an exercise protocol, there was no measurement of performance, so future research should indeed include varying exercise protocols to include performance testing.

In conclusion, the present study replicated the results of prior research in that meal sequence impacts PPG. Further, we demonstrated that there is an effect of meal sequence on perceptions of satiety and on substrate oxidation—specifically, a carbohydrate-last meal order reduces carbohydrate oxidation and increases fat oxidation at rest and during moderate-intensity exercise carried out one hour after eating. Future research conducted in this area should continue to utilize healthy populations to further examine the possible effects of meal sequence on exercise of varying intensity. Additionally, researchers should attempt to implement an ordered eating protocol in a free-living environment to observe its possible usefulness beyond a laboratory setting.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Ordered eating is a fairly new concept in the literature that has only begun to be explored as recently as 2010 (Imai et al., 2010)—though, anecdotally, the concept of ordered eating may be an intuitive one. Since that time, the depth of research has not extended much beyond a handful of studies and labs with a heavy focus on T2DM, T1DM, and prediabetes (Faber et al., 2018; Imai et al., 2010; Kuwata et al., 2016; Lee et al., 2021; Nishino et al., 2018; Shukla et al., 2015, 2017, 2018, 2019; Sun et al., 2020). The primary outcome of interest across the literature that does exist has been PPG. Almost universally, researchers have found that altering meal sequence—specifically carbohydrate-dense food placement in meal sequence, leads to a significant difference in PPG (Faber et al., 2018; Imai et al., 2010; Kuwata et al., 2016; Lee et al., 2021; Nishino et al., 2018; Shukla et al., 2015, 2017, 2018, 2019; Sun et al., 2020). More specifically, carbohydrate-dense foods being placed last in meal sequence tends to lead to a significant decrease in PPG when compared to carbohydrate-dense foods being placed first in meal sequence (Faber et al., 2018; Imai et al., 2010; Kuwata et al., 2016; Lee et al., 2021; Nishino et al., 2018; Shukla et al., 2015, 2017, 2018, 2019; Sun et al., 2020). While this phenomenon may initially appear somewhat intuitive, there is interestingly not a well understood mechanism as to why this happens. Imai and colleagues have speculated that the high fiber content of vegetables eaten before carbohydrate-dense foods may delay gastric emptying and slow digestion such that less insulin is secreted by the pancreas (Imai et al., 2010). While it is certainly likely that high fiber vegetables first in meal sequence will have this effect, the mechanisms that underly this phenomenon are poorly studied in the current literature. Another

potential mechanism is the secretion of incretins when protein is ingested prior to carbohydrate (Ma et al., 2009). This ingestion of protein increases GLP-1 release which, in turn would increase circulating insulin, thereby reducing overall PPG response.

The purpose of this dissertation was to, through both a systematic review and a laboratory study, determine the efficacy and ecological realities of ordered eating relating to the management of T2DM, its effects on blood glucose maintenance in an acute setting, and its effects on the glycemic, substrate utilization, and perceptual responses to exercise. This was accomplished through two separate studies; the first, a systematic review of the literature on the topic of ordered eating, and a second experimental intervention study. The systematic review focused on the current state of the literature regarding ordered eating and its potential efficacy as an acute intervention to improve PPG and related markers. The experimental intervention study involved examining the effects of ordered eating on responses to exercise. Outcomes of interest in the experimental study included PPG excursions, substrate utilization, and perceptual responses (hunger, satiety, gastrointestinal symptoms). Participants performed steady state exercise for 30 minutes, and these outcomes were measured before, during, and after exercise. This experiment took the form of a crossover design with participants exercising under both a carbohydrate-first and a carbohydrate-last condition.

It was hypothesized that altering meal order would result in variations in PPG and PPI excursions. More specifically, the timing of carbohydrate-dense foods relative to other foods included in the meal will have the greatest effect on PPG and PPI. This was evaluated through a systematic review of the literature relating to ordered eating. It was found that there is not a large amount of literature relating to the topic of ordered eating, and the total number of studies included in the review totaled at 11, including 9 randomized controlled trials. Glucose was the

primary outcome of interest and certainly is as it relates to this dissertation. Out of the 11 studies included in the review, 10 included PPG while the single study that did not include it was a secondary analysis of the same author's work. In all of the studies that assessed PPG, it was found that carbohydrate-dense foods eaten last in meal sequence lowered glucose AUC, peak glucose, and/or glucose at specific time points. Most studies that did examine AUC showed significant difference by condition (Imai et al., 2013; Lee et al., 2021; Sukla et al., 2017; Shukla et al., 2019; Sun et al., 2020). Secondarily to glucose was PPI. Insulin was included as an outcome in seven of the 11 reports. Unsurprisingly, PPI followed a very similar pattern to PPG in the studies which it was measured. All of the seven reports that included PPI found that it was lower after eating carbohydrate-dense foods at the end of a meal sequence (Imai et al., 2010; Kuwata et al., 2016; Nishino et al., 2018; Shukla et al., 2015, 2017, 2019; Sun et al., 2020).

A second hypothesis that contained four parts was the basis for the laboratory study included in this dissertation. The first part of that hypothesis was that peak PPG would be smaller in a carbohydrate-last meal sequence compared to a carbohydrate-first meal sequence. In our lab study that included 18 complete data sets, peak PPG in the RF condition was at 30-minutes after meal consumption, $M = 132.6$, $SD = 19.9$. Peak PPG in the RL condition was at 120 minutes after meal consumption and was $M = 119.1$, $SD = 18.5$. From this data, we can conclude that peak PPG was indeed larger in the RF condition compared to the RL condition. It is important to note that at least some of this difference at the 120-minute mark is attributable to the exercise protocol.

Part 2 of this hypothesis was that there would be a larger delta change in peak-nadir glucose in the RF condition. This was found to be partially supported. In the RF condition, average delta change in peak-nadir glucose was $M = 52.3$, $SD = 22.6$, while average delta change

in peak-nadir glucose was smaller in the RL condition, $M = 40.7$, $SD = 13.0$. Importantly, this difference was not statistically significant. However, the change in glucose from pre-exercise to post-exercise ($M = -16.8$, $SD = 31.2$) was significantly different; $t = -2.29$, $p = .035$.

Part 3 of the hypothesis was that carbohydrate utilization at rest and during exercise would be higher, and fat oxidation would be lower, following a RF meal sequence. Again, this was found to be true. While there was no statistically significant difference at any one time period during exercise in terms of carbohydrate oxidation, the total oxidation was significantly higher in the RF condition, $F(1, 14) = 5.0$, $p = .043$. At rest, the difference, based on meal sequence was even larger than during exercise and was significantly different at multiple timepoints, $F(1, 14) = 5.5$, $p = .034$.

The final portion of this hypothesis focused on perceptual outcomes, more specifically, satiety. We hypothesized that participants would report higher levels of satiety in a RF condition. There was a significant effect of meal sequence on perceptions of satiety. In this case, the hypothesis was also correct. Participants experienced higher levels of satiety in the RF condition, based on AUC, $M = 587.5$, $SD = 306.8$, compared to RL, $M = 366.5$, $SD = 278.9$.

On a more global scale, the aims of this dissertation were to evaluate the current state of the literature related to ordered eating and its acute effects on postprandial measures well as its effects on other measures associated with T2DM and to evaluate the effects of ordered eating on PPG excursions, substrate utilization, and perceptual responses during an acute exercise bout. To the first aim, the systematic review demonstrated that there was a meaningful effect of meal sequence on a number of PP measurements, and it elucidated on gaps in the existing literature. Relating to the second aim, the laboratory study included in this dissertation addressed, at least in some capacity, the gaps in the existing literature on ordered eating as it relates to substrate

utilization, exercise, and perceptions. It should be noted that significantly more research is needed with ordered eating and its effects on substrate utilization.

