The Effects of Pre-Exercise Modified Starch Ingestion on Adipose Tissue Lipolysis and Running Performance

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THE EFFECTS OF PRE-EXERCISE MODIFIED STARCH INGESTION ON ADIPOSE TISSUE LIPOLYSIS AND RUNNING PERFORMANCE

By

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ABSTRACT

BACKGROUND: It is well-documented that ingesting carbohydrate prior to exercise attenuates fat oxidation. However, it is yet to be established whether this effect is primarily the result of alterations in the mobilization of free fatty acids (FFA) from adipose tissue (i.e. lipolysis). Additionally, there is evidence suggesting that the glycemic index of carbohydrate influences the magnitude of the attenuation in fat oxidation. Specifically, low glycemic index carbohydrate increases fat oxidation relative to high glycemic index carbohydrate. Whether this effect is also due to alterations in adipose tissue metabolism is unknown. Finally, as increasing fat oxidation results in sparing of endogenous carbohydrate, it is possible that pre-exercise low glycemic index carbohydrate may enhance overall energy availability, particularly late in exercise, thereby enhancing endurance performance.

PURPOSE: To determine the impact of pre-exercise carbohydrate of different glycemic indices on subcutaneous abdominal adipose tissue (SCAAT) metabolism and running performance.

METHODS: Ten trained male runners (mass = 67.1 ± 7.4 kg, VO$_{2\text{max}}$ = 63.5 ± 5.3 ml·kg$^{-1}$·min$^{-1}$, 5-km personal best = 15.9 ± 3.3 min) completed three experimental trials consisting of 30 min at 60% VO$_{2\text{max}}$, 30 min at 75% VO$_{2\text{max}}$, and a 5-km time trial (TT). Thirty min prior to exercise, participants consumed one of three treatment beverages: 1) 75 g low glycemic index modified waxy maize starch supplement (UCAN), 2) 75 g high glycemic index sucrose- and glucose-based supplement (G), or 3) a flavor-, color-, and texture-matched non-caloric placebo (PL). SCAAT lipolysis was assessed via microdialysis. Resting and exercise gas exchange (i.e. VO$_2$ and fuel selection patterns) were assessed via indirect calorimetry. Glucose, insulin, catecholamine, FFA, and glycerol concentrations were analyzed in whole blood and/or plasma at rest and during exercise. Perceptual responses (i.e. gastrointestinal comfort and perceived exertion) during exercise were measured via visual analog scales. Data were analyzed via magnitude-based inferences (i.e. performance, gas exchange, and perceptual responses) and null hypothesis testing (i.e. plasma and interstitial variables; $p < 0.05$).
RESULTS: Immediately prior to exercise, blood glucose was elevated with G vs. PL (+53.0 ± 21.3 mg·dL$^{-1}$ [SD]; $p = 0.000$) and G vs. UCAN (+36.6 ± 24.9 mg·dL$^{-1}$; $p = 0.00007$). Additionally, insulin was increased prior to exercise with G vs. PL (+33.9 ± 11.0 µU·mL$^{-1}$; $p = 0.000$), UCAN vs. PL (+8.7 ± 4.4 µU·mL$^{-1}$; $p = 0.039$), and G vs. UCAN (+25.2 ± 11.0 µU·mL$^{-1}$; $p = 0.000$). VO$_2$ was increased prior to exercise with G vs. PL (+19.6% ± 12.5; likelihoods [%] increase/trivial/decrease: 98/1/0) and UCAN vs PL (10.9 ± 12.2%; 86/11/2). Carbohydrate oxidation was elevated prior to exercise with G vs. PL (+200.1 ± 89.9%; 100/0/0) and G vs. UCAN (+75.5 ± 20.0%; 99/0/0). In addition, carbohydrate oxidation was enhanced at 65% VO$_{2\text{max}}$ with G vs. PL (22.9 ± 17.5%; 95/5/0) and UCAN vs. PL (+75.5 ± 20.0%; 75/24/1). Fat oxidation was reduced with G vs PL (-50.1 ± 26.4%; 1/2/97) and G vs. UCAN (-121 ± 74.2%; 0/0/100) prior to exercise, and with G vs. PL (-14.6 ± 9.9%; 1/5/94) and UCAN vs. PL (-9.9 ± 6.8%; 0/10/90) during exercise at 65% VO$_{2\text{max}}$. While SCAAT lipolysis increased over time during exercise, there was no treatment effect. Similarly, plasma catecholamines and glycerol also increased over time but were unaffected by treatment. There was a main effect for time ($p = 0.00001$) and a treatment x time interaction ($p = 0.002$) for plasma FFA; however, post hoc testing revealed no significant differences between treatments. While UCAN likely attenuated abdominal cramping (-18.2 ± 14.3 units vs. PL; -10.0 ± 10.4 units vs. G), tiredness (-6.5 ± 6.6 units vs. PL), and the effort of running (-6.2 ± 5.7 units vs. G) following 60 min running, differences in TT performance (PL = 18.7 ± 1.8 min; G = 18.4 ± 2.0 min; UCAN = 18.3 ± 1.9 min) were unclear and/or trivial.

CONCLUSIONS: In conclusion, pre-exercise ingestion of low glycemic index modified starch attenuated the blood glucose and insulin response to feeding. Additionally, carbohydrate ingestion reduced fat oxidation, and this effect was attenuated at rest with low glycemic index carbohydrate. Despite these effects, adipose tissue metabolism and running performance were not influenced by pre-exercise carbohydrate regardless of glycemic index.
CHAPTER 1
INTRODUCTION

Most endurance events are completed at intensities (60-90% maximal oxygen consumption \([\text{VO}_2\text{max}]\)) that require the majority of energy expended to be derived from carbohydrate. Unfortunately, endogenous carbohydrate storage is limited [1]. As such, maximizing carbohydrate availability during endurance exercise is essential. This can be accomplished in two ways: 1) ingest carbohydrates either before and/or during exercise, and/or 2) augment utilization of alternative fuels (i.e. fat) in order to reduce the rate of carbohydrate utilization.

In certain ways, these goals are mutually exclusive. The ingestion of carbohydrates has physiological effects that attenuate the utilization of fat. Specifically, carbohydrate ingestion increases blood glucose levels causing the release of insulin from the pancreas [2]. Insulin binding to the muscle and adipose tissue substantially reduces the oxidation of fat [3]. Conversely, most nutritional methods designed to enhance fat oxidation, directly or indirectly reduce carbohydrate availability. For instance, ingesting a high fat diet reduces glycogen storage [4,5], but also attenuates the activity of enzymes on the mitochondrial membrane responsible for carbohydrate uptake (i.e. pyruvate dehydrogenase) [6].

Importantly, research suggests that simultaneous attainment of both goals (maintaining sufficient carbohydrate availability and maximizing fat utilization) may be achieved by ingestion of specific starch-based carbohydrates. Starches are the most common form of carbohydrate ingested by humans. Depending on their botanical origin, starch macromolecular and physicochemical structures vary widely, which affects starch digestion. Importantly, the rate at which starches are digested determines the magnitude of change in blood glucose, and thus, insulin. As such, starch digestion rates mediate effects on fat metabolism with slow-digesting (i.e. low glycemic index) and fast-digesting (i.e. high glycemic index) starches being positive and negative regulators of fat utilization, respectively. Of interest, starches can be molecularly modified by various processing methods in order to reduce digestion rates, and thus, enhance exercise fat utilization relative to high glycemic index carbohydrates/starches [7]. Worth noting, modification processes can also enhance starch solubility allowing for the development of starch-
based supplements. As such, modified starches could represent a novel low glycemic index supplement for endurance athletes.

Nevertheless, numerous questions remain to be answered. The specific effects of ingesting carbohydrate, generally, and low glycemic index starches, specifically, on fat metabolism are not fully elucidated. While carbohydrates have a well-documented inhibitory effect on fat oxidation, the mechanism of this effect is still somewhat equivocal. For instance, research is conflicting on the impact of carbohydrate ingestion on fat degradation (i.e. lipolysis) in the two primary storage depots, intramuscular triglycerides (IMTG) [8,9] and adipose tissue [3,10]. As adipose tissue is a vastly larger energy store relative to IMTG, the impact of carbohydrate ingestion on this fuel depot likely has major consequences for fuel availability during exercise and thus requires further research. Along these same lines, the impact of low glycemic index carbohydrate/starches on adipose tissue metabolism has yet to be investigated. Additionally, the precise impact that ingestion of low glycemic index carbohydrate/starches has on endurance performance is still equivocal. Numerous studies have reported endurance performance, (i.e. exercise capacity or time trial performance in running or cycling), to be either enhanced [11–17] or unchanged [7,18–32] with ingestion of low glycemic index relative to high glycemic index of carbohydrates/starches. These studies widely differ in their methodologies. As such, drawing firm conclusions is challenging, and further research is warranted.

**Aims**

1. To determine the degree to which pre-exercise carbohydrate ingestion attenuates adipose tissue lipolysis relative to a non-caloric placebo

2. To evaluate the impact of ingesting a low glycemic index modified starch supplement before exercise on adipose tissue lipolysis relative to a high glycemic index supplement

3. To assess the degree to which pre-exercise ingestion of a low glycemic index modified starch enhances running time trial performance.
Hypotheses

1. Pre-exercise carbohydrate ingestion will inhibit adipose tissue lipolysis relative to a non-caloric placebo in trained, male runners.
2. Adipose tissue lipolysis will be enhanced with pre-exercise ingestion of a low glycemic index modified starch supplement relative to a high glycemic index carbohydrate supplement in trained, male runners.
3. Running performance will be enhanced following ingestion of a low glycemic index modified starch supplement relative to a high glycemic index carbohydrate supplement in trained, male runners.

Assumptions

1. All participants accurately reported their training/racing history.
2. All participants followed instructions for dietary and training standardization between trials.
3. All participants completed the 5-km time trial at the maximum of their abilities.
4. All participants rated subjective feelings of gastrointestinal distress and perceived exertion honestly.
5. Participant fitness levels did not change appreciably over the course of the study.
6. Laboratory equipment gave accurate measurements during experimental trials.

Delimitations

1. Participants were restricted to 18-40 yr. Thus, findings may not be generalizable to younger or older populations.
2. Participants were trained runners from the Tallahassee area. Thus, findings may not be generalizable to untrained runners from Tallahassee or different geographical areas.
3. Only men were recruited to participate in this study. As such, results may not be generalizable to the female endurance athlete population.
4. The intensities prescribed during the exercise protocol were 60% and 75% VO$_{2\text{max}}$. Thus, findings may not be generalizable to other intensities.
5. All nutrients were consumed in liquid form pre-exercise. Thus, results may not be generalizable for solid foods.

**Limitations**

1. Blood sampling was accomplished via venous blood draw. As such, sampling required participants to stop exercising briefly, which may impact levels of metabolites and hormones in the blood.

2. Participants were recruited through personal contacts, flyers, and social media postings. The participants may not represent a truly random sample of trained runners.

**Definition of Terms**

**Adipose tissue**: fat stored subcutaneously or viscerally. In this paper, the term means subcutaneous adipose tissue unless otherwise specified.

**Glycemic index**: a 0-100 scale which classifies carbohydrates based on the area under the curve for glucose in the 2 hr following ingestion [33]. In this paper, the glycemic index will generally be used to describe high (80-100) or low glycemic index carbohydrates (< 50). Moderate glycemic index carbohydrates (50-80) will not be examined.

**Lipolysis**: the liberation of free fatty acids from an adipocyte. Lipolysis is generally measured via concentrations or appearance of glycerol. Thus, the term is generally in reference to changes in glycerol [34].

**Microdialysis**: a technique to assess metabolism in which a probe with a semi-permeable membrane is inserted into an area of interest to determine nutritive flow [35]. This paper will only examine microdialysis assessment of glycerol appearance in the muscle or subcutaneous adipose tissue.

**Modified starch**: a starch that has undergone chemical, enzymatic, or hydrothermal processing. Modification may or may not alter starch physical structure [36]

**Pre-exercise**: occurring before the start of exercise. In this paper, pre-exercise generally means ≥ 30 min prior to exercise.
**Performance**: a measure of physical function in a given task [37]. In this paper, performance will be used to describe outcomes in time trials, work completed in a given time trials, and time to exhaustion.

**Supplement**: a form of nutrients consumed additional to one’s diet. In this paper, supplements will primarily describe carbohydrate in powder form, which is dissolved in water (unless otherwise specified).

**VO\(_{2}\text{max}\)**: maximal aerobic capacity as measured by oxygen consumption.
CHAPTER 2
REVIEW OF LITERATURE

Low glycemic Index Modified Starches and Their Influence on Fat Metabolism and Endurance Performance

Endurance exercise has extreme energy demands that stress fuel availability. As such, efficient utilization of available fuels is required for optimal performance. Fuel utilization patterns vary based on a number of elements including sex, training status, and body composition [38]. Importantly, nutrition also has robust effects on fuel use and, in contrast to the other factors, which are difficult or impossible to alter, is easily manipulated. As a consequence, decades of research and experimentation have sought to optimize nutritional intake to enhance exercise metabolism and performance. In the continued search for a competitive edge, new strategies and products continue to be developed. One area of recent interest is nutritional upregulation of fat metabolism to augment endurance via the pre- and/or during-exercise ingestion of starches that have undergone specific processing techniques to alter their digestion. The following will examine the importance of how fat is used as a fuel source for endurance exercise, how carbohydrates and modified starches alter fat metabolism, and how this translates to endurance performance.

Metabolic Determinants of Endurance Performance

Primary exercise fuels are carbohydrate and fat. An examination of carbohydrate and fat storage locations and amounts reveal that carbohydrate is more readily available, but also much less abundant than fat. Carbohydrate is stored in the muscles (~350-700 g; 1,400-2,800 kilocalories [kcals]) and liver (~100 g; 400 kcals) as glycogen [1]. Small amounts of glucose also circulate in the blood (~20 g; 80 kcals), levels of which can be altered by the release of glucose from the liver via the hydrolysis of glycogen or generation of glucose from other sources (i.e. gluconeogenesis). Conversely, fat is primarily stored in adipose tissue (~8,000 g; 72,000 kcals) and in small amounts in the muscle (200 g; 1,800 kcals) [39].
The relative and absolute contribution of carbohydrate and fat to total energy expenditure during exercise varies with intensity. With increasing exercise intensity, there is a greater reliance on carbohydrate for fuel [40,41]. A stable isotope study by Van Loon et al., [41] revealed that at intensities corresponding to 40%, 55%, and 75% $W_{\text{max}}$, the relative contribution of carbohydrate to total energy expenditure was 45%, 51%, and 76%, respectively. Coinciding with an increased relative contribution, the absolute amount of energy (kcals) contributed by carbohydrate increased with increasing exercise intensity to meet higher energy demands. As such, increases in intensity corresponded to carbohydrate energy contributions of 5.76, 8.16, and 15.60 kcals·min$^{-1}$. Conversely, the fat energy contribution increased from 6.12 to 7.20 kcals·min$^{-1}$ at 40% and 55% $W_{\text{max}}$, respectively, but was reduced to 4.59 kcals at 75% $W_{\text{max}}$. Thus, from a moderate to high intensity, carbohydrate is more heavily relied upon and contributes more energy to total energy expenditure while fat oxidation is maximized at moderate intensities (55-65% $V_{O_2\text{max}}$), but reduced as intensity is increased.

Importantly, most endurance exercise is completed at intensities that depend heavily on carbohydrate fuels. In the marathon, for example, elite runners typically run at intensities eliciting ~80-90% $V_{O_2\text{max}}$ while non-elite trained runners often complete the race at 60-75% $V_{O_2\text{max}}$ [42,43]. Considering the reliance on carbohydrate and its relative scarcity compared to fat, endurance events such as marathon running will severely stress carbohydrate availability. Unfortunately, while the contribution of fat will increase over time during prolonged (≥2 hr) exercise [40], carbohydrate is required to maintain exercise intensity. Coggan and Coyle [44] elegantly illustrated this point. In this study, cyclists exercised at 70% $V_{O_2\text{max}}$ until exhaustion (i.e. the point at which they could no longer maintain the intensity). The authors found that infusion of glucose at a high rate (~1.1 g·min$^{-1}$) allowed the cyclists to continue exercising at the same intensity for an additional 43 ± 5 min vs. 10 ± 1 min when placebo was ingested ($p < 0.001$). This study reveals that carbohydrate availability is a primary limiter to endurance performance, and this conclusion has been consistently reaffirmed by studies in which performance was enhanced by augmentation of carbohydrate availability via pre- and/or during- exercise carbohydrate supplementation [e.g. reviewed in Ref # 9,10]. As such, maximizing carbohydrate availability is essential to endurance performance.

One possible method of augmenting carbohydrate availability during prolonged exercise is to reduce the rate at which endogenous carbohydrate is utilized (i.e. preservation or sparing). This
can be accomplished by increasing one’s reliance on fat as an exercise fuel. Indeed, increasing fat availability results in increased oxidation of fat during exercise. In a number of studies, participants were infused with heparin to artificially raise plasma free fatty acid (FFA) concentrations [47,48]. This resulted in increased fat oxidation and reduced carbohydrate oxidation. Importantly, these metabolic effects have also translated to enhanced endurance capacity [49]. While intriguing, the results of nutritional methods to augment fat utilization have been mixed. Ingesting fat before and/or during exercise induces gastrointestinal distress, which impairs performance [50–52]. Moreover, high fat diets increase fat utilization, but compromise carbohydrate stores. As a consequence, performance following such diets is, at best, maintained [5,53–56], and, at worst, impaired [57–61]. However, one area that has shown promise for augmenting fat utilization without necessarily compromising carbohydrate storage is ingestion of slow-absorbing carbohydrates [14,22,23,62,63], a primary source of these being starches.

**Starches and Their Modification**

*Starch sources and physical properties*

Starches are the primary storage form of carbohydrate in plants and can be found in pollen, leaves, fruits, tubers, bulbs, stems, roots, etc. Humans typically consume starches following extraction most commonly in the form of maize (corn). However, starches can be found in a variety of other forms including wheat, oats, potatoes, peas, and barley [64]. The botanical origin of starches plays an important role in determining a number of starch characteristics. Specifically, starch origin informs their structure and physiochemical properties. These characteristics are fundamental to starch digestibility and metabolism [65].

Starches are composed of two primary polymers, amylose and amylopectin. Amylose molecules are essentially linear with limited branching, while amylopectin is highly branched. Being mostly linear, amylose is primarily (99%) composed of α-(1-4) linkages with only a small (<1%) amount of α-(1-6) bonds at branch points [65]. Conversely, a relatively large amount of branching in amylopectin results in a greater proportion (5%) of linkages being derived from α-(1-6) bonds. Each amylose molecule contains ~1000 glucose units while amylopectin typically contains 3 x 10^5 or greater glucose units [65,66]. The ratio of amylose to amylopectin is used for classification of starches. Specifically, starches are classified as waxy, normal or high amylose.
based on amylose contents of ~15%, ~16-35%, and >36%, respectively [64]. Amylose and amylopectin are typically arranged in a semi-crystalline granule (Figure 1). This semi-crystalline structure allows for the maximization of energy storage for a given amount of space [64]. Within each granule are a number of concentric “light” and “dark” rings. The “dark” rings are composed of amylopectin double-helices, which are primarily responsible for the crystalline structure of the starch. Branching between “dark” amylopectin rings results in the less-structured amorphous lighter rings [67]. The specific location of amylose relative to amylopectin has been the subject of much investigation [68–70]. While the location is highly dependent of starch botanical origin, amylose is generally thought to be more concentrated in amorphous regions [68]. Amylose also tends to arrange in helical form and forms double helixes in high-amylose starches [71]. In contrast to amylopectin, amylose helices contain hydrogen atoms in their interior making them hydrophobic [72]. This characteristic permits the binding of amylose to lipids, iodine, and some alcohols, which has important implications for digestibility (discussed below). Variability in the crystallinity and location of amylose relative to amylopectin results in different starch classifications based on X-ray diffraction profiles (i.e. the diffraction angles of X-rays when passed through a starch granule). There are three basic starch types based on diffraction angle. Generally, A-type comprises cereal-type starches (e.g. maize), B-type comprises tuber starches (i.e. potato), and C-type is a mixture of A and B (common among legumes) [73,74].

The physical organization of starches into semi-crystalline granules prevents humans from digesting most native starches. As such, almost all starches consumed by humans undergo processing (e.g. cooking) to make these starches digestible. This processing typically involves suspending starches in water at high temperatures. The result of this processing is an almost complete breakdown of starch physical structures and an unwinding of amylopectin double-helices resulting in what is termed gelatinization [64]. During food storage, gelatinized starches undergo retrogradation, which is the partial re-association of amylose and amylopectin to form double helices and a semi-crystalline structure [64,75].
The influences of the above properties alter the digestion of starches allowing for categorization based on digestion rate. Starch digestibility is typically determined \textit{in vitro} as it is cheaper and less-labor intensive than \textit{in vivo} methods like the glycemic index (discussed below). The \textit{in vitro} method developed by Englyst et al. [76] is widely used and categorizes starches as either rapidly digesting starch (RDS), slowly digesting starch (SDS), or resistant starch (RS). With the Englyst method, enzymatic conditions present in the stomach and intestine are simulated to determine the rate of glucose release following starch exposure to this medium. The composition of a starch is determined based on the timeline of glucose release as follows: RDS, the amount of glucose released in 20 min; SDS, glucose released from 20-120 min; and RS, total starch minus glucose.

\textbf{Figure 1.} Schematic representation of starch granule structure [67,351]. (A) A single granule with alternating amorphous and semicrystalline layers, representing growth rings; (B) expanded view of the semicrystalline layer of a growth ring, consisting of alternating crystalline and amorphous lamellae; (C) the cluster structure of amylopectin within the semicrystalline layer of the growth ring.
the amount of glucose released in 120 min (i.e. all glucose released following 120 min) [76]. As the name signifies, RS resist digestion in the small intestine. These starches, more typically termed dietary fibers, are fermented in the colon to produce small chain fatty acids, which can be absorbed and utilized as an energy source [77]. Starches are composed of varying amounts of RDS, SDS, and RS. RDS are typically highly processed and/or freshly cooked starches such as breads or gelatinized waxy maize and SDS are common to native (raw) maize and legumes. RS are typically raw tubers like uncooked potato [76]. It should be noted that the Englyst method is not without certain drawbacks. For example, the exceedingly slow, but complete, digestion of certain SDS starches results in digestion occurring beyond 120 min. As such, the SDS content and RS content of certain starches are underestimated and overestimated, respectively [78].

The glycemic index

Starch digestibility in vivo is determined by the carbohydrate glycemic index. Following ingestion of a carbohydrate, glucose is absorbed in the small intestine and released into the blood stream. The degree to which a carbohydrate elevates blood glucose levels can be measured a categorized using this scale. Specifically, the glycemic index describes carbohydrate quality based on measurement of the incremental area under the curve of blood glucose responses following ingestion [33]. The greater the elevation in blood glucose, the greater is the glycemic index of the carbohydrate. Fast-digesting/absorbing starches and carbohydrates like white bread and glucose rapidly elevate blood glucose to high concentrations and thus have very high glycemic indices (~100). Slower digesting/absorbing foods like lentils have moderate and prolonged effects on blood glucose and thus have low glycemic indices (~30).

Like the Englyst method, the glycemic index is also an imperfect method for the measurement of carbohydrate digestibility. This is because, in contrast to in vitro methods, the glycemic index is influenced by a number of factors post-absorption. Specifically, co-ingestion of starches with other nutrients can substantially alter glycemic effects. For example, protein, which is insulino-tropic, can enhance the disappearance of blood glucose effectively lowering the glycemic index of a meal [79,80]. Moreover, the addition of dietary fiber (RS) to a meal can result in a low glycemic index response despite a high absorbability of ingested carbohydrate [81,82]. This may explain why in vitro measurements (RDS, SDS, and RS content) of starch-based foods may not always entirely correlate with glycemic index measurements [83].
Nevertheless, starches generally have “true” glycemic effects. That is, the glycemic effects of starches are almost entirely a result of their digestibility. As such, the composition of starches informs their glycemic index. For instance, starches high in RDS have been reported to have a higher glycemic than starches containing a large proportion of SDS and/or RS [84,85]. In this way, SDS starches can be viewed as an ideal low glycemic index starch as their slow, but complete, digestion results in a gradual rise and sustained elevation of blood glucose [78]. With this in mind, “glycemic index” terminology will be used consistently below to describe the effects of starch digestibility on physiology.