Overall, this dissertation did accomplish what it set out to do with a comprehensive, systematic review of the literature on ordered eating and a well-conducted laboratory-based study. All hypotheses were supported--at least partially. The concept of ordered eating shows promise as an approachable lifestyle modification that could have an effect on risk of chronic disease. In our lab study, specifically, there was a significant difference in PPG at the 30-minute mark that could be extrapolated and repeated over time. Future research should address gaps that still exist. At this point, the body of literature surrounding ordered eating is generally small and somewhat biased in its sampling. Additionally, outside of the study conducted in our laboratory, there is no literature that exists examining the potential effects of ordered eating on substrate utilization which may indeed have implications for body composition or exercise performance. Further, there is very little literature concerning the efficacy of this sort of intervention in free-living conditions, which is a large gap that needs to be addressed. In sum, while the area of ordered eating shows promise as a lifestyle intervention, much more robust research is needed to determine if it is an intervention that can be used in a real-life scenario and if it has significant effects with less-than-standardized meals.

REFERENCES

Acar, N., Ozcelik, H., Cevik, A. A., Ozakin, E., Yorulmaz, G., Kebapci, N., Bilge, U., & Bilgin, M. (2014). Low perfusion index affects the difference in glucose level between capillary and venous blood. *Therapeutics and Clinical Risk Management, 10*, 985-991. https://doi.org/10.2147/TCRM.S73359

Achten, J., Gleeson, M., & Jeukendrup, A. E. (2002). Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine & Science in Sports & Exercise, 34*(1), 92-97. https://doi.org/10.1097/00005768-200201000-00015

Adams, O. P. (2013). The impact of brief high-intensity exercise on blood glucose levels. *Diabetes, Metabolic Syndrome and Obesity, 6*, 113-122. https://doi.org/10.2147/DMSO.S29222

Aggarwal, R., Vaduganathan, M., Chiu, N., & Bhatt, D. L. (2022). Out-of-pocket costs for SGLT-2 (sodium-glucose transport protein-2) inhibitors in the United States. *Circulation: Heart Failure, 15*(3), e009099. https://doi.org/10.1161/CIRCHEARTFAILURE.121.009099.

Agiostratidou, G., Anhalt, H., Ball, D., Blonde, L., Gourgari, E., Harriman, K. N., Kowalski, A. J., Madden, P., McAuliffe-Fogarty, A. H., & McElwee-Malloy, M. (2017). Standardizing clinically meaningful outcome measures beyond HbA1c for type 1 diabetes: A consensus report of the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society, JDRF International, the Leona M. and Harry B. Helmsley Charitable Trust, the Pediatric Endocrine Society, and the T1D Exchange. *Diabetes Care, 40*(12), 1622-1630. https://doi.org/10.2337/dc17-1624

Ajala, O., English, P., & Pinkney, J. (2013). Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *American Journal of Clinical Nutrition, 97*(3), 505-516. https://doi.org/10.3945/ajcn.112.042457

Alwafi, H., Alsharif, A. A., Wei, L., Langan, D., Naser, A. Y., Mongkhon, P., Bell, J. S., Ilomaki, J., Al Metwazi, M. S., Man, K. K. C., Fang, G., & Wong, I. C. K. (2020). Incidence and prevalence of hypoglycaemia in type 1 and type 2 diabetes individuals: A systematic review and meta-analysis. *Diabetes Research and Clinical Practice, 170*, 108522. https://doi.org/10.1016/j.diabres.2020.108522

American Diabetes Association (ADA). (2001). Postprandial blood glucose. *Diabetes Care, 24*(4), 775–778. https://doi.org/10.2337/diacare.24.4.775

American Diabetes Association (ADA). (2015). Classification and diagnosis of diabetes. *Diabetes Care, 38*(Supplement 1), S13-S22. https://doi.org/10.2337/dc16-S005

American Diabetes Association (ADA). (2018). Economic costs of diabetes in the U.S. in 2017. *Diabetes Care, 41*(5), 917–928. https://doi.org/10.2337/dci18-0007

American Diabetes Association (ADA). (2020). Classification and diagnosis of diabetes: Standards of medical care in diabetes-2020. *Diabetes Care, 43*(Supplement 1), S14-S31. https://doi.org/10.2337/dc20-S002

American Diabetes Association (ADA). (2021). *Statistics About Diabetes*. Retrieved from https://diabetes.org/about-diabetes/statistics/about-diabetes

Astrup, A. (2011). The relevance of increased fat oxidation for body-weight management: metabolic inflexibility in the predisposition to weight gain. *Obesity Reviews*, *12*(10), 859-865. https://doi.org/https://doi.org/10.1111/j.1467-789X.2011.00894.x

Bani, I. (2015). Prevalence, knowledge, attitude, and practices of diabetes mellitus among the Jazan population, Kingdom of Saudi Arabia (KSA). *Journal of Diabetes Mellitus, 5*, 115–122. https://doi.org/10.4236/jdm.2015.52014

Bashir, M., Ahmed, S., & Aslam, S. (2019). Outcomes of type 1 diabetes mellitus in pregnancy: Effect of excessive gestational weight gain and hyperglycemia on fetal growth. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 13*(1), 84-88. https://doi.org/10.1016/j.dsx.2018.08.003

Blaslov, K., Naranđa, F. S., Kruljac, I., & Renar, I. P. (2018). Treatment approach to type 2 diabetes: Past, present, and future. *World Journal of Diabetes, 9*(12), 209–219. https://doi.org/10.4239/wjd.v9.i12.209

Bluestone, J. A., Herold, K., & Eisenbarth, G. (2010). Genetics, pathogenesis, and clinical interventions in type 1 diabetes. *Nature, 464*(7293), 1293-1300. https://doi.org/10.1038/nature08933

Blüher, M. (2019). Obesity: Global epidemiology and pathogenesis. *Nature Reviews Endocrinology, 15*(5), 288-298. https://doi.org/10.1038/s41574-019-0176-8

Boje, O. (1940). Arbeitshypoglykämie nach Glucose Eingabe. *Skandinavisches Archiv für Physiologie, 83*, 308-312.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise, 14*(5), 377-381. https://doi.org/10.1249/00005768-198205000-00012

Boulé, N. G., Kenny, G. P., Haddad, E., Wells, G. A., & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus. *Diabetologia, 46*(8), 1071–1081. https://doi.org/10.1007/s00125-003-1160-2

Burdon, C. A., Spronk, I., Cheng, H. L., & O'Connor, H. T. (2017). Effect of glycemic index of a pre-exercise meal on endurance exercise performance: A systematic review and meta-analysis. *Sports Medicine, 47*(6), 1087-1101. https://doi.org/10.1007/s40279-016-0632-8

Campbell, R., & White, J. (2003). *Medications for the treatment of diabetes*. American Diabetes Association.