It is important to note that there are a number of non-starch carbohydrates with a low glycemic index. For instance, carbohydrates like isomalutose, trehalose, and fructose have unique glycosidic bonds and intestinal transport mechanisms that slow their digestion and absorption [25,86,87]. However, while slow to digest, ingestion of these types of carbohydrates may have different, and potentially undesirable, physiological effects relative to SDS. This is because these carbohydrate moieties either consist of, or are partly composed of, fructose [78]. Fructose is uniquely absorbed via the GLUT5 transporter in the small intestine [88]. Following absorption, the vast majority of fructose travels directly to the liver where fructose is phosphorylated to fructose-1-phosphate, which stimulates pyruvate production via pyruvate kinase [89]. Greater availability of pyruvate enhances anaerobic production of lactate, which is released into the blood [90–92]. Importantly, lactate downregulates lipolysis in adipose tissue (see Section: Adipose tissue). As such, starches may be a preferable source of low glycemic index carbohydrate.

**Starch digestion and modulating factors**

Starch digestion begins in the oral cavity. After consuming a starch, α-amylase present in saliva begins the initial breakdown of starch chains to shorter oligosaccharides. This process continues in the duodenum under the control of pancreatic α-amylase, which cleaves α-(1–4) bonds to produce maltose and branched dextrins [93]. Conversion to glucose requires diffusion of these products from the intestinal lumen into the brush border membrane. Here, final hydrolytic processes are carried out by sucrase-isomaltase, maltase-glucoamylase, and amyloglucosidase to produce glucose, which can then be absorbed via the sodium-dependent glucose transporter 1 (SGLT1) into the blood stream [94–96]. At the macromolecular level, starch hydrolysis occurs in two primary ways depending on granular surface properties. Some starches including waxy maize
have pores present on their surface permitting enzyme infiltration (Figure 2D) [97,98]. In these starches, enzyme hydrolysis occurs via an inside out and side-by-side pattern (Figure 3) [99]. In this mechanism, hydrolytic enzymes enter surface pores and begin to degrade the starch molecule from the walls of the pores outward (i.e. inside out). The hydrolysis of α-(1→4) bonds requires the binding of α-amylase. This enzyme is maximally stimulated with the binding of glucose at five subsites [100,101]. As relatively few glucose units are exposed when facing the head of an amylopectin double helix, binding and cleaving via α-amylase requires access to the sides of crystalline amylopectin branches. Surface pores permit this side-by-side hydrolysis to occur [99]. In the absence of surface pores (Figure 2A), amolytic hydrolysis must proceed via exocorrosion [102]. This mechanism involves the gradual hydrolysis of a starch granule starting at its surface and moving inward. Due to the above-noted binding characteristics of α-amylase, it follows that exocorrosion is a much slower process thereby limiting digestibility.

Figure 2. Scanning electron micrographs of starch granules from (A) potato, (B) wheat, (C) maize, magnitude × 600 [352], and (D) surface pores from waxy maize starch granules [98]. Reproduced from [93].
According to Colonna, Leloup, and Buléon [103], starch digestion is primarily limited by the diffusion of enzymes to the starch surface, the adhesion of the enzymes to the starch surface, and the hydrolysis itself. All of these limiters are influenced by factors both intrinsic and extrinsic to the starch. Intrinsic factors that influence starch digestion include the physical macromolecular size and structure, granular surface characteristics, the amylose to amyllopectin ratio, and the molecular structure of amyllopectin. Larger starch molecules have a smaller surface area to volume ratio, which slows $\alpha$-amylase hydrolysis [103–106]. Additionally, the type of crystallinity (A, B, or C) affects digestibility. Native cereal starches, which are mostly SDS, are composed of primarily A-type crystals [73,99,107]. Conversely, B-type crystals are more common in RS like raw potatoes [73,107]. Also common to A-type starches are granular surface pores [97,98]. As described above, this permits infiltration of $\alpha$-amylase thereby enhancing digestibility. In B-type starches, these pores are not present which increases the resistance to digestion [99,108]. A combination of these digestive properties is typically found with high C-type starches. A high ratio of amylose to amyllopectin is associated with a greater degree of resistance to digestion [102,109,110]. As such,

**Figure 3.** Schematic diagram to show the structure of cereal starch granule and the process of enzymatic digestion [99]. (A) Crystalline and amorphous layer structure, (B) the inside-out layer-by-layer digestion starts at the channel and always ends at the crystalline layer (black), (C) side-by-side digestion to enlarge the internal channel (top) and the resulting pyramidal shape residuals with less and less layers after fragmentation of starch granules (bottom).
most RS have high levels of amylose [111]. One potential explanation for the relative indigestibility of high amylose starches is that, following gelatinization and retrogradation, there is increased crystallinity in amorphous regions owing to increased amylose chain interactions preventing infiltration by α-amylase [112]. Additionally, it may also be due to the propensity of amylose to bind lipids and/or protein, which makes the starch granule less accessible to α-amylase [113–115].

The influence of starch fine structure on digestion becomes more apparent following gelatinization as this process more or less demolishes the starch macromolecular structure. When this occurs, starch digestibility is determined primarily by amylopectin chain length. Specifically, Zhang et al. [99] noted that amylopectin chains either long or short in length were slower to digest in gelatinized starches. This was likely explained by an increase in crystalline imperfections during retrogradation in the case of long chain amylopectin. With short chains, increased branching results in greater amounts of α-(1-6) bonds, the breakdown of which by amyloglucosidase is much slower than α-amylase breakdown of α-(1-4) bonds [101].

Starch digestibility is also influenced by a number of extrinsic factors. Specifically, gastric emptying rate regulates starch delivery to the small intestine. Reductions in the gastric emptying rate, which may occur with the ingestion of foods containing acids, vinegar, or high in fat content will slow starch digestion [116–118]. Digestion may also be altered by prior meals. In the well-documented “second meal effect,” digestion of starches in a lunch is slowed following a breakfast composed of primarily SDS and RS [119,120]. This is likely a consequence of RS fermentation products (i.e. short chain fatty acids) stimulating hormones which slow gastric motility [121]. Food viscosity also mediates digestibility as highly viscous fluids can slow transit time through the stomach and/or intestine, inhibit access of digestive enzymes, and/or impair diffusion to the mucosal membrane. Viscosity is altered by a number of food additives like guar gum and β-glucan [122,123].

Hydrothermal starch modification

Starches can be modified in a number of ways to alter their digestibility and glycemic index. A common modification method is hydrothermal treatment. Unlike the processing (e.g. milling, cooking, etc.) resulting in gelatinization, hydrothermal treatments such as heat-moisture treatment and annealing alter the fine structures of starch without altering its physical structure.
[65]. Heat-moisture treatment and annealing differ in their use of water and temperature. Heat-moisture treatment utilizes low moisture levels (10-30% w/w) and high temperatures (90-120°C) for an extended period of time, the precise amount being determined by the native starch type and desired effect [124,125]. In contrast, annealing requires heating a starch in an excess of water (>60% w/w) for a given amount of time [126]. Both hydrothermal treatments require the use of temperatures that fall below the gelatinization point for the given starch to prevent degradation of the starch granular structure [65].

Depending on the treatment conditions and starches in question, hydrothermal modification results in a wide variety of effects on digestibility. For example, heat-moisture treatment has been reported to enhance digestibility in maize, potato, rice, and cocoyam, but inhibit digestion in plantain flour [127]. Similarly, annealing has been reported by some to decrease the digestibility of wheat and lentils [112], while others have reported an increased digestibility of lentils as well as corn and peas [124]. Changes in digestibility may be explained by compositional changes. For instance, increases in the digestibility of potato starches following heat-moisture treatment corresponded with a doubling of SDS content [128]. Similarly, Chung et al. [124] reported that a heat-moisture treatment reduced the RDS content while increasing SDS and RS levels in maize, peas, and lentils. These changes in starch composition seem to be the result of physicochemical changes. For instance, reduced digestibility following heat-moisture treatment and annealing has been associated with an increase in crystallinity and a greater degree of crystallite packing [112,129]. Additionally, increases in digestibility have been reported following modification in conjunction with an increased appearance of surface pores and cracks facilitating greater enzyme penetration [130]. Finally, heat-moisture treatment-induced increases in A-type crystals relative to B- and C-type have also been noted corresponding to enhanced digestibility [112,128].

**Chemical and enzymatic starch modification**

Starches can also be modified enzymatically or chemically. Enzymatic treatment typically involves the application of either an amylase or branching enzymes in order to remove RDS, increase branching density, or shorten chains [131]. Chemical modifications include acid treatment, cross-linking, oxidation, esterification, and etherification. These treatments are utilized
to add or remove functional groups from starch molecules, which alter starch physical structure and digestibility [132,133].

Like hydrothermal modification, chemical and enzymatic modification can alter starch digestibility in various ways. For example, acid hydrolysis has been reported to increase digestibility as evidenced by large increases in RDS content and reductions in RS [108]. Other chemical modification techniques (oxidation, esterification, and dextrinization) have been shown to increase starch resistance to digestion [134]. Alternatively, enzymatic treatment can also be used to slow starch digestion (i.e. increase SDS content) [131].

These changes in digestibility can be attributed to a number of structural and/or physicochemical alterations. For example, acid treatment reduces α-(1-6) branching points, which typically slow digestion, and reduces the chain density in amorphous regions thereby potentiating increased α-amylase infiltration within the starch granule [74]. Octenyl succinic anhydride treatment results in the formation of esters which inhibit enzyme activity and digestion [135]. Conversely, treatment with branching enzymes can increase branching density thereby reducing the rate of starch digestion [131].

Modified starch applications

Modified starches have a number of potential applications. For example, modified starches are common in the food industry. As modification processes can enhance starch stability and enhance resistance to retrogradation, modification can improve starch shelf-life and improve cooking outcomes [136,137]. Modified starches are also useful in the production of paper and packaging materials due to their biodegradable nature [138]. Importantly, the modification of starches to produce low glycemic index carbohydrates is an application that is gaining popularity and has numerous potential benefits for various populations.

Recently, a starch was developed as a treatment for glycogen storage disease, a disorder which impairs endogenous glucose production from glycogen due to impairments in glycogen synthesis or breakdown (i.e. glycogenolysis) [139]. Individuals with this condition rely on blood glucose to maintain normal function. As such, low glycemic index starches are commonly utilized to maintain euglycemia in these patients. Importantly, it was discovered that heat-moisture treatment (20% moisture w/w at 105 °C for 16 hr) of a waxy maize starch dramatically altered its digestibility and glucose release profile relative to uncooked starch [140]. Specifically, compared
to an uncooked cornstarch with a glycemic index of 70, the hydrothermal treatment of the waxy (~100% amylopectin) maize reduced its glycemic index to 30. As a consequence, the modification of the starch extended the duration of glucose release. Indeed, Bhattacharya et al. [141] and Correia et al. [142] reported that ingestion of the modified starch maintained euglycemia (≥60 mg/dL) for a longer duration relative to the uncooked cornstarch suggesting a prolonged and moderate release of glucose (9 hr vs. 7 hr) [141]. In addition to patients with glycogen storage disease, modified starches may also benefit individuals with diabetes. As diabetics struggle to maintain consistent glucose levels, it is recommended that they consume low glycemic index carbohydrates [143]. The addition of low glycemic index modified starches to their meals may thus be beneficial for their blood glucose homeostasis.

Modified low glycemic index starches may also have applications for athletes. Native (i.e. unprocessed/modified) starches are insoluble in water. Starch modification can increase solubility allowing for development of starch-based supplements that are soluble in water, which can be consumed during exercise. Additionally, the complex and dense structure of starches results in a high molecular weight. As high molecular weight molecules have a low osmolarity in fluid, modified starch-based sport drinks would presumably not impair, and may enhance, gastric emptying and/or fluid absorption relative to traditional high-osmolarity sport drinks [144]. Finally, the slow glucose release of a modified low glycemic index starch would potentially provide a sustained energy source with less of the hormonal perturbations associated with ingestion of traditional very high glycemic index sport supplements (discussed below). These effects have important implications for fat metabolism and endurance performance.

**Fatty Acid Mobilization and Oxidation**

The mobilization of FFA and its oxidation in the muscle is a complex process involving multiple potentially limiting steps subject to modulation via nutritional stimuli. Specifically, FFA must be liberated from adipocytes (i.e. lipolysis), delivered to the muscle and/or mitochondria, transported into the mitochondria, and ultimately oxidized. Importantly, these steps have unique features depending on the origin of the FFA. As mentioned, primary storage depots for fat are in adipose tissue and IMTG. However, fat can also be found on the outer surface of muscle fibers (extramuscular triglycerides, EMTG) and circulating in the blood associated with lipoproteins.
Finally, the rate of oxidation of FFA from the various depots is partially governed by interactions between them; greater utilization of FFA from one source may spare FFA from elsewhere. As such, the potential impact of nutrition must be understood not only as it pertains to whole body fat oxidation, but in how it affects depot-specific FFA utilization. The following will focus primarily on adipose tissue and IMTG as EMTG metabolism is not well-understood and the contribution of lipoprotein-derived FFA seems to be relatively small [147].