Centers for Disease Control and Prevention (CDC). (2022). *Obesity and disability*. Centers for Disease Control and Prevention. https://www.cdc.gov/ncbddd/disabilityandhealth/obesity.html

Cerami, A., Stevens, V. J., & Monnier, V. M. (1979). Role of nonenzymatic glycosylation in the development of the sequelae of diabetes mellitus. *Metabolism, 28*(4 Suppl 1), 431–437. https://doi.org/10.1016/0026-0495(79)90051-9

Choi, L., Liu, Z., Matthews, C. E., & Buchowski, M. S. (2011). Validation of accelerometer wear and nonwear time classification algorithm. *Medicine & Science in Sports & Exercise, 43*(2), 357-364. https://doi.org/10.1249/MSS.0b013e3181ed61a3

Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in sports and exercise medicine research? *European Journal of Sport Science, 14*(8), 847-851. https://doi.org/10.1080/17461391.2014.911354

Coyle, E. F., Coggan, A. R., Hemmert, M. K., Lowe, R. C., & Walters, T. J. (1985). Substrate usage during prolonged exercise following a preexercise meal. *Journal of Applied Physiology, 59*(2), 429-433. https://doi.org/10.1152/jappl.1985.59.2.429

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., & Sallis, J. F. (2003). International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Medicine & Science in Sports & Exercise, 35*(8), 1381-1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Cryer, P. E., & Gerich, J. E. (1985). Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. *The New England Journal of Medicine, 313*(4), 232–241. https://doi.org/10.1056/NEJM198507253130405

Cullmann, M., Hilding, A., & Östenson, C. G. (2012). Alcohol consumption and risk of pre-diabetes and type 2 diabetes development in a Swedish population. *Diabetic Medicine, 29*(4), 441-452. https://doi.org/10.1111/j.1464-5491.2011.03450.x

DeFronzo, R. A. (1988). The triumvirate: β-cell, muscle, liver: A collusion responsible for NIDDM. *Diabetes, 37*(6), 667-687. https://doi.org/10.2337/diab.37.6.667

DeMarco, H. M., Sucher, K. P., Cisar, C. J., & Butterfield, G. E. (1999). Pre-exercise carbohydrate meals: Application of glycemic index. *Medicine & Science in Sports & Exercise, 31*(1), 164-170. https://doi.org/10.1097/00005768-199901000-00025

Ding, H., Hu, G. L., Zheng, X. Y., Chen, Q., Threapleton, D. E., & Zhou, Z. H. (2015). The method quality of cross-over studies involved in Cochrane Systematic Reviews. *PLoS ONE, 10*(4), e0120519. https://doi.org/10.1371/journal.pone.0120519

Donaldson, C. M., Perry, T. L., & Rose, M. C. (2010). Glycemic index and endurance performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(2), 154-165. https://doi.org/10.1123/ijsnem.20.2.154

D'Souza, A. C., Wageh, M., Williams, J. S., Colenso-Semple, L. M., McCarthy, D. G., McKay, A. K. A., Elliott-Sale, K. J., Burke, L. M., Parise, G., MacDonald, M. J., Tarnopolsky, M. A., & Phillips, S. M. (2023). Menstrual cycle hormones and oral contraceptives: A multimethod systems physiology-based review of their impact on key aspects of female physiology. *Journal of Applied Physiology (1985), 135*(6), 1284-1299. https://doi.org/10.1152/japplphysiol.00346.2023

Dyck, D. J., Putman, C. T., Heigenhauser, G. J., Hultman, E., & Spriet, L. L. (1993). Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. *American Journal of Physiology-Endocrinology and Metabolism, 265*(6), E852-E859. https://doi.org/10.1152/ajpendo.1993.265.6.E852

Edwards, C. M., Todd, J. F., Mahmoudi, M., Wang, Z., Wang, R. M., Ghatei, M. A., & Bloom, S. R. (1999). Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39. *Diabetes*, *48*(1), 86-93. https://doi.org/10.2337/diabetes.48.1.86

Faber, E. M., van Kampen, P. M., Clement-de Boers, A., Houdijk, E. C. A. M., & van der Kaay, D. C. M. (2018). The influence of food order on postprandial glucose levels in children with type 1 diabetes. *Pediatric Diabetes, 19*(4), 809-815. https://doi.org/10.1111/pedi.12640

Faria, V. C., Lima, L. M., & Pereira, D. A. G. (2018). Glycemic index of pre-exercise meal in diabetes mellitus: A systematic review. *Revista Brasileira de Medicina do Esporte, 24*(5), 515-520. https://doi.org/10.1590/1517-869220182405170370

Fava, M. C., Agius, R., & Fava, S. (2019). Obesity and cardio-metabolic health. *British Journal of Hospital Medicine (Lond.), 80*(8), 466-471. https://doi.org/10.12968/hmed.2019.80.8.466

Ferland, A., Brassard, P., Lemieux, S., Bergeron, J., Bogaty, P., Bertrand, F., Simard, S., & Poirier, P. (2009). Impact of high-fat/low-carbohydrate, high-, low-glycaemic index or low-caloric meals on glucose regulation during aerobic exercise in Type 2 diabetes. *Diabetic Medicine, 26*(6), 589-595. https://doi.org/10.1111/j.1464-5491.2009.02734.x

Fitchett, D., Butler, J., van de Borne, P., Zinman, B., Lachin, J. M., Wanner, C., Woerle, H. J., Hantel, S., George, J. T., Johansen, O. E., & Inzucchi, S. E.; EMPA-REG OUTCOME® trial investigators. (2018). Effects of empagliflozin on risk for cardiovascular death and heart failure hospitalization across the spectrum of heart failure risk in the EMPA-REG OUTCOME® trial. *European Heart Journal, 39*(5), 363-370. https://doi.org/10.1093/eurheartj/ehx511

Fletcher, G., Eves, F. F., Glover, E. I., Robinson, S. L., Vernooij, C. A., Thompson, J. L., & Wallis, G. A. (2017). Dietary intake is independently associated with the maximal capacity for fat oxidation during exercise. *American Journal of Clinical Nutrition, 105,* (4), 864-872. https://doi.org/10.3945/ajcn.116.133520

Flint, A., Gregersen, N. T., Gluud, L. L., Møller, B. K., Raben, A., Tetens, I., Verdich, C., & Astrup, A. (2007). Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *British Journal of Nutrition*, *98*(1), 17-25. https://doi.org/10.1017/S000711450768297X

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power, and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity, 24(*1), 38-48. https://doi.org/10.1038/sj.ijo.0801083

Flores-Opazo, M., Boland, E., Garnham, A., Murphy, R. M., McGee, S. L., & Hargreaves, M. (2018). Exercise and GLUT4 in human subcutaneous adipose tissue. *Physiological Reports, 6*(22), e13918. https://doi.org/10.14814/phy2.13918

Flores-Opazo, M., McGee, S. L., & Hargreaves, M. (2020). Exercise and GLUT4. *Exercise and Sport Sciences Reviews, 48*(3), 110-118. https://doi.org/10.1249/jes.0000000000000224

Forouhi, N., Misra, A., Mohan, V., Taylor, R., & Yancy, W. (2018). Dietary and nutritional approaches for prevention and management of type 2 diabetes. *BMJ, 361*, k2234. https://doi.org/10.1136/bmj.k2234

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology, 55*(2), 628-634. https://doi.org/10.1152/jappl.1983.55.2.628

Freychet, L., Desplanque, N., Zirinis, P., Rizkalla, S., Basdevant, A., Tchobroutsky, G., & Slama, G. (1988). Effect of intranasal glucagon on blood glucose levels in healthy subjects and hypoglycaemic patients with insulin-dependent diabetes. *The Lancet, 331*(8599), 1364-1366. https://doi.org/10.1016/s0140-6736(88)92181-2

Gangji, A. S., Cukierman, T., Gerstein, H. C., Goldsmith, C. H., & Clase, C. M. (2007). A systematic review and meta-analysis of hypoglycemia and cardiovascular events: A comparison of glyburide with other secretagogues and with insulin. *Diabetes Care, 30*(2), 389-394. https://doi.org/10.2337/dc06-1789