Adipose tissue

Oxidation of FFA derived from adipose tissue begins with lipolysis. Adipose tissue lipolysis is the result of the actions of specific lipases. Following a signaling cascade, adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoglyceride lipase (MGL) break down adipocytes to produce triglycerides, diacylglycerides, and monoacylglycerols, respectively. Each reaction results in the release of an FFA [148–150].

There are numerous neurohormonal stimuli which regulate lipolysis in adipose tissue, the impact of which is dependent upon receptor type and density on adipocyte membranes [151]. Primary positive regulators are catecholamines (i.e. epinephrine and norepinephrine) and natriuretic peptides (i.e. atrial and β-type). Catecholamines upregulate lipolysis by binding to β-adrenergic receptors (β₁, and β₂) on the adipocyte surface [152,153]. Binding at β₁/2 receptors results in the disassociation of Gαₙ resulting in the activation of adenylyl cyclase and the formation of cyclic-AMP (cAMP). Production of cAMP activates protein kinase A resulting in the phosphorylation of HSL and perilipin proteins, specifically PLIN1, on the adipocyte surface [154–156]. PLIN1 phosphorylation results in fragmentation of the adipocyte increasing the available surface area for the action of lipases [157]. Moreover, it also results in the release of comparative gene identification-58, which activates ATGL [158]. HSL phosphorylation stimulates the lipase to move to the adipocyte surface and dock with PLIN1 whereby it acts on diacylglyceride [159]. Natriuretic peptides act through similar yet distinct pathways. These peptides bind to guanylyl cyclase-linked type-A receptors resulting in guanylyl cyclase-induced conversion of GTP to cGMP. Production of cGMP activates protein kinase G which, similar to protein kinase A, phosphorylates HSL and PLIN1 to commence lipolysis [160].

The rate-limiting step for adipose tissue lipolysis is somewhat uncertain but is clearly mediated by ATGL and HSL. Langin et al. [161] inhibited HSL activity in isolated human
adipocytes and examined basal and catecholamine-stimulated lipolytic responses. Importantly, HSL inhibition resulted in total inhibition of catecholamine-stimulated lipolysis. In addition, basal lipolysis was reduced 50%. These results suggest that during catecholamine stimulation, HSL is the primary limiter of adipose tissue lipolysis, but basally other lipases like ATGL may also play a significant role. This central role of HSL to stimulated lipolysis is supported by data suggesting a correlation between lipolytic capacity and HSL expression [162]. In contrast, Bezaire et al. [163] found that inhibition of ATGL reduced basal lipolysis and nearly completely ablated stimulated lipolysis whereas HSL inhibition had little effect in either condition. Taken together, determining the relative importance of ATGL and HSL for basal and stimulated lipolysis requires further research. However, it is clear that both lipases are required for achieving maximal lipolytic capacity [163,164].

Catecholamines are also responsible for downregulation of lipolysis. This occurs via the actions of $\alpha$-adrenergic receptors, specifically $\alpha_2$. Following binding of catecholamines, $G_{o_i}$ protein disassociation from the $\alpha_2$ receptor complex results in inhibition of adenylyl cyclase thereby reducing the production of cAMP [153]. Another primary negative regulator of lipolysis is insulin. Insulin binding to its receptor on the adipocyte membrane downregulates lipolysis via multiple pathways. In the first, insulin binding initiates a well-described signaling cascade ending in phosphorylation of Akt [165]. This results in the activation of phosphodiesterase 3B (PDE3B) which degrades cAMP. This pathway seems to dominate with maximal adrenergic stimulation. In contrast, with submaximal stimulation, insulin binding seems to inhibit lipolysis via an Akt-independent pathway which inhibits PLIN1 phosphorylation [166]. Interestingly, the antilypolytic actions of insulin seem to be partially mediated by lactate, which is itself an antilypolytic agent [167,168]. Indeed, insulin binding at either muscle or adipose results in increased glucose metabolism, which increases lactate production [169]. Lactate released from adipocytes can bind to the lactate GPR81 receptor on adipocyte membranes [168]. This receptor is coupled to $G_{o_i}$, and binding inhibits cAMP production [170]. Thus, as a result of insulin binding, lipolysis is inhibited not only by the above-described PDE3B and PLIN1 mechanisms, but as a result of increased lactate production leading to $G_{o_i}$-induced modulation of cAMP. This suggests maximal lipolytic suppression is the result of additive inhibitory signals. Indeed, Ahmed et al. [171] found that in mice lacking the GPR81 receptor, the drop in FFA levels following a rise in insulin was substantially attenuated suggesting these mechanisms work in concert to reduce lipolysis.
Once FFA have been produced, they are subject to various fates which may, or may not, include delivery to the muscle for uptake and oxidation. Lipolysis is constantly occurring to some degree even when FFA demand is low. As a consequence, a majority of FFA produced are re-esterified. Wolfe et al. [172] noted that, at rest, ~70% of whole body FFA were re-esterified. Alternatively, some FFA may be released into the circulation and taken up by the liver only to be converted via glycerol 3-phosphate back into triglycerides. Following conversion, these new triglycerides are often released back into the circulation bound to very low-density lipoproteins [173]. If liberated FFA escape these two fates, they are available to be transported (via albumin) to the muscle where ~50% of circulating FFA are taken up into the muscle during rest [174,175]. Importantly, this process is limited by blood flow and/or perfusion of the adipose tissue which is variable depending on the physiological state (i.e. rest/exercise, fasted/fed, etc.) [176].

At the sarcolemma, FFA entry into the muscle is largely dependent on the availability of protein transporters. The two most highly-investigated transporters to date are fatty acid binding protein (FABP) and fatty acid translocase CD36 (FAT/CD36) [177]. Interestingly, these proteins appear to act similarly to GLUT4, the transport protein responsible for glucose uptake. Indeed, FABP and FAT/CD36 translocate from intracellular compartments in response to stimuli (e.g. contraction or insulin binding) to facilitate FFA uptake into the muscle [177,178].

Once in the cytosol, FFA can either be directed towards oxidation or re-esterified to IMTG. At rest, a majority (50-60%) of incoming FFA are incorporated into IMTG [174,179]. FFA directed to oxidation must undergo a number of reactions in order to cross the mitochondrial membrane. First, a coenzyme A (CoA) is attached to the FFA via acetyl CoA synthetase, which is likely located on the mitochondrial membrane, to produce fatty acyl-CoA [180]. This molecule then reacts with the carnitine palmitoyltransferase 1 (CPT1) complex on the outer mitochondrial membrane. This reaction produces acetylcarnitine, which crosses the outer mitochondrial membrane to react with carnitine palmitoyltransferase 2 (CPT2) at the inner mitochondrial membrane. CPT2 converts acetylcarnitine to acyl-CoA, which can then enter β oxidation [181].

Mobilization of FFA from adipose tissue during exercise

During exercise, there is a dramatic and rapid upregulation of fat metabolism allowing for a 5- to 10-fold increase in fat oxidation [182]. Importantly, the majority of this increase is derived from adipose tissue FFA (~40-60%) [40,183]. This increased contribution is due to a number of
factors. When going from rest to exercise, there is an increase in circulating catecholamine levels, which rise almost exponentially as intensity increases [40]. As a result, stimulation of $\beta_{1/2}$ receptors increases. This overrides any inhibitory influence of $\alpha_2$, which is the dominant receptor during resting conditions [184]. As a consequence, lipolysis increases 2-3-fold [40,41,185]. Additionally, there are substantial changes in rates of re-esterification and adipose tissue blood flow. Specifically, the rate of FFA re-esterification drops to 25% or lower [172], and blood flow increases 2-3-fold [186,187]. These combined effects increase FFA release into the circulation.

In skeletal muscle, a number of changes occur which also support increased adipose tissue-derived FFA oxidation. First, muscle blood flow increases 10- to 15-fold during exercise [185]. Additionally, muscle contractions increase FAT/CD36 and FABP translocation to the sarcolemma, which is likely mediated by calcium and calmodulin-dependent kinases and extracellular signal regulated kinase 1/2 [188,189]. While these changes would presumably enhanced FFA delivery and uptake, the fractional rate of plasma FFA uptake actually decreases to ~20% (from ~50%) possibly owing to the increased velocity of blood flow [147,174,175]. However, as a result of the increased concentration of FFA in the plasma resulting from enhanced lipolysis and the increased blood flow, absolute FFA uptake increases substantially. Importantly, the FFA fractional extraction rate remains stable among endurance-trained individuals even as FFA delivery increases with exercise duration allowing for increased total FFA uptake late in exercise [174,190]. Once inside the skeletal muscle, FFA handling is also unique during exercise. In contrast to rest where large amounts of FFA are re-esterified to IMTG, 85-100% of FFA taken up is directly oxidized within the mitochondria during exercise [147,174,175].

The oxidation of adipose-derived FFA is seemingly limited by different mechanisms depending on the intensity of exercise. At low-to-moderate intensities (25-65% VO$_{2\text{max}}$), FFA delivery to the muscle seems to be the primary limiter of FFA oxidation. Indeed, fat oxidation increases with duration during moderate intensity exercise, and this coincides with increased FFA delivery [40,175]. Furthermore, manipulation of plasma FFA levels results in predictable changes in fat oxidation at these intensities. Various studies have utilized nicotinic acid administration to abolish lipolysis resulting in dramatic reductions in plasma FFA [191,192]. As a consequence, total fat oxidation rates have been reported to drop upwards of ~35% during exercise following nicotinic acid administration [191]. Conversely, ingestion or infusion of fat emulsion in
combination with heparin administration significantly increases plasma FFA levels resulting in concomitant increases in fat oxidation [47,48,192,193].

Fat oxidation rates at high intensities drop substantially relative to moderate intensity exercise [40,41]. Indeed, van Loon et al. [41] noted fat oxidation levels at 75% VO\(_{2\text{max}}\) to be lower than those at 40% VO\(_{2\text{max}}\). It is tempting to suggest that this reduction in fat oxidation is the result of the same factors which limit FFA oxidation at moderate exercise intensities (i.e. FFA delivery). In fact, FFA delivery during high-intensity exercise is dramatically reduced [40,41] likely as a result of reduced adipose tissue blood flow owing to sympatho-adrenal-mediated blood flow redistribution to the working muscle [194,195]. Nevertheless, oxidation of adipose-derived FFA at high intensities may not be limited primarily by FFA delivery. In a study by Romijn et al. [48], plasma FFA levels during high-intensity exercise (85% VO\(_{2\text{max}}\)) were manipulated via fat emulsion and heparin in order to match concentrations typically observed at 65% VO\(_{2\text{max}}\). Interestingly, the authors reported this to increase fat oxidation rates by 27%. However, this increase did not fully compensate for the drop in fat oxidation (50%) when exercise increased in intensity from 65% to 85% VO\(_{2\text{max}}\) in the control condition. Thus, this study suggests factors other than delivery limit FFA oxidation during high-intensity exercise. This is supported by others who reported the accumulation of intramuscular FFA during high-intensity exercise (90% VO\(_{2\text{max}}\)) [196]. Taken together, these studies indicate that intramuscular factors limit FFA oxidation during high-intensity exercise. One potential explanation is reduced transport of FFA into the mitochondria owing to inhibition of CPT1. During high-intensity exercise, enhanced glycolytic flux enhances acetyl CoA formation. To buffer rising acetyl CoA level and thereby attenuate any feedback inhibition, acetyl CoA can be converted to malonyl CoA and/or combined with carnitine to form acetylcarnitine + CoASH [34,197,198]. Both products inhibit CPT1 activity (see section: Carbohydrate ingestion and mitochondrial FFA transport).

**Intramuscular triglycerides**

IMTG metabolism is similar in many ways to adipose tissue. Like adipose tissue, IMTG lipolysis is mediated by ATGL, HSL, and MGL [199]. Moreover, regulation of IMTG lipolysis shares many of the same features as adipose. For instance, catecholamines are potent stimulators of these intramuscular lipases [200,201]. Additionally, IMTG are also re-esterified at high rates during rest [174]. Similarities are also present during exercise. IMTG are readily oxidized during
exercise. A number of studies have reported that with long duration (2-3 hr), moderate intensity exercise, IMTG stores can be depleted by as much as 50-70% [8,9,202–205]. Moreover, oxidation of IMTG FFA may constitute upwards of 50% of total fat oxidation during exercise [41].

However, the regulation of IMTG lipolysis and re-esterification has important distinctions from adipose, which impact its availability as an exercise fuel. First, the roles and relative importance of HSL and ATGL in IMTG lipolysis seem to differ from adipose. HSL and ATGL are both heavily involved in IMTG lipolysis. Alsted et al. [206] reported that 98% of skeletal muscle lipase activity is explained by HSL and ATGL. Additionally, the actions of these lipases appear to be mediated by perilipins [207,208]. However, HSL may play a more secondary role relative to ATGL. With catecholamine stimulation and/or exercise, HSL translocates to the IMTG surface, and IMTG levels are reduced [209]. However, IMTG lipolysis in mouse skeletal muscle is unaffected by pharmacological or genetic knockout of HSL [206]. Moreover, IMTG lipolysis independent of HSL activity has also been reported in humans [210]. Thus, these data suggest that ATGL plays a more prominent role in IMTG lipolysis. This notion is supported by Haemmerle et al. [211], where a 20-fold increase in myocyte IMTG content was reported in ATGL knockout mice.

Another unique aspect of IMTG regulation is the lipolytic response to insulin. Unlike in adipose tissue, where insulin binding has well-documented inhibitory effects, the influence of insulin on IMTG lipolysis is less clear. The earliest study to examine this question reported insulin to downregulate IMTG lipolysis [212]. Moreover, this study found the effects of insulin at physiologic levels to be substantially more potent at the muscle relative to adipose. While this finding has been supported by some [213], others have reported insulin to have no effect on IMTG lipolysis [214–216]. These divergent findings may be explained by methodological differences (e.g. IMTG lipolysis measurement via radioactive isotope, biopsy, spectroscopy, etc.). It is also possible that IMTG lipolysis regulation is heavily influenced by factors other than hormonal stimuli.

For instance, rates of IMTG utilization seem to change based on FFA availability. Watt et al. [210] had participants cycle for 3 hr following nicotinic acid ingestion which completely suppressed the exercise-induced increase in plasma FFA. As a consequence, IMTG utilization increased 3-fold with nicotinic acid relative to the control condition. Importantly, others have confirmed this finding that low plasma FFA is associated with increased IMTG breakdown [191].
Conversely, when plasma FFA levels are high, the opposite also seems to be true. Utilizing heparin combined with fat emulsion ingestion significantly elevates plasma FFA levels and results in IMTG accumulation (i.e. more synthesis than degradation) [217,218]. This phenomenon also seems to apply without pharmacological intervention. High fat diets and starvation increase plasma FFA, both of which coincide with IMTG accumulation [219]. Furthermore, in the latter stages of prolonged exercise when FFA delivery from adipose rises, IMTG utilization is reduced [40].

Skeletal muscle lipolysis is also distinct from adipose in terms of glycerol turnover and re-esterification, particularly during exercise. Glycerol, which is produced along with FFA during triglyceride lipolysis, is used as a marker of lipolysis. This is because, unlike FFA, glycerol cannot be reincorporated into triglyceride or metabolized without the availability of the enzyme glycerol kinase [220]. As glycerol kinase is predominantly found in the liver, it was assumed that the vast majority of glycerol, if not all, released from adipose tissue or muscle was metabolized in the liver, and that little glycerol was taken up by other tissues [221]. However, more recent research indicates that other tissues including skeletal muscle and adipose tissue contain small amounts of glycerol kinase and that glycerol can be taken up by these tissues [220,222,223]. Importantly, this capacity for glycerol uptake in skeletal muscle seems high relative to adipose tissue as evidenced by glycerol cycling (i.e. simultaneous release and uptake of glycerol during exercise). For instance, Stallknecht et al. [223] found that with moderate to high-intensity exercise, adipose tissue net glycerol release increased 10-fold. In contrast, there was no net release of glycerol from skeletal muscle at the same intensities despite IMTG lipolysis, which indicated significant glycerol cycling [222]. Interestingly, glycerol taken up by skeletal muscles has been reported to be incorporated into IMTG of mice [224]. Furthermore, significant glycerol uptake combined with no reductions in IMTG levels during exercise have been reported in humans supporting the notion of glycerol incorporation into triglycerides [222].

This potential glycerol incorporation likely explains the high rates of IMTG re-esterification during exercise. As noted above, IMTG are re-esterified at a high rate at rest [174,225]. However, unlike adipose tissue, this rate of re-esterification is not substantially reduced during exercise [174,225]. Sacchetti et al. [174] measured IMTG turnover using labelled palmitate tracers during 5 hr of one-legged extensor exercise. The authors noted that IMTG synthesis, although reduced from 3.8% to 1.0% per hour due to a reduced incorporation of plasma-derived FFA, was maintained during exercise. IMTG esterification also remains high when FFA demand is
artificially elevated by lowering plasma FFA availability. In the above-mentioned study by Watt et al. [210], nicotinic acid suppressed plasma FFA and this was associated with increased utilization of IMTG. Nevertheless, the authors noted that despite this increased IMTG utilization, rates of re-esterification as assessed by the activity of enzymes responsible for IMTG synthesis, (glycerol-3-phosphate acyltransferase), were unchanged.

These high rates of IMTG synthesis may impact the utility of IMTG as a source of FFA during exercise as it likely inhibits the capacity of IMTG to rapidly respond to changes in energy demand [226]. Indeed, while IMTG are a proximal source of FFA in the muscle, the capacity for oxidation of IMTG FFA seems limited. Nicotinic acid-induced suppression of plasma FFA levels increases IMTG utilization. However, IMTG cannot completely compensate for the loss of plasma FFA as evidenced by reductions in total fat oxidation [191,210]. As a result, there is a compensatory increase in the oxidation of carbohydrates, which will reduce carbohydrate availability during prolonged exercise [191,210]. Thus, it is clear that adequate adipose tissue delivery of FFA is essential to maintain total fat oxidation and to attenuate declines in endogenous carbohydrate levels. Importantly, carbohydrate ingestion may have more potent effects on adipose tissue versus IMTG.

**Effects of Carbohydrate and/or Starch Ingestion on Exercise Fat Metabolism**

Ingesting carbohydrates in any form has robust physiological effects which alter fat metabolism. Following ingestion, carbohydrate is absorbed in the small intestine via facilitated diffusion. Carbohydrate immediately enters the portal vein resulting in elevations of blood glucose. High concentrations of glucose in the blood increase glucose binding at the β cells of the pancreas stimulating the release of insulin [2]. As mentioned above, insulin plays a primary role in the downregulation of various mechanisms involved in fat metabolism. As a result, carbohydrate ingestion before and/or during exercise (relative to fasting) results in substantial reductions in fat oxidation [3,11,19,22,227–244]. Indeed, Achten and Jeukendrup [245] reported that pre-exercise glucose ingestion (75 g) reduced maximal fat oxidation rates by 28% and reduced the intensity that elicits that maximal fat oxidation rates by 14% (60.1 ± 1.9% to 52.0 ± 3.0% VO$_{2\text{max}}$). Importantly, these reductions are the result of alterations in FFA delivery to the muscle and changes in FFA metabolism within the muscle itself (e.g. IMTG utilization and FFA entry into the
mitochondria). Furthermore, these effects are partially mediated by the timing of carbohydrate intake (e.g. before versus during exercise).

**Effects of carbohydrate ingestion on FFA mobilization from adipose tissue**

Carbohydrate ingestion reduces FFA delivery to the muscle. Indeed, numerous studies have reported pre- and or during-exercise carbohydrate ingestion to reduce plasma levels of FFA [for example see Ref #18,22,235,249]. There are three possible explanations for this: 1) reduced adipose tissue lipolysis resulting in less FFA availability, 2) increased re-esterification of FFA, and/or 3) reduced adipose tissue blood flow preventing the delivery of liberated FFA to muscle. While relatively few studies have directly examined this question, it seems likely that the explanation for reduced FFA delivery is the two former mechanisms as carbohydrate ingestion does not impair adipose tissue blood flow [247,248]. However, the evidence for alterations in lipolysis and/or re-esterification is conflicting. Furthermore, the impact of carbohydrate-induced alterations in FFA delivery on total fat oxidation is yet to be fully elucidated.

Before investigating the effects of carbohydrate intake on adipose tissue metabolism, it is important to note that interpreting the data of many studies in this area require caution due to the methods utilized to quantify lipolysis. Most studies utilize either glycerol balance methods or stable isotope tracers to determine the rate of glycerol appearance in the blood. As mentioned above, adipose tissue and skeletal muscle possess small amounts of glycerol kinase, which allows for the reuptake of glycerol following lipolysis. As such, measuring the rate of appearance of glycerol in the plasma may lead to underestimation of lipolysis. Nevertheless, Stallknecht and colleagues [223] revealed that during moderate exercise, the net release of glycerol from muscle is negligible, and the reuptake of glycerol by adipose tissue is likewise negligible. Thus, glycerol appearance is assumed to be an accurate means of assessing lipolysis, and, due to the zero net release of glycerol from muscle, is indicative of adipose tissue lipolysis. Also worth noting is the possibility that plasma glycerol levels can be contaminated by the hydrolysis of triglycerides from other sources including EMTG and/or circulating triglycerides associated with lipoproteins (e.g. chylomicrons and very low density lipoproteins). It is commonly argued that these sources of FFA contribute minimally to plasma FFA and glycerol levels during exercise [147]. However, lipolysis of alternative sources of triglycerides may actually be more substantial than originally thought [249], and it is not possible to delineate between adipose-derived FFA/glycerol and FFA/glycerol
from other sources [146]. For these reasons, assessment of glycerol appearance in the interstitial space of adipose tissue via microdialysis is preferred for accurate determination of lipolysis [35]. However, studies utilizing alternative techniques are useful in assessing the larger trends of lipolysis and fat oxidation.

Two studies have examined the influence of pre-exercise carbohydrate ingestion on rates of whole body lipolysis during exercise. In the first, Horowitz et al. [3] utilized stable isotope tracers to measure the appearance of glycerol in the plasma as a marker of whole body lipolysis. Active male participants cycled for 1 hr at 44% VO$_{2\text{peak}}$ fasted or following carbohydrate (0.8 g·kg$^{-1}$ glucose) ingestion. Glucose ingestion resulted in a ~5-fold increase in insulin, and this was associated with reductions in lipolysis, plasma FFA, and fat oxidation. Interestingly, it was also found that lipolysis in the fed condition limited fat oxidation as evidenced by an approximate equaling of lipolysis and fat oxidation. While at rest or during fasted exercise, lipolysis exceeded fat oxidation. That is, more FFA were liberated than were oxidized. Overall, this study suggests carbohydrate-induced reductions in fat oxidation during exercise are primarily the result of insulin-mediated reductions in lipolysis. This finding is supported by a number of studies noting inhibition of lipolysis following carbohydrate and/or insulin administration at rest [250–252].

The other study to investigate the lipolytic response to pre-exercise carbohydrate feeding does not support this finding. Enevoldsen et al. [10] measured adipose tissue lipolysis responses during cycling at 55% VO$_{2\text{max}}$ 1 hr following a mixed meal (60% carbohydrate, 20% fat, 20% protein) via glycerol balance methods (i.e. aterio-venous concentration of glycerol difference x blood flow [xenon washout technique]). Similar to Horowitz et al. [3], the authors noted substantial reductions in fat oxidation (~50%) that were coupled with reduced levels of FFA in the plasma following the high-carbohydrate meal. However, the reduction in plasma FFA was not found to be the result of alterations in lipolysis, which increased to the same degree in the fasted and fed state, nor the result of reductions in adipose tissue blood flow. Rather, the reduction in plasma FFA levels was likely due to ~35% increase in FFA re-esterification. The reason for the discrepancy in findings between the two studies is not immediately clear, but may be a function of catecholamines. While both studies utilized low-moderate intensity exercise, the intensity in the Enevoldsen et al. study was slightly higher versus the Horowitz et al. study (55% vs. 44% VO$_{2\text{max}}$). As catecholamines respond exponentially to exercise intensity [40], it stands to reason that catecholamine levels were higher for participants in the study by Enevoldsen et al. If this was the
case, it is possible that catecholamine levels in the study by Enevoldsen et al. were sufficient to override the effects of insulin on lipolysis, but perhaps not re-esterification. Indeed, the anti-lipolytic effects of insulin can be overridden when sympathetic nervous stimulation of adipose tissue lipolysis is enhanced via lower body negative pressure [253]. Taken together, it is clear that the effects of pre-exercise carbohydrate on adipose tissue FFA mobilization require further study.