Gentilcore, D., Chaikomin, R., Jones, K. L., Russo, A., Feinle-Bisset, C., Wishart, J. M., Rayner, C. K., & Horowitz, M. (2006). Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*, *91*(6), 2062-2067. https://doi.org/10.1210/jc.2005-2644

Giacco, R., Parillo, M., Rivellese, A. A., Lasorella, G., Giacco, A., D'Episcopo, L., & Riccardi, G. (2000). Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 2 diabetes. *Diabetes Care, 23*(10), 1461-1466. https://doi.org/10.2337/diacare.23.10.1461

Ginsberg, B. H. (2009). Factors affecting blood glucose monitoring: Sources of errors in measurement. *Journal of Diabetes Science and Technology, 3*(4), 903-913. https://doi.org/10.1177/193229680900300411

Goyal, R., Singhal, M., & Jialal, I. (2023, June 23). *Type 2 diabetes*. In *StatPearls* [Internet]. StatPearls Publishing. Available from https://www.ncbi.nlm.nih.gov/books/NBK513253/

Grace, A., Chan, E., Giallauria, F., Graham, P. L., & Smart, N. A. (2017). Clinical outcomes and glycaemic responses to different aerobic exercise training intensities in type II diabetes: A systematic review and meta-analysis. *Cardiovascular Diabetology, 16*(1), 37. https://doi.org/10.1186/s12933-017-0518-6

Grant, S., Aitchison, T., Henderson, E., Christie, J., Zare, S., McMurray, J., & Dargie, H. (1999). A comparison of the reproducibility and the sensitivity to change of visual analogue scales, Borg scales, and Likert scales in normal subjects during submaximal exercise. *Chest, 116*(5), 1208-1217. https://doi.org/10.1378/chest.116.5.1208

Gray, N., Picone, G., Sloan, F., & Yashkin, A. (2015). Relation between BMI and diabetes mellitus and its complications among US older adults. *Southern Medical Journal, 108*(1), 29-36. https://doi.org/10.14423/SMJ.0000000000000214

Greenwood, D. C., Threapleton, D. E., Evans, C. E., Cleghorn, C. L., Nykjaer, C., Woodhead, C., & Burley, V. J. (2013). Glycemic index, glycemic load, carbohydrates, and type 2

diabetes: Systematic review and dose-response meta-analysis of prospective studies. *Diabetes Care, 36*(12), 4166-4171. https://doi.org/10.2337/dc13-0325

Guh, D. P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C. L., & Anis, A. H. (2009). The incidence of comorbidities related to obesity and overweight: A systematic review and meta-analysis. *BMC Public Health, 9*, 88. https://doi.org/10.1186/1471-2458-9-88

Guo, Y., Huang, Z., & et al. (2020). The role of nutrition in the prevention and intervention of type 2 diabetes. *Frontiers in Bioengineering and Biotechnology, 8*, 576. https://doi.org/10.3389/fbioe.2020.00576

Guo, Y., Huang, Z., Sang, D., Gao, Q., & Li, Q. (2020). The role of nutrition in the prevention and intervention of type 2 diabetes. *Frontiers in Bioengineering and Biotechnology, 8*, 575442. https://doi.org/10.3389/fbioe.2020.575442

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2017). Prevalence of obesity among adults and youth: United States, 2015-2016. *National Center for Health Statistics Data Brief, No. 288*. https://www.cdc.gov/nchs/products/databriefs/db288.htm

Hardy, C. J., & Rejeski, W. J. (1989). Not what, but how one feels: The measurement of affect during exercise. *Journal of Sport and Exercise Psychology, 11*(3), 304-317. https://doi.org/10.1123/jsep.11.3.304

Hawley, J. A., Hargreaves, M., & Zierath, J. R. (2006). Signalling mechanisms in skeletal muscle: Role in substrate selection and muscle adaptation. *Essays in Biochemistry, 42*, 1-12. https://doi.org/10.1042/bse0420001

Hellmund, R., Weitgasser, R., & Blissett, D. (2018). Cost calculation for a flash glucose monitoring system for adults with type 2 diabetes mellitus using intensive insulin: A UK perspective. *European Endocrinology, 14*(2), 86-92. https://doi.org/10.17925/EE.2018.14.2.86

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., Savović, J., Schulz, K. F., Weeks, L., & Sterne, J. A. C. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ, 343*, d5928. https://doi.org/10.1136/bmj.d5928

Holesh, J. E., Aslam, S., & Martin, A. (2023, May 12). *Physiology, carbohydrates*. In *StatPearls* [Internet]. StatPearls Publishing. Available from https://www.ncbi.nlm.nih.gov/books/NBK459280/

Holliday, A., Johnson, K. O., Kaiseler, M., & Crabtree, D. R. (2021). APPetite: Validation of a smartphone app-based tool for the remote measure of free-living subjective appetite. *British Journal of Nutrition, 129*(9), 1615-1625. https://doi.org/10.1017/S0007114521003512

Holloszy, J. O. (2005). Exercise-induced increase in muscle insulin sensitivity. *Journal of Applied Physiology (1985), 99*(1), 338–343. https://doi.org/10.1152/japplphysiol.00123.2005

Holmes, B. F., Sparling, D. P., Olson, A. L., Winder, W. W., & Dohm, G. L. (2005). Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated

protein kinase. *American Journal of Physiology: Endocrinology and Metabolism, 289*(6), E1071-E1076. https://doi.org/10.1152/ajpendo.00606.2004

Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., & Willett, W. C. (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine, 345*(11), 790-797. https://doi.org/10.1056/NEJMoa010492

Hussey, S. E., McGee, S. L., Garnham, A., Wentworth, J. M., Jeukendrup, A. E., & Hargreaves, M. (2011). Exercise training increases adipose tissue GLUT4 expression in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism, 13*(10), 959-962. https://doi.org/10.1111/j.1463-1326.2011.01426.x

International Hypoglycaemia Study Group (IHSG). (2015). Minimizing hypoglycemia in diabetes. *Diabetes Care, 38*(8), 1583-1591. https://doi.org/10.2337/dc15-0279

International Diabetes Federation (IDF). (2017). *IDF diabetes atlas* (8th ed.). International Diabetes Federation.

Imai, S., Fukui, M., & Kajiyama, S. (2014). Effect of eating vegetables before carbohydrates on glucose excursions in patients with type 2 diabetes. *Journal of Clinical Biochemistry and Nutrition, 54*(1), 7–11. https://doi.org/10.3164/jcbn.13-67

Imai, S., Fukui, M., Ozasa, N., Ozeki, T., Kurokawa, M., Komatsu, T., & Kajiyama, S. (2013). Eating vegetables before carbohydrates improves postprandial glucose excursions. *Diabetic Medicine, 30*(3), 370–372. https://doi.org/10.1111/dme.12073

Jenkins, D. J., Wolever, T., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., Jenkins, A. L., & Goff, D. V. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition, 34*(3), 362-366. https://doi.org/10.1093/ajcn/34.3.362

Jeukendrup, A. E. (2010). Carbohydrate and exercise performance: The role of multiple transportable carbohydrates. *Current Opinion in Clinical Nutrition and Metabolic Care, 13*(4), 452-457. https://doi.org/10.1097/MCO.0b013e328339de9f

Jeukendrup, A. E., & Killer, S. C. (2011). The myths surrounding pre-exercise carbohydrate feeding. *Annals of Nutrition and Metabolism, 57*(Suppl. 2), 18-25. https://doi.org/10.1159/000322698

Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International Journal of Sports Medicine, 26*(Suppl 1), S28-S37. https://doi.org/10.1055/s-2004-830512