Ingestion of during-exercise carbohydrate also seems to reduce FFA delivery to the muscle, and this seems to be the result of reductions in adipose tissue lipolysis. A study by de Glisezinski et al. [254] utilized the microdialysis technique to measure glycerol appearance in the interstitial space of subcutaneous adipose tissue (SCAAT) during exercise in trained men who consumed either water or sucrose (0.75 g·kg\(^{-1}\)) 50 min after commencing a 100-min bout of cycling (50% \(\text{VO}_{2\max}\)). Following sucrose ingestion, blood glucose and insulin levels rose, and the exercise-induced rise in epinephrine was attenuated. Additionally, glycerol appearance in SCAAT was reduced at 100 min of exercise indicating reduced lipolysis. This finding is supported by Horowitz et al. [255] who reported reductions in plasma glycerol appearance following during-cycling ingestion of carbohydrate relative to fasting at two exercise intensities (low: 25% \(\text{VO}_{2\text{peak}}\) and moderate: 68% \(\text{VO}_{2\text{peak}}\)). Interestingly, the authors of this study reported that the magnitude of the reduction in glycerol appearance was attenuated at the higher intensity, and this was associated with an attenuated rise in insulin suggesting the impact of carbohydrate on lipolysis may be attenuated at higher intensities.

Despite this clear evidence for a reduction in adipose tissue lipolysis with during-exercise carbohydrate ingestion, the impact of reduced FFA delivery on fat oxidation is less predictable relative to pre-exercise data. In the same study by Horowitz et al. [255], fat oxidation at the low exercise intensity was not reduced until more than 1 hr following carbohydrate ingestion. Moreover, at this point when fat oxidation rates were reduced, lipolysis exceeded fat oxidation rates by \(\sim 25\%\) meaning that FFA delivery to the muscle was not limiting fat oxidation. Similarly, during moderate intensity exercise, fat oxidation was unaffected despite a \(\sim 25\%\) reduction in lipolysis. These findings indicate that fat oxidation rates are maintained by oxidation of FFA from sources alternative to adipose tissue with during-exercise carbohydrate ingestion. Another interesting finding of this study was that, during low intensity exercise, the drop in fat oxidation rates late in exercise mirrored an increase in plasma glucose uptake. Clearly, this suggests that
interactions between carbohydrate and fat play an important role in mediating fat oxidation (see section: *Carbohydrate ingestion and mitochondrial FFA transport*).

**Effects of carbohydrate ingestion on intramuscular triglyceride FFA mobilization**

Similar to adipose tissue, relatively few studies have examined the impact of carbohydrate ingestion on IMTG metabolism. Additionally, from the available evidence, it appears that the effects seem to be mediated by the timing of intake (before vs. during exercise). Pre-exercise carbohydrate ingestion seems to attenuate IMTG oxidation. Indeed, Coyle et al. [256] investigated the effects of glucose (1.4 g·kg⁻¹) ingested 60 min and 10 min before 40 min of cycling at 50% VO₂max. Relative to fasting, glucose ingestion reduced fat oxidation, which was explained by both reduced plasma FFA oxidation and a 27% decrease in IMTG oxidation. Likewise, De Bock et al. [9] examined IMTG utilization with both pre- (150 g) and during-exercise (1 g·kg⁻¹·hr⁻¹) carbohydrate ingestion versus fasting. In the fasted condition, IMTG content was reduced ~65% (from pre-exercise levels). However, with pre- and during-exercise carbohydrate, IMTG content was unchanged following exercise. Thus, in both studies, IMTG utilization seems to be inhibited with pre-exercise carbohydrate ingestion.

Two studies have investigated the effects of ingesting carbohydrate during exercise on IMTG utilization. In the first, Watt et al. [257] calculated IMTG utilization during 2 hr of cycling at 65% VO₂max with participants ingesting either glucose (60 g·hr⁻¹; every 15 min) or a non-caloric placebo. Predictably, glucose ingestion increased insulin and attenuated the rise in epinephrine typically observed during exercise. Also, like the study by Horowitz et al. [255], fat oxidation was unaffected by glucose ingestion despite late-exercise reductions in lipolysis and plasma FFA oxidation. Importantly, Watt and colleagues reported no changes in non-plasma FFA (IMTG) oxidation despite reduced late-exercise oxidation of plasma-derived FFA. These findings are seemingly paradoxical as fat oxidation was maintained despite reductions in plasma FFA oxidation and unchanged non-plasma FFA oxidation. It is possible that a reduction in fat oxidation was prevented by increased oxidation of plasma triglycerides (e.g. very low-density lipoproteins) [249]. Regardless, this indicates that IMTG utilization does not appear to be affected by during-exercise carbohydrate ingestion, and this finding is supported by others [8]. Stellingwerff and colleagues [8] quantified changes in IMTG following exercise using oil red staining and microscopy techniques. In this study, participants cycled for 3 hr at 63% VO₂max while ingesting
glucose (0.7 g·hr⁻¹; every 20 min) or water. IMTG utilization was unchanged in this study despite reductions in adipose tissue-derived FFA oxidation starting in the first hour of exercise. In contrast to Watt et al. [257], the reductions in plasma FFA oxidation and maintenance of IMTG oxidation resulted in reduced total fat oxidation.

A possible explanation for this clear impact of timing may be the magnitude and timing of the insulin response. In both studies examining pre-exercise carbohydrate intake, insulin levels rose to ~40 μU·ml⁻¹ [9,256]. Conversely, with carbohydrate ingestion starting during exercise, insulin levels did not exceed ~15 μU·ml⁻¹ likely as a result of catecholamine-induced inhibition of insulin release from the pancreatic β cells [8,257,258]. Thus, pre-exercise carbohydrate ingestion resulted in insulin levels that rose both substantially higher and earlier relative to during-exercise intake. Moreover, this insulin spike was not accompanied by elevated catecholamine levels that might partially override insulin signaling as would be present with during-exercise ingestion. In these conditions, insulin would be expected to exert more potent effects both in adipose tissue, but also in the muscle. These effects at the muscle likely contribute to impaired mitochondrial FFA transport, which would inhibit FFA oxidation from any source.

**Carbohydrate ingestion and mitochondrial FFA transport**

Carbohydrate ingestion may also limit fat oxidation by limiting mitochondrial membrane FFA transport. This is likely mediated by insulin, which has two effects at the muscle that can impact transport. First, insulin enhances glucose uptake and storage resulting in an overall increase in carbohydrate, specifically glycogen, availability. Importantly, carbohydrate oxidation during exercise is stimulated by increased glycogen storage prior to exercise [259]. Second, insulin binding to its receptor stimulates hexokinase and phosphofructokinase activity resulting in increased glycolysis [260]. Thus, both effects, via increasing glycolytic flux, ultimately enhance acetyl CoA production. This reduces FFA transport because CPT1 activity seems to be heavily influenced by acetyl CoA. Indeed, early evidence suggested that malonyl CoA, the production of which is driven by acetyl CoA concentrations, is a potent inhibitor of CPT1 activity [197], and that there is a negative correlation between malonyl CoA levels and fat oxidation [198]. Worth noting, fat metabolism also drives acetyl CoA production. However, metabolic intermediates of fat metabolism including fatty acyl-CoA pre-emptively inhibit malonyl CoA generation from FFA-derived acetyl CoA [261]. As such, malonyl CoA production is primarily driven by carbohydrate
metabolism. Importantly, more recent studies have shown a disassociation between malonyl CoA levels and mitochondrial FFA uptake and fat oxidation suggesting that fat metabolism is modulated by other mechanisms [193,262,263]. Specifically, studies suggest that acetyl CoA levels may modulate CPT1 activity via a mechanism involving carnitine. In the first step of FFA transport across the mitochondrial membrane via CPT1, fatty acyl CoA reacts with free carnitine to produce acylcarnitine, which can diffuse across the outer mitochondrial membrane [181]. As such, free carnitine is required for this step. Importantly, when feeding-induced insulin binding or high-intensity exercise induces high acetyl CoA levels, free carnitine can act as an acceptor of the acetyl molecule of acetyl CoA to form acetyl carnitine + CoASH via the enzyme carnitine acetyl transferase [34,264]. As the acetyl CoA/CoA ratio can modulate pyruvate dehydrogenase activity [265], this reaction is important to keep cytosolic levels of acetyl CoA relatively low and thereby attenuate feedback inhibition. However, this reaction reduces levels of free carnitine thereby inhibiting the first step in the CPT1 reaction, and ultimately, reducing fat oxidation. This mechanism seems a likely contributor to carbohydrate-ingestion-induced downregulation of FFA transport into the mitochondria based on studies showing that increasing glycogen storage via carbohydrate ingestion is associated with reductions in free carnitine levels and fat oxidation [263]. Moreover, high free carnitine levels resulting from supplementation has been reported to reduce pyruvate dehydrogenase complex activity during moderate exercise, which suggests enhanced fat oxidation [266].

Regardless of the precise mechanism, it is apparent that carbohydrate ingestion modulates FFA entry into the mitochondria. Indeed, Coyle et al. [256] investigated the effects of pre-exercise carbohydrate ingestion on mitochondrial FFA transport by infusing long chain FFA (palmitate) and short chain FFA (octanoate) during exercise (50% VO2max cycling). FFA derived from adipose tissue and IMTG are mostly long chain FFA, which are limited by CPT1 transport. In contrast, short chain fatty acids, which, as described above, are produced via the fermentation of RS in the colon, diffuse across the mitochondrial membrane and are thus not limited by CPT1 [267]. In this study, pre-exercise glucose ingestion (1.4 g·kg\(^{-1}\)) resulted in a 34% reduction in fat oxidation. Importantly, this effect seemed to be the result of reduced FFA uptake into the mitochondria. This is evidenced by the significantly reduced oxidation of palmitate as a percentage of the amount infused. However, the oxidation of short chain FFA was unchanged. These results strongly suggest that carbohydrate ingestion and the corresponding increase in glycolytic flux inhibit FFA
mitochondrial uptake. These results also suggest an alternative mechanism for the increased fat oxidation reported by some studies following ingestion of starches containing RS (e.g. lentils) [11,22].

Low glycemic index carbohydrate and starches and fat metabolism

As mentioned, the absorption/digestion rate of carbohydrates and/or starches has important implications for the postprandial physiological responses. Slower digesting carbohydrates attenuate the postprandial rise in blood glucose, and thus, reduce the magnitude of the insulin response [143]. Due to the aforementioned effects of insulin on lipolysis, FFA re-esterification, and/or mitochondrial transport [3,10,256], ingestion of slow-absorbing carbohydrate would seemingly promote fat utilization relative to fast-absorbing high glycemic index sugars like glucose.

Indeed, a number of studies investigating pre-exercise ingestion of a wide variety of low glycemic index carbohydrate sources have reported enhanced fat oxidation (see Table 1). For instance, enhanced fat oxidation during exercise has been noted following ingestion of isolated low glycemic index starches (i.e. lentils) relative to high glycemic index starches (i.e. cooked potato) and/or carbohydrates (i.e. glucose) [11,22]. Enhanced fat oxidation has also been reported following ingestion of mixed meals with a low glycemic index relative to high [12,14,23,62,63,268–270], the glycemic indices of which were determined using the weighted-means formula [271]. Nevertheless, a number of studies have also reported that ingestion of slow absorbing carbohydrates and/or starches have no effect on fat oxidation rates relative to high glycemic index carbohydrate [13,15,16,18,19,21,24,25,28–30,272,273].

Conflicting results for fat oxidation are also apparent when examining the impact of during-exercise ingestion of slow absorbing carbohydrate (see Table 2). Too et al. [32] reported enhanced fat oxidation during running (70% VO2max) with the ingestion of raisins every 20 min versus a sports gel. Additionally, Oosthuyse et al. [87] recently found fat oxidation to be elevated with during-cycling (60% Wmax) ingestion of isomalutose versus a glucose-fructose beverage. Conversely, Leijssen and colleagues [274] reported galactose to have no impact on fat oxidation versus glucose during cycling (65% Wmax).

Based on the evidence, it is clear that the absorption/digestion rate of carbohydrate can impact fat oxidation. However, the wide-ranging diversity in the carbohydrate source examined
and the methodology utilized prevents firm conclusions. For example, in pre-exercise studies, the timing of intake prior to exercise varies widely from 15 min to 3 hr. Moreover, the impact of timing is difficult to elucidate as separate studies have reported either increased fat oxidation or no effect at the same various time points before exercise. The difficulty in determining what, if any, role the timing of intake plays is also complicated by the variety of carbohydrate sources examined. While lentils versus potatoes has been investigated a number of times (with conflicting results) [11,16,19,22,28,29], mixed meal studies typically differ drastically in the foods chosen. Moreover, while mixed meal studies are certainly advantageous in terms of real world application, the inclusion of non-carbohydrate sources in these meals complicates interpretation of the metabolic effects. A variety of non-starch carbohydrates have also been investigated ranging from fructose to trehalose. As mentioned, these fructose-containing carbohydrates can reduce fat oxidation via insulin-independent mechanisms and are thus not truly representative of a slow digesting, low glycemic index starch. Finally, the mode and intensity of exercise is different across studies. It is well-established that different forms of exercise (i.e. running vs. cycling) have unique metabolic demands [275–277]. Moreover, the effects of carbohydrate ingestion on exercise metabolism is different with running versus cycling [278]. The intensity of exercise is also highly variable among these studies. Importantly, the effects of carbohydrate ingestion on fat metabolism are difficult to determine when the intensity of exercise is high (>75% VO\(_{2\text{max}}\)), and thus, primarily dependent on carbohydrate. Jentjens and Jeukendrup [25] reported no effects of pre-exercise trehalose ingestion on fat oxidation. However, this conclusion was based on respiratory exchange rate (RER) data collected during cycling at 65% \(W_{\text{max}}\), which elicited RER values of 0.96-1.01. As these values correspond to carbohydrate contributions of 87-100% to total energy expenditure, it seems unlikely that changes in fat oxidation would be readily apparent. Likewise, two studies by Little et al. [28,29] utilized an intermittent running protocol incorporating a number of sprints. This variability and intensity would also likely complicate interpretation of changes in fat oxidation rates.

Determining the precise nature and mechanisms responsible for alterations in fat metabolism with pre- and/or during-exercise ingestion of low glycemic index carbohydrates and starches is also difficult primarily due to a lack of data. No studies have directly investigated adipose tissue lipolysis responses to acute low glycemic index carbohydrate feeding via the microdialysis technique, nor has IMTG utilization been quantified. Nevertheless, there is some indirect evidence for enhanced adipose tissue lipolysis stemming from pre- or during-exercise
ingestion of slow absorbing carbohydrate. Specifically, plasma glycerol levels have been measured in a number of studies [13,16,21,23,27,32,63,268,279,280]. Interestingly, the majority, but not all [13,16,27], of these studies suggest that pre-exercise [14,21,23,63,268] slow absorbing carbohydrate ingestion enhances lipolysis relative to fast absorbing carbohydrate. Furthermore, in all but one study [21], the increase in glycerol levels corresponded with an increase in fat oxidation suggesting that any potential alterations in FFA re-esterification mirrored changes in lipolysis. During exercise, the evidence is clearer. Lipolysis seems unaffected when the high or low glycemic index carbohydrate/starch is consumed during exercise [32,274]. Nevertheless, increases in fat oxidation with during-exercise low glycemic index carbohydrate has been reported by some [32,87], but not all [274], despite no change in plasma glycerol relative to high glycemic index carbohydrate. Clearly, more research is needed.

The best available evidence for determination of depot-specific alterations in fat metabolism resulting from low glycemic index carbohydrate/starch ingestion comes from dietary studies. Stevenson et al. [281] and Trenell et al. [282] investigated the impact of high or low glycemic index recovery diets on exercise fat metabolism. In this experiment, trained cyclists depleted their muscle glycogen stores by exercising 90 min at 70% VO\(_{2\text{peak}}\). Thereafter, participants consumed, in a crossover design, a 24-hr diet consisting primarily (~60% carbohydrate, 20% fat, 20% protein) of either high or low glycemic index carbohydrates/starches. The following morning, fasted participants again cycled for 90 min at 70% VO\(_{2\text{peak}}\) while assessments of plasma FFA, substrate utilization, and IMTG (via magnetic resonance spectroscopy) were made. Of interest, the low glycemic index recovery diet increased fat oxidation the next morning. Moreover, it also increased plasma FFA levels and attenuated the utilization of IMTG [281,282]. This suggests that lipolysis was enhanced, (or not inhibited), allowing for greater FFA delivery resulting in sparing of IMTG. This reduced reliance on IMTG due to increased FFA use is supported by others. Solomon et al. [283] investigated the effects of a 3-month lifestyle intervention in which diets (~55% carbohydrate, ~25% fat, 20% protein) were provided that consisted of high or low glycemic index carbohydrates and starches. Following the intervention, the authors noted increased fat oxidation per kilogram of body mass during exercise (65% VO\(_{2\text{max}}\) walking). Interestingly, pre- and post-intervention measurements of IMTG were unchanged, but EMTG levels were significantly decreased following the low glycemic index diet. This suggests that extra-muscular sources of fat were being utilized to a greater extent throughout the
intervention. However, the contribution of fat from various sites (EMTG or adipose tissue) during exercise was not assessed, so this is purely speculative. Regardless, the impact of acute pre- and/or during-exercise ingestion of slow absorbing carbohydrates and starches on site-specific fat metabolism is yet to be determined.

Modified starches and fat metabolism

Modified starches provide an excellent model for examining the impact of starch digestion and absorption on exercise fat metabolism as these can be designed to be nearly 100% SDS and thus “truly” low glycemic index without the confounding influence of insoluble fiber and/or non-carbohydrate foods. Nevertheless, relatively few studies have examined this topic (see Table 3). Moreover, only two types of modified starches have been investigated. Johannsen and Sharp [284] studied exercise metabolism following ingestion of an acid/alcohol modified high amylose starch in trained cyclists. Although this starch is only 62% digestible (i.e. 38% RS) before modification, modification results in a 92% digestible starch in rats [285]. Thus, modification increases the SDS content (based on the amylose content) and reduces the RS content. Not surprisingly, results indicated that this pre-exercise ingestion of this starch substantially attenuated both the blood glucose and insulin responses early in exercise relative to glucose. Unexpectedly, this did not translate to differences in fat utilization. Fat oxidation rates were not different between the starch and glucose. Moreover, plasma glycerol was also unchanged suggesting no differences in lipolysis. This finding is difficult to reconcile as the vast majority of studies which report differences in the plasma glucose and insulin responses to carbohydrate ingestion also report differences in plasma FFA/glycerol and/or fat oxidation [11,12,14,19–23,26,27,62,63,268,270,286]. It is possible that the high digestibility of this starch, presumably the result of low RS content, attenuated short chain fatty acid formation in the colon. However, more research is needed to confirm this conclusion.

More recently, a modified starch, originally developed for the treatment of glycogen storage disease [141,142], has been investigated for potential application as a sport supplement. As described above, this starch was developed via hydrothermal modification of waxy maize resulting in a low glycemic index starch [140]. Unlike the above acid/alcohol modified starch, ingestion of this slow absorbing starch seems to augment fat utilization. Roberts et al. [7] studied trained cyclists that ingested either the modified waxy maize starch or maltodextrin (1 g·kg\(^{-1}\)) 30 min prior to 2.5 hr of exercise at 65% VO\(_{2\text{max}}\). During exercise, glucose and insulin responses were
attenuated, and serum FFA and glycerol levels were enhanced with the modified starch relative to maltodextrin. Importantly, these effects seemed to translate to enhanced fat oxidation (although not statistically significant, \( p = 0.07 \)). More recently, Baur et al. [287] confirmed an increase in fat oxidation following ingestion of the same starch (0.60 ± 0.16 vs. 0.46 ± 0.14 g·min\(^{-1}\); 99% likelihood). In this study, trained cyclists similarly ingested pre-exercise modified starch (60 g, 30 min prior), but also ingested starch during exercise (60 g·hr\(^{-1}\)). The authors noted an attenuated blood glucose response and enhanced ("very likely" based on confidence intervals and pre-determined thresholds for meaningful change) fat oxidation both pre-exercise and during the first hr of exercise (50% \( W_{\text{max}} \)). However, no measurements of plasma FFA/glycerol were made. Taken together, it is clear that ingestion of this modified waxy maize starch alters fat utilization. However, more research is needed to determine whether this effect is mediated primarily by changes in adipose tissue or IMTG metabolism.

**Low Glycemic Index Carbohydrates and Starches and Endurance Performance**

*Methodological considerations in the assessment of performance*

Measuring the effects of nutritional interventions on endurance performance require careful consideration of exercise protocol methodology. Specifically, the value of any measurement of performance requires a high degree of reliability and validity. Reliability is defined as the consistency of performance when measured repeatedly [288]. When a performance protocol has low reliability (i.e. a large amount of within-subject variation from trial-to-trial), a larger sample size is required to provide adequate statistical power to avoid Type I errors [289]. As the population of trained endurance athletes is typically limited, designing protocols for maximum reliability is required to ensure the accuracy of measurement and/or that the results are indicative of the “true” effect for the average participant. Unfortunately, a common performance protocol utilized in studies assessing the performance effects of slow-absorbing carbohydrates and starches is the time to exhaustion (TTE) protocol. This protocol requires participants to exercise at a constant intensity until volitional exhaustion. Various studies have assessed the reliability of this protocol in comparison to time trial protocols, which require participants to complete a given distance as quickly as possible. Consistently, TTE reliability is reported to be substantially lower (13-22% coefficient of variation [CV]) versus time trials (TT; 1-5% CV) [37,290]. Moreover, the
reliability of TTE protocols may be attenuated further with protocols utilizing lower exercise intensities (60-75% VO$_{2\text{max}}$), which, consequently, are longer in duration [288]. This may be due to reduced motivation and/or boredom. For these reasons, interpretation of studies utilizing TTE protocols to assess the impact of nutritional interventions is difficult due to low reliability.

An additional concern with TTE protocols is a lack of external and/or internal validity. Assessments of endurance performance are not externally valid if results do not translate to real-world events. Endurance athletes are generally required to complete a given distance as quickly as possible, rather than maintain a constant intensity indefinitely. As such, the external validity of TTE for the assessment of endurance performance is questionable [291]. Worth noting, TTE results can be utilized for prediction of TT performance [290,292]. Thus, while not optimal, TTE results may still be informative as to real-world endurance performance.

External validity can also be impaired by the nutritional protocol utilized. With prolonged (>2 hr) endurance exercise, athletes commonly consume nutrients both before and during exercise [293]. As such, studies in which participants consume a meal prior to prolonged exercise, but do not consume nutrients during exercise, lack external validity. Moreover, it is also possible that any metabolic alterations incurred by a pre-exercise meal may be abolished late in prolonged exercise without during-exercise ingestion of similar nutrients. Obviously, this would make assessing late-exercise performance effects more challenging.

Another major concern in the assessment of endurance is the internal validity of a measure. Internal validity refers to the inherent “truth” of conclusions regarding causal relationships. In studies that have confounding variables, the validity of conclusions may be limited. Exercise protocols can have confounding variables when their design is improper for determining the nature and magnitude of certain effects. For example, in a study seeking to determine the effect that nutrition-mediated alterations in metabolism have on endurance performance, the internal validity may be flawed if the duration and/or intensity of the exercise protocol utilized is insufficient for performance to be limited by endogenous fuel stores. Therefore, sufficiently challenging exercise protocols must be utilized in order to draw valid conclusions regarding the true impact of a nutritional intervention.
Pre-exercise slow-absorbing carbohydrate/starch and endurance performance

Unfortunately, studies evaluating the effects of pre-exercise low glycemic index carbohydrate/starches on endurance performance often suffer from low reliability and/or validity (see Table 1). Indeed, of the 20 studies that assessed performance, eight of them utilized TTE exercise protocols [11–13,16–18,23,24]. Furthermore, several of these studies utilized moderate intensity exercise TTE protocol [11,13,17]. As mentioned, this potentiates an even greater reduction in reliability relative to high-intensity and shorter duration TTE protocols. The validity of many pre-exercise meal studies also seems low. This is mostly a consequence of the exercise duration prescribed. Specifically, regardless of performance protocol (i.e. TTE or TT), exercise duration in many studies is often too short to stress endogenous fuel stores [22,25–27], or too long for the nutritional protocol to correspond with real-world practices (i.e. nutrient consumption before and during exercise) [12,19,20]. Taken together, interpretation of pre-exercise low glycemic index carbohydrate/starch performance data is challenging.

Nevertheless, a compelling case can be made for performance benefits stemming from pre-exercise low glycemic index carbohydrate/starch ingestion. As discussed above, TTE can be utilized for prediction of TT performance [290]. However, the effect size required in a TTE protocol required to detect a subtle change in TT performance is large. For example, using prediction equations, a 15% improvement in TTE would equate to a 1% change in TT performance [37]. With this in mind, TTE data can indicate performance enhancement in a TT assuming a substantial enhancement in TTE performance. Importantly, of the eight studies to assess TTE performance following pre-exercise low glycemic index carbohydrate/starch ingestion, five of them report enhanced performance [11–13,16,17]. Moreover, each of these studies reported increases in TTE exceeding 15%, which suggests enhanced TT performance. In contrast, three studies reported no change in TTE performance with low versus high glycemic index carbohydrates/starches. However, Hargreaves et al. [18] assessed the effects of fructose relative to glucose. As noted, fructose enhances lactate production, which inhibits lipolysis [171]. Metabolic benefits due to a slower absorption rate are thus likely attenuated with fructose. Stannard et al. [24] also reported no change in TTE performance with an incremental exercise protocol (50 W increase·3 min⁻¹). However, the short duration and high-intensity of this protocol would likely prevent detection of performance changes resulting from alterations in fat metabolism.
Cumulatively, the majority of studies which utilized TTE protocols support ingesting low glycemic index carbohydrate and starches prior to exercise to enhance performance.