Jentjens, R. L., & Jeukendrup, A. E. (2002). Prevalence of hypoglycemia following pre-exercise carbohydrate ingestion is not accompanied by higher insulin sensitivity. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(4), 398-413. https://doi.org/10.1123/ijsnem.12.4.398

Joseph, J. S., Ayeleso, A. O., & Mukwevho, E. (2017). Exercise increases hyper-acetylation of histones on the cis-element of NRF-1 binding to the Mef2a promoter: Implications on type 2 diabetes. *Biochemical and Biophysical Research Communications, 486*(1), 83-87. https://doi.org/10.1016/j.bbrc.2017.03.002

Kashima, H., Uemoto, S., Eguchi, K., Endo, M. Y., Miura, A., Kobayashi, T., & Fukuba, Y. (2016). Effect of soy protein isolate preload on postprandial glycemic control in healthy humans. *Nutrition*, *32*(9), 965-969. https://doi.org/10.1016/j.nut.2016.02.014

Kaviani, M., Chilibeck, P. D., Gall, S., Jochim, J., & Zello, G. A. (2020). The effects of low- and high-glycemic index sport nutrition bars on metabolism and performance in recreational soccer players. *Nutrients, 12*(4), 982. https://doi.org/10.3390/nu12040982

Khan, L. A., & Khan, S. A. (2000). Level of knowledge and self-care in diabetics in a community hospital in Najran. *Annals of Saudi Medicine, 20*(3-4), 300-301. https://doi.org/10.5144/0256-4947.2000.300

Khan, A. H., Thurmond, D. C., Yang, C., Ceresa, B. P., Sigmund, C. D., & Pessin, J. E. (2001). Munc18c regulates insulin-stimulated GLUT4 translocation to the transverse tubules in skeletal muscle. *Journal of Biological Chemistry, 276*(6), 4063-4069. https://doi.org/10.1074/jbc.M007419200

Kheir, N., Greer, W., Yousif, A., Al Geed, H., & Al Okkah, R. (2011). Knowledge, attitude, and practices of Qatari patients with type 2 diabetes mellitus. *International Journal of Pharmacy Practice, 19*(3), 185-191. https://doi.org/10.1111/j.2042-7174.2011.00118.x

Kirwan, J. P., Sacks, J., & Nieuwoudt, S. (2017). The essential role of exercise in the management of type 2 diabetes. *Cleveland Clinic Journal of Medicine, 84*(7 Suppl 1), S15-S21. https://doi.org/10.3949/ccjm.84.s1.03

Knight, J. B., Eyster, C. A., Griesel, B. A., & Olson, A. L. (2003). Regulation of the human GLUT4 gene promoter: Interaction between a transcriptional activator and myocyte enhancer factor 2A. *Proceedings of the National Academy of Sciences U.S.A., 100*(25), 14725-14730. https://doi.org/10.1073/pnas.2432756100

König, D., Theis, S., Kozianowski, G., & Berg, A. (2012). Postprandial substrate use in overweight subjects with the metabolic syndrome after isomaltulose (Palatinose™) ingestion. *Nutrition, 28*(6), 651-656. https://doi.org/10.1016/j.nut.2011.09.019

Kriska, A. M., Saremi, A., Hanson, R. L., Bennett, P. H., Kobes, S., Williams, D. E., & Knowler, W. C. (2003). Physical activity, obesity, and the incidence of type 2 diabetes in a high-risk population. *American Journal of Epidemiology, 158*(7), 669-675. https://doi.org/10.1093/aje/kwg191

Kueh, Y. C., Morris, T., Borkoles, E., & Shee, H. (2015). Modelling of diabetes knowledge, attitudes, self-management, and quality of life: A cross-sectional study with an Australian sample. *Health and Quality of Life Outcomes, 13*, 129. https://doi.org/10.1186/s12955-015-0303-8

Kuwata, H., Iwasaki, M., Shimizu, S., Minami, K., Maeda, H., Seino, S., Nakada, K., Nosaka, C., Murotani, K., Kurose, T., Seino, Y., & Yabe, D. (2016). Meal sequence and glucose excursion, gastric emptying, and incretin secretion in type 2 diabetes: A randomised, controlled crossover, exploratory trial. *Diabetologia, 59*(3), 453-461. https://doi.org/10.1007/s00125-015-3841-z

Lee, C. L., Shyam, S., Lee, Z. Y., & Tan, J. L. (2021). Food order and glucose excursion in Indian adults with normal and overweight/obese body mass index: A randomised

crossover pilot trial. *Nutritional Health, 27*(2), 161–169. https://doi.org/10.1177/0260106020975573

Ley, S. H., Hamdy, O., Mohan, V., & Hu, F. B. (2014). Prevention and management of type 2 diabetes: Dietary components and nutritional strategies. *The Lancet, 383*(9933), 1999-2007. https://doi.org/10.1016/S0140-6736(14)60613-9

Liese, A. D., Roach, A. K., Sparks, K. C., Marquart, L., D'Agostino, R. B., Jr., & Mayer-Davis, E. J. (2003). Whole-grain intake and insulin sensitivity: The Insulin Resistance Atherosclerosis Study. *American Journal of Clinical Nutrition, 78*(5), 965-971. https://doi.org/10.1093/ajcn/78.5.965

Lund, S., Holman, G., Schmitz, O., & Pedersen, O. (1995). Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proceedings of the National Academy of Sciences, 92*(13), 5817-5821. https://doi.org/10.1073/pnas.92.13.5817

Ma, J., Stevens, J. E., Cukier, K., Maddox, A. F., Wishart, J. M., Jones, K. L., Clifton, P. M., Horowitz, M., & Rayner, C. K. (2009). Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care, 32*(9), 1600–1602. https://doi.org/10.2337/dc09-0723

MacDonald, P. E., El-Kholy, W., Riedel, M. J., Salapatek, A. M., Light, P. E., & Wheeler, M. B. (2002). The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes, 51*(Suppl 3), S434–S442. https://doi.org/10.2337/diabetes.51.2007.s434

Magkos, F., Tsekouras, Y., Kavouras, S. A., Mittendorfer, B., & Sidossis, L. S. (2008). Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure. *Clinical Science (London), 114*(1), 59-64. https://doi.org/10.1042/CS20070134

Magurová, D., Majerníková, Ľ., Hloch, S., Tozan, H., & Goztepe, K. (2012). Knowledge of diabetes in patients with type 2 diabetes on insulin therapy from Eastern Slovakia. *Diabetologia Croatia, 41*, 95-102.

Malone, J. I., & Hansen, B. C. (2019). Does obesity cause type 2 diabetes mellitus (T2DM)? Or is it the opposite? *Pediatric Diabetes, 20*(1), 5-9. https://doi.org/10.1111/pedi.12787

Manson, J. E., Ajani, U. A., Liu, S., Nathan, D. M., & Hennekens, C. H. (2000). A prospective study of cigarette smoking and the incidence of diabetes mellitus among US male physicians. *American Journal of Medicine, 109*(7), 538-542. https://doi.org/10.1016/s0002-9343(00)00568-4

Marzban, S., Najafi, M., Agolli, A., & Ashrafi, E. (2022). Impact of patient engagement on healthcare quality: A scoping review. *Journal of Patient Experience, 9*, 23743735221125439. https://doi.org/10.1177/23743735221125439

Mathew, T. K., & Tadi, P. (2022). Blood glucose monitoring. In *StatPearls*. StatPearls Publishing. http://europepmc.org/abstract/MED/32310436

Matthews, J. N., Altman, D. G., Campbell, M. J., & Royston, P. (1990). Analysis of serial measurements in medical research. *BMJ, 300*(6719), 230-235. https://doi.org/10.1136/bmj.300.6719.230

McAulay, V., Deary, I. J., & Frier, B. M. (2001). Symptoms of hypoglycaemia in people with diabetes. *Diabetes Medicine, 18*(9), 690-705. https://doi.org/10.1046/j.1464-5491.2001.00620.x

McGee, S. L., Sparling, D., Olson, A. L., & Hargreaves, M. (2006). Exercise increases MEF2- and GEF DNA-binding activity in human skeletal muscle. *FASEB Journal, 20*(2), 348-349. https://doi.org/10.1096/fj.05-4671fje

McGee, S. L., van Denderen, B. J., Howlett, K. F., Mollica, J., Schertzer, J. D., Kemp, B. E., & Hargreaves, M. (2008). AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes, 57*(4), 860-867. https://doi.org/10.2337/db07-0843

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Annals of Internal Medicine, 151*(4), 264-269. https://doi.org/10.7326/0003-4819-151-4-200908180-00135

Monnier, L., & Colette, C. (2015). Postprandial and basal hyperglycaemia in type 2 diabetes: Contributions to overall glucose exposure and diabetic complications. *Diabetes & Metabolism, 41*(6 Suppl 1), 6S9-6S15. https://doi.org/10.1016/S1262-3636(16)30003-9

Mouri, M., & Badireddy, M. (2023, April 24). *Hyperglycemia*. In *StatPearls* [Internet]. StatPearls Publishing. Available from https://www.ncbi.nlm.nih.gov/books/NBK586214/

Muñoz Fabra, E., Díez, J.-L., Bondia, J., & Laguna Sanz, A. J. (2021). A comprehensive review of continuous glucose monitoring accuracy during exercise periods. *Sensors, 21*(2), 479. https://doi.org/10.3390/s21020479

Murad, M. H., Mustafa, R. A., Schünemann, H. J., Sultan, S., & Santesso, N. (2017). Rating the certainty in evidence in the absence of a single estimate of effect. *Evidence-Based Medicine, 22*(3), 85-87. https://doi.org/10.1136/ebmed-2017-110668

Nagoro, D. S., Tamtomo, D. G., & Indarto, D. (2019). A randomized control trial related to meal order of fruit, vegetable and high glycaemic carbohydrate in healthy adults and its effects on blood glucose levels and waist circumference. *Bali Medical Journal*, *8*(1), 247-254.

Nakhleh, A., & Shehadeh, N. (2021). Hypoglycemia in diabetes: An update on pathophysiology, treatment, and prevention. *World Journal of Diabetes, 12*(12), 2036-2049. https://doi.org/10.4239/wjd.v12.i12.2036

Nikolaidis, P. T., Rosemann, T., & Knechtle, B. (2018). Age-predicted maximal heart rate in recreational marathon runners: A cross-sectional study on Fox's and Tanaka's equations. *Frontiers in Physiology, 9*, 226. https://doi.org/10.3389/fphys.2018.00226

Nishino, K., Sakurai, M., Takeshita, Y., & Takamura, T. (2018). Consuming carbohydrates after meat or vegetables lowers postprandial excursions of glucose and insulin in nondiabetic subjects. *Journal of Nutritional Science and Vitaminology (Tokyo), 64*(5), 316–320. https://doi.org/10.3177/jnsv.64.316

Ormsbee, M. J., Bach, C. W., & Baur, D. A. (2014). Pre-exercise nutrition: The role of macronutrients, modified starches, and supplements on metabolism and endurance performance. *Nutrients, 6*(5), 1782-1808. https://doi.org/10.3390/nu6051782

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., et al. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Systematic Reviews, 10*(1), 89. https://doi.org/10.1186/s13643-021-01626-4

Palmer, M. K., & Toth, P. P. (2019). Trends in lipids, obesity, metabolic syndrome, and diabetes mellitus in the United States: An NHANES analysis (2003-2004 to 2013-2014). *Obesity (Silver Spring), 27*(2), 309-314. https://doi.org/10.1002/oby.22370

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological Reviews, 98*(4), 2133-2223. https://doi.org/10.1152/physrev.00063.2017

Pettitt, D. J., Knowler, W. C., Lisse, J. R., & Bennett, P. H. (1980). Development of retinopathy and proteinuria in relation to plasma-glucose concentrations in Pima Indians. *The Lancet, 2*(8203), 1050-1052. https://doi.org/10.1016/s0140-6736(80)92274-6

Pettus, J. H., Zhou, F. L., Shepherd, L., Preblick, R., Hunt, P. R., Paranjape, S., Miller, K. M., & Edelman, S. V. (2019). Incidences of severe hypoglycemia and diabetic ketoacidosis and prevalence of microvascular complications stratified by age and glycemic control in U.S. adult patients with type 1 diabetes: A real-world study. *Diabetes Care, 42*(12), 2220-2227. https://doi.org/10.2337/dc19-0830

Ploug, T., van Deurs, B., Ai, H., Cushman, S. W., & Ralston, E. (1998). Analysis of GLUT4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. *The Journal of Cell Biology, 142*(6), 1429-1446. https://doi.org/10.1083/jcb.142.6.1429

Pociot, F., & Lernmark, Å. (2016). Genetic risk factors for type 1 diabetes. *The Lancet, 387*(10035), 2331-2339. https://doi.org/10.1016/S0140-6736(16)30582-7

Quianzon, C. C., & Cheikh, I. E. (2012). History of current non-insulin medications for diabetes mellitus. *Journal of Community Hospital Internal Medicine Perspectives, 2*(3), 19081. https://doi.org/10.3402/jchimp.v2i3.19081

Rewers, M., & Ludvigsson, J. (2016). Environmental risk factors for type 1 diabetes. *The Lancet, 387*(10035), 2340-2348. https://doi.org/10.1016/S0140-6736(16)30507-4

Rico-Campà, A., Martínez-González, M. A., Alvarez-Alvarez, I., de Deus Mendonça, R., de la Fuente-Arrillaga, C., Gómez-Donoso, C., & Bes-Rastrollo, M. (2019). Association between consumption of ultra-processed foods and all-cause mortality: SUN prospective cohort study. *BMJ, 365*, l1949. https://doi.org/10.1136/bmj.l1949

Rodbard, H. W., Blonde, L., Braithwaite, S. S., Brett, E. M., Cobin, R. H., Handelsman, Y., Hellman, R., Jellinger, P. S., Jovanovic, L. G., Levy, P., Mechanick, J. I., & Zangeneh, F.; AACE Diabetes Mellitus Clinical Practice Guidelines Task Force. (2007). American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of diabetes mellitus. *Endocrine Practice, 13*(Suppl 1), 1-68. https://doi.org/10.4158/EP.13.S1.1

Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine, 48*(3), e219. https://doi.org/10.1038/emm.2016.6

Rodnick, K. J., Haskell, W. L., Swislocki, A., Foley, J. E., & Reaven, G. M. (1987). Improved insulin action in muscle, liver, and adipose tissue in physically trained human subjects. *American Journal of Physiology-Endocrinology and Metabolism, 253*(5), E489-E495. https://doi.org/10.1152/ajpendo.1987.253.5.E489

Rodriguez, N. R., Di Marco, N. M., & Langley, S. (2009). American College of Sports Medicine position stand. Nutrition and athletic performance. *Medicine and Science in Sports and Exercise, 41*(3), 709-731. https://doi.org/10.1249/MSS.0b013e31890eb86

Rossi, M. C., Nicolucci, A., Ozzello, A., Gentile, S., Aglialoro, A., Chiambretti, A., Baccetti, F., Gentile, F. M., Romeo, F., Lucisano, G., & Giorda, C. B.; HYPOS-1 Study Group of AMD. (2019). Impact of severe and symptomatic hypoglycemia on quality of life and fear of hypoglycemia in type 1 and type 2 diabetes: Results of the Hypos-1 observational study. *Nutrition, Metabolism and Cardiovascular Diseases, 29*(7), 736-743. https://doi.org/10.1016/j.numecd.2019.04.009

Ruderman, N. B., Carling, D., Prentki, M., & Cacicedo, J. M. (2013). AMPK, insulin resistance, and the metabolic syndrome. *The Journal of Clinical Investigation, 123*(7), 2764-2772. https://doi.org/10.1172/JCI67227

Sakaguchi, K., Takeda, K., Maeda, M., Ogawa, W., Sato, T., Okada, S., Ohnishi, Y., Nakajima, H., & Kashiwagi, A. (2016). Glucose area under the curve during oral glucose tolerance test as an index of glucose intolerance. *Diabetology International, 7*(1), 53–58. https://doi.org/10.1007/s13340-015-0212-4

Samson, S. L., Vellanki, P., Blonde, L., Christofides, E. A., Galindo, R. J., Hirsch, I. B., Isaacs, S. D., Izuora, K. E., Low Wang, C. C., Twining, C. L., Umpierrez, G. E., & Valencia, W. M. (2023). American Association of Clinical Endocrinology Consensus Statement: Comprehensive Type 2 Diabetes Management Algorithm – 2023 Update. *Endocrine Practice, 29*, 305-340. https://doi.org/10.1016/j.eprac.2023.02.001

Schwartz, M. W., & Porte, D., Jr. (2005). Diabetes, obesity, and the brain. *Science, 307*(5708), 375-379. https://doi.org/10.1126/science.1104344

Seino, Y., Fukushima, M., & Yabe, D. (2010). GIP and GLP-1, the two incretin hormones: Similarities and differences. *Journal of Diabetes Investigation, 1*(1–2), 8–23. https://doi.org/10.1111/j.2040-1124.2010.00022.x

Silbert, R., Salcido-Montenegro, A., Rodriguez-Gutierrez, R., Katabi, A., & McCoy, R. G. (2018). Hypoglycemia among patients with type 2 diabetes: Epidemiology, risk factors, and prevention strategies. *Current Diabetes Reports, 18*(8), 53. https://doi.org/10.1007/s11892-018-1018-0

Spriet, L. L. (2014). New insights into the interaction of carbohydrate and fat metabolism during exercise. *Sports Medicine, 44*(Suppl 1), S87-S96. https://doi.org/10.1007/s40279-014-0154-1

Shukla, A., Dickinson, M., & Jones, A. (2019). The impact of food order on postprandial glycemic excursions in prediabetes. *Diabetes, Obesity and Metabolism, 21*(2), 377–381. https://doi.org/10.1111/dom.13503

Shukla, A., Illiescu, R., & McDonald, T. (2015). Food order has a significant impact on postprandial glucose and insulin levels. *Diabetes Care, 38*(7), e98-e99. https://doi.org/10.2337/dc15-0429

Shukla, A. P., Andono, J., Touhamy, S. H., & et al. (2017). Carbohydrate-last meal pattern lowers postprandial glucose and insulin excursions in type 2 diabetes. *BMJ Open Diabetes Research & Care, 5*, e000440. https://doi.org/10.1136/bmjdrc-2017-000440

Shukla, A. P., Mauer, E., Igel, L. I., Truong, W., Casper, A., Kumar, R. B., Saunders, K. H., & Aronne, L. J. (2018). Effect of food order on ghrelin suppression. *Diabetes Care, 41*(5), e76-e77. https://doi.org/10.2337/dc17-2244

Stanojevic, V., & Habener, J. F. (2015). Evolving function and potential of pancreatic alpha cells. *Best Practice & Research Clinical Endocrinology & Metabolism, 29*(6), 859-871. https://doi.org/10.1016/j.beem.2015.10.002

Stevenson, E. J., Astbury, N. M., Simpson, E. J., Taylor, M. A., & Macdonald, I. A. (2009). Fat oxidation during exercise and satiety during recovery are increased following a low-glycemic index breakfast in sedentary women. *The Journal of Nutrition, 139*(5), 890-897. https://doi.org/10.3945/jn.108.101956

Sun, F.-H., Wong, S. H.-S., Huang, Y.-J., Chen, Y.-J., & Tsang, K.-F. (2012). Substrate utilization during brisk walking is affected by glycemic index and fructose content of a pre-exercise meal. *European Journal of Applied Physiology, 112*(7), 2565-2574. https://doi.org/10.1007/s00421-011-2231-6

Sun, L., Goh, H. J., Govindharajulu, P., Leow, M. K., & Henry, C. J. (2020). Postprandial glucose, insulin, and incretin responses differ by test meal macronutrient ingestion sequence (PATTERN study). *Clinical Nutrition, 39*(3), 950-957. https://doi.org/10.1016/j.clnu.2019.04.001

Tack, C., Pohlmeier, H., Behnke, T., Schmid, V., Grenningloh, M., Forst, T., & Pfützner, A. (2012). Accuracy evaluation of five blood glucose monitoring systems obtained from the pharmacy: A European multicenter study with 453 subjects. *Diabetes Technology & Therapeutics, 14*(4), 330-337. https://doi.org/10.1089/dia.2011.0170

Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology, 37*(1), 153-156. https://doi.org/10.1016/S0735-1097(00)01054-8

Tanamas, S. K., Permatahati, V., Ng, W. L., Backholer, K., Wolfe, R., Shaw, J. E., & Peeters, A. (2016). Estimating the proportion of metabolic health outcomes attributable to obesity: A cross-sectional exploration of body mass index and waist circumference combinations. *BMC Obesity, 3*, 4. https://doi.org/10.1186/s40608-016-0085-5

Taylor, S. I., Yazdi, Z. S., & Beitelshees, A. L. (2021). Pharmacological treatment of hyperglycemia in type 2 diabetes. *Journal of Clinical Investigation, 131*(2), e142243. https://doi.org/10.1172/JCI142243

Thomas, D. E., Brotherhood, J. R., & Brand-Miller, J. (1991). Carbohydrate feeding before exercise: Effect of glycemic index. *International Journal of Sports Medicine, 12*, 180-186. https://doi.org/10.1055/s-2007-1024664

Thomas, D. E., Brotherhood, J. R., & Miller, J. B. (1994). Plasma glucose levels after prolonged strenuous exercise correlate inversely with glycemic response to food consumed before exercise. *International Journal of Sport Nutrition, 4*(4), 361-373. https://doi.org/10.1123/ijsn.4.4.361

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Medicine and Science in Sports and Exercise, 48*(3), 543–568. https://doi.org/10.1249/MSS.0000000000000852

Tirone, T. A., & Brunicardi, F. C. (2001). Overview of glucose regulation. *World Journal of Surgery, 25*(4), 461-467. https://doi.org/10.1007/s002680020338

Tricò, D., Filice, E., Trifirò, S., & Natali, A. (2016). Manipulating the sequence of food ingestion improves glycemic control in type 2 diabetic patients under free-living conditions. *Nutritional Diabetes, 6*(8), e226. https://doi.org/10.1038/nutd.2016.33

Troiano, R. P., Berrigan, D., Dodd, K. W., Mâsse, L. C., Tilert, T., & McDowell, M. (2008). Physical activity in the United States measured by accelerometer. *Medicine and Science in Sports and Exercise, 40*(1), 181-188. https://doi.org/10.1249/mss.0b013e31815a51b3

Trüeb, R. M. (2020). Brief history of human nutrition. In *Nutrition for healthy hair* (pp. 15-25). Springer. https://doi.org/10.1007/978-3-030-59920-1_2

van Exel, E., Gussekloo, J., de Craen, A. J., Frölich, M., Bootsma-Van Der Wiel, A., & Westendorp, R. G.; Leiden 85 Plus Study. (2002). Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: The Leiden 85-Plus Study. *Diabetes, 51*(4), 1088-1092. https://doi.org/10.2337/diabetes.51.4.1088

Verma, S., Mazer, C. D., Fitchett, D., Inzucchi, S. E., Pfarr, E., George, J. T., & Zinman, B. (2018). Empagliflozin reduces cardiovascular events, mortality, and renal events in participants with type 2 diabetes after coronary artery bypass graft surgery: Subanalysis of the EMPA-REG OUTCOME® randomized trial. *Diabetologia, 61*(8), 1712-1723. https://doi.org/10.1007/s00125-018-4644-9

Verma, S., & McMurray, J. J. V. (2018). SGLT2 inhibitors and mechanisms of cardiovascular benefit: A state-of-the-art review. *Diabetologia, 61*(10), 2108-2117. https://doi.org/10.1007/s00125-018-4670-7

Villegas-Valverde, C., Kokuina, E., & Breff-Fonseca, M. (2018). Strengthening national health priorities for diabetes prevention and management. *MEDICC Review, 20*(4), 5. https://doi.org/10.37757/MR2018.V20.N4.2

Wei, M., Gibbons, L. W., Kampert, J. B., Nichaman, M. Z., & Blair, S. N. (2000). Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. *Annals of Internal Medicine, 132*(8), 605-611. https://doi.org/10.7326/0003-4819-132-8-200004180-00002

Wei, H., Lan, F., He, Q., Li, H., Zhang, F., Qin, X., & Li, S. (2017). A comparison study between point-of-care testing systems and central laboratory for determining blood glucose in venous blood. *Journal of Clinical Laboratory Analysis, 31*(3), e22051. https://doi.org/10.1002/jcla.22051

Whitley, H. A., Humphreys, S. M., Campbell, I. T., Keegan, M. A., Jayanetti, T. D., Sperry, D. A., MacLaren, D. P., Reilly, T., & Frayn, K. N. (1998). Metabolic and performance responses during endurance exercise after high-fat and high-carbohydrate meals. *Journal of Applied Physiology (1985), 85*(2), 418-424. https://doi.org/10.1152/jappl.1998.85.2.418

Wilson, P. B. (2017). Frequency of chronic gastrointestinal distress in runners: Validity and reliability of a retrospective questionnaire. *International Journal of Sport Nutrition and Exercise Metabolism, 27*(4), 370-376. https://doi.org/10.1123/ijsnem.2016-0305

Wing, R. R., Reboussin, D., & Lewis, C. E.; Look AHEAD Research Group. (2013). Intensive lifestyle intervention in type 2 diabetes. *New England Journal of Medicine, 369*(24), 2358-2359. https://doi.org/10.1056/NEJMc1312802

Wong, J. M., & Jenkins, D. J. (2007). Carbohydrate digestibility and metabolic effects. *Journal of Nutrition, 137*(11 Suppl), 2539S-2546S. https://doi.org/10.1093/jn/137.11.2539S

World Health Organization. (2011). *Diabetes fact sheet*. World Health Organization. https://www.who.int/europe/news-room/fact-sheets/item/diabetes

Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences, 11*(11), 1185-1200. https://doi.org/10.7150/ijms.10001

Wu, C. L., Nicholas, C., Williams, C., Took, A., & Hardy, L. (2003). The influence of high-carbohydrate meals with different glycaemic indices on substrate utilization during subsequent exercise. *British Journal of Nutrition, 90*(6), 1049-1056. https://doi.org/10.1079/bjn20031006

Yale, J.-F., Paty, B., & Senior, P. A. (2018). Hypoglycemia. *Canadian Journal of Diabetes, 42*, S104-S108. https://doi.org/10.1016/j.jcjd.2017.10.010

Zakrzewski, J. K., Stevenson, E. J., & Tolfrey, K. (2012). Effect of breakfast glycemic index on metabolic responses during rest and exercise in overweight and non-overweight adolescent girls. *European Journal of Clinical Nutrition, 66*(4), 436-442. https://doi.org/10.1038/ejcn.2011.175

Zhang, S., Li, W., Jia, X., Zhang, J., Jiang, H., Wang, L., Wang, H., Zhang, B., Wang, Z., & Ding, G. (2022). Association of obesity profiles with type 2 diabetes in Chinese adults: Findings from the China Health and Nutrition Survey. *Frontiers in Nutrition, 9*, 922824. https://doi.org/10.3389/fnut.2022.922824

Zignoli, A., Fontana, F. Y., Lipman, D. J., Skroce, K., Maturana, F. M., & Zisser, H. C. (2023). Association between pre-exercise food ingestion timing and reactive hypoglycemia: Insights from a large database of continuous glucose monitoring data. *European Journal of Sport Science*, *23*(12), 2340-2348. https://doi.org/10.1080/17461391.2023.2233468

Zinman, B., Wanner, C., Lachin, J. M., Fitchett, D., Bluhmki, E., Hantel, S., ... & Inzucchi, S. E. (2015). Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *New England Journal of Medicine, 373*(22), 2117-2128. https://doi.org/10.1056/NEJMoa1504720

VITA

Brian Ferguson

Department of Human Movement Sciences

4700 Powhatan Ave, Norfolk, VA 23529

Doctor of Philosophy (PhD) - Human Movement Science **2024**
Old Dominion University (Norfolk, VA)
Focus: Applied Physiology and Nutrition

Master of Science (M.S.) – Exercise Science **2020**
Auburn University (Auburn, AL)
Focus: Applied Physiology and Motor Behavior

Bachelor of Science (B.S) – Exercise Science **2018**
Auburn University (Auburn, AL)

Publications

Winter, I, **Ferguson, B**, Wilson, P. (2024). Associations between urine specific gravity and race/ethnicity at the population level: implications for hydration status categorization. *American Journal of Human Biology*. ;Epub ahead of print.

Ferguson, B., Wilson, P. (2023). Trunk-to-leg-volume ratio is not associated with bone density or fracture risk in middle-aged adults: results from the National Health and Nutrition Examination Survey. *Archives of Osteoporosis.* 18:118.

Wilson, P, **Ferguson, B,** Mavins, M, Ehlert, A. (2023). Anxiety and visceral sensitivity relate to gastrointestinal symptoms in runners but not pre- or during-event nutrition intake. *The Journal of Sports Medicine and Physical Fitness.* 63(7): 846-851.

Ferguson, B., Wilson, P. (2022). Ordered eating and its effects on various postprandial health markers: A systematic review. *Journal of the American Nutrition Association.* 1-12.

Vann C, Morton R, Brooks C, **Ferguson B**, Chetti I, Haun C, Osburn S, Sexton C, Fox C, Romero M, Roberson P, Oikawa S, Young K, McCarthy J, McGlory C, Phillips S, Roberts, M. (2021). An intron variant of the GLI Family Zinc Finger 3 (GLI3) gene differentiates resistance training-induced muscle fibre hypertrophy in younger men. *The FASEB Journal.* 35(5).

Vann C, Morton R, **Ferguson B**, Osburn S, Sexton C, Oikawa S, McGlory C, Young K, Phillips S, Roberts M. (2020). Targeted SNP interrogation to determine if select polymorphisms are associated with skeletal muscle hypertrophy following 12 weeks of resistance training. *The FASEB Journal.* 34(S1): 1-11.