A close examination of studies utilizing TT protocols also suggests potential benefits of pre-exercise slow-absorbing carbohydrates and starches, but the evidence is more equivocal. Of the 10 studies to utilize TT protocols, three report enhanced performance [14,294,295], and seven report no differences [19–22,25–27]. While the weight of the evidence seems to be on the side of no differences, various methodological considerations complicate interpretation. For example, three of these seven studies utilize short (~60 min) and/or high-intensity pre-loaded (65% $W_{\text{max}}$) exercise protocols in which performance is not likely to be limited by fuel and/or fat availability [22,25,26]. This factor also likely contributed to null findings in two studies which assessed repeated sprint performance following intermittent running [28,29]. Interpretation of the studies reporting enhanced performance stemming from pre-exercise slow-absorbing carbohydrate/starch ingestion is also challenging. Moore et al. [294] found a 3.2% improvement in 40-km TT performance in trained cyclists following ingestion of high- or low-glycemic index mixed meals. However, the metabolic effects of the two meals were opposite to what would be expected. The low glycemic index meal elevated blood glucose and insulin relative to the high-glycemic index meal. Moreover, plasma FFA and fat oxidation were higher with the high versus low glycemic index meal. As such, it is difficult to draw conclusions regarding performance from this study. Wong et al. [14] reported a 2.8% improvement in running performance in a 16-km TT following a 5-km pre-load (70% $VO_{\text{2max}}$) following ingestion of a low glycemic index mixed meal relative to a high glycemic index meal. Importantly, this was associated with increased concentrations of FFA/glycerol in the blood and enhanced fat oxidation. As such, this study seems more representative of the expected effects of a low glycemic index carbohydrate pre-exercise meal. Finally, Konig et al. [295] recently investigated the impact of pre-exercise isomaltulose ingestion relative to maltodextrin (75 g, 45 min prior). Of interest, the authors reported enhanced fat oxidation during steady state cycling and a likely 2.7% improvement in TT performance. These results seem to confirm those of Wong et al. [14]. Also worth noting, the duration of exercise in these studies hold high external validity as the exercise duration in both seem to be long enough to warrant careful consideration of a pre-exercise meal, but also short enough that athletes may choose not to consume any nutrients during the race of that length. Taken together, it is clear that methodological considerations limit drawing firm conclusions as to the impact of pre-exercise
slow-absorbing carbohydrates and starches. More research is warranted to resolve some of these issues and elucidate the ergogenic potential of pre-exercise slow-absorbing carbohydrates/starches.

**During-exercise ingestion of slow-absorbing carbohydrate/starch and performance**

Consumption of low glycemic index carbohydrates and starches during exercise does not appear to enhance performance; although there is a lack of data in this area (see Table 2). Earnest et al. [31] first investigated this area. In this study, trained cyclists consumed either honey or glucose (15 g·carbohydrate·16 km⁻¹) during a 64-km TT. Honey ingestion had no influence on TT performance. However, it should again be noted that honey is primarily composed of fructose, and as such, may actually impair FFA delivery and therefore attenuate any metabolic benefits [171]. However, the subtle changes in fat oxidation resulting from during-exercise ingestion of “true” low glycemic index carbohydrates do not seem to translate to performance gains. In a study by Too et al. [32], trained runners consumed either raisins or commercially available high glycemic index sport chews (0.5 g·carbohydrate·kg⁻¹ every 20 min). While fat oxidation was slightly elevated with raisins during 80 min of running at 75% VO₂max, 5-km performance was unchanged.

Recently, Oosthuyse et al. [87] reported enhanced fat oxidation with during-cycling ingestion of isomalutose versus a glucose/fructose composite, but impaired 20-km performance. Participants in this study had substantially increased ratings of stomach cramping and bloating with isomalutose ingestion, which likely explains the reduction in performance. This study highlights an important risk associated with consuming low glycemic index carbohydrate/starches during exercise. Due to the longer transit time in the stomach/small intestine, low glycemic index carbohydrate/starches may pull water into the gastrointestinal track via osmotic drag and/or saturate enzymes responsible for their degradation leading to carbohydrate accumulation in the gut (i.e. malabsorption) [296]. As such, the potential metabolic benefits of during-exercise consumption of these carbohydrates and starches must be weighed against the potential for gastrointestinal distress. Importantly, the metabolic effects of pre-exercise ingestion of low glycemic index carbohydrate/starch (e.g. enhanced fat oxidation) are abolished when high glycemic index carbohydrates are consuming during exercise [297]. Thus, for prolonged exercise (>2 hr) limited by fuel availability, the benefits of a low glycemic index pre-exercise carbohydrate/starch meal seem to require continued ingestion of low glycemic index
carbohydrate/starch during exercise to realize the metabolic benefits. More research is needed to determine the feasibility of this approach. It is possible that gastrointestinal tolerance may be highly dependent on inter-individual variability and thus is only beneficial among “responders.” Furthermore, “training the gut” with chronic slow-absorbing carbohydrate/starch intake during exercise may be required to reduce symptoms of gastrointestinal distress [298].

Modified starches and endurance performance

Only two studies have investigated the impact of ingesting modified starches on endurance performance (see Table 3). In the first study, Roberts et al. [7] assessed the metabolic and performance effects of consuming a hydrothermally modified waxy maize starch (1 g·kg⁻¹) 30 min prior to cycling (2.5 hr at 70% VO₂max followed by TTE at 100% VO₂max). The authors reported no changes in TTE performance. However, considering that participants exercised for 2.5 hr without supplemental nutrients, it is possible that any early-exercise differences in endogenous fuel utilization were minimized late in exercise. As such, endogenous fuel availability may have been similar to start the TTE despite substantial differences earlier in exercise.

To address this limitation, Baur et al. [287] recently investigated the effects of ingesting this same modified starch versus a commercially available glucose-based supplement both before (30 min) and during (15 g every 15 min) an intermittent high-intensity 100-km cycling race simulation. A third experiment was also conducted in which a lower dose of the modified starch (30 g given at the end of each hour of exercise) was consumed during exercise following the same 60 g pre-exercise beverage. Importantly, this modified starch is known to have a low osmolarity (44 mosml·kg⁻¹) and thus likely to empty quickly from the stomach and presumably have a low osmotic force in the small intestine. These characteristics would seem to attenuate the risks for gastrointestinal distress that are likely increased with ingestion of higher osmolarity carbohydrates like isomalutose (278 mosml·kg⁻¹) [87]. Nevertheless, Baur and colleagues reported substantially higher ratings of nausea during late-exercise repeated sprints with either an isocaloric or the low dose of during-exercise modified starch [287]. It is possible that this is due to starch accumulation resulting from saturation of amolytic enzymes. Additionally, other have suggested that extremely high molecular weight, low osmolarity, beverages are potentially more prone to gelling in the small intestine, which slows transit from the intestinal lumen to the microvilli for carbohydrate absorption [299–301]. Importantly, increased ratings of gastrointestinal distress may have
influenced performance. Indeed, mean sprint power was unchanged between isocaloric trials despite enhanced fat oxidation early in exercise. Performance was impaired with the low dose modified starch versus isocaloric treatments. This finding would seem to suggest that the dose-response effect found with glucose and glucose/fructose composites may also apply to low glycemic index carbohydrate/starches [302–304].

Taken together, these studies seem to suggest that the applications of modified starches for endurance performance may be limited. Indeed, the risk of gastrointestinal distress when consumed during exercise may preclude its use in prolonged endurance events where traditional high glycemic index carbohydrates are easily accessible (e.g. cycling). However, the metabolic effects of ingesting modified starches before exercise may be ergogenic given certain conditions. For example, athletes competing in events in which during-exercise access to carbohydrate is limited (e.g. long distance swimming) may benefit from pre-exercise modified starch consumption. Additionally, in some running events (in excess of 10 km, but shorter than the marathon), the risk of gastrointestinal distress combined with the shorter duration may preclude consuming nutrients during the event. It is also possible that ingesting modified starches as a training supplement enhances metabolic flexibility, which could translate to enhanced performance. Ultimately, more research is warranted to determine the potential benefits, if any, of consuming modified starches before and/or during endurance exercise.

**Conclusions and Future Directions**

Endurance events have extreme energy demands that place a premium on accessing nearly inexhaustible fat energy stores. Ingesting carbohydrate reduces the utilization of fat during exercise. Starches are the primary source of carbohydrate consumed by humans. The digestion rates of starches vary depending on their physical and macromolecular structure, and these traits can be modified through chemical, enzymatic, and/or hydrothermal processing. Ingesting low glycemic index carbohydrates and/or starches before and/or during exercise seems to enhance fat oxidation, and there is some evidence suggesting this can benefit endurance performance.
Table 1. The effect of pre-exercise low versus high glycemic index carbohydrate/starch on fat oxidation and performance/exercise capacity.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>n</th>
<th>Treatments</th>
<th>Timing Prior</th>
<th>Protocol</th>
<th>Lipid biomarkers</th>
<th>FatOx</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hargreaves et al. [18]</td>
<td>1987</td>
<td>6</td>
<td>75% G or F</td>
<td>45 min</td>
<td>75% VO\textsubscript{2max} TTE, Cycle</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas et al. [11]</td>
<td>1991</td>
<td>8</td>
<td>L, P, G 1 g·kg\textsuperscript{-1}</td>
<td>1 hr</td>
<td>65-70% VO\textsubscript{2max} TTE, Cycle</td>
<td>FFA ↔</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Febbraio et al. [19]</td>
<td>1996</td>
<td>6</td>
<td>L or P 1 g·kg\textsuperscript{-1}</td>
<td>45 min</td>
<td>2 hr 70% VO\textsubscript{2max}, 15 min TT, Cycle</td>
<td>FFA ↑</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Goodpaster et al. [21]</td>
<td>1996</td>
<td>10</td>
<td>WS, RS, G 1 g·kg\textsuperscript{-1}</td>
<td>30 min</td>
<td>1.5 hr 66% VO\textsubscript{2max}, 30 min TT, Cycle</td>
<td>Glycerol ↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Sparks et al. [22]</td>
<td>1998</td>
<td>8</td>
<td>L or P 1 g·kg\textsuperscript{-1}</td>
<td>45 min**</td>
<td>50 min 67% VO\textsubscript{2max}, 15 min TT, Cycle</td>
<td>FFA ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demarco et al. [12]</td>
<td>1999</td>
<td>10</td>
<td>Mixed HGI vs. LGI 1.5 g·kg\textsuperscript{-1}</td>
<td>30 min</td>
<td>2 hr 70% VO\textsubscript{2max}, 100% VO\textsubscript{2max} TTE, Cycle</td>
<td>N/A</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Wee et al. [23]</td>
<td>1999</td>
<td>8</td>
<td>Mixed HGI vs. LGI 2.0 g·kg\textsuperscript{-1}</td>
<td>3 hr</td>
<td>70% VO\textsubscript{2max} TTE, Run</td>
<td>FFA ↑ Glycerol ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Febbraio et al. [20]</td>
<td>2000</td>
<td>8</td>
<td>Muesli, P 1 g·kg\textsuperscript{-1}</td>
<td>30 min</td>
<td>2 hr 70% VO\textsubscript{2max}, 30 min TT, Cycle</td>
<td>FFA ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stannard et al. [24]</td>
<td>2000</td>
<td>10</td>
<td>Pasta, G</td>
<td>65 min</td>
<td>Incremental cycle, 3 min stages, 50 watt step</td>
<td>N/A</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Kirwan et al. [13]</td>
<td>2001</td>
<td>6</td>
<td>Rolled oats or puffed rice</td>
<td>45 min</td>
<td>60% VO\textsubscript{2peak} TTE, Cycle</td>
<td>FFA ↔ Glycerol ↔</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al. [268]</td>
<td>2003</td>
<td>9</td>
<td>Mixed HGI vs. LGI 2.0 g·kg\textsuperscript{-1}</td>
<td>3 hr</td>
<td>1 hr 60% VO\textsubscript{2max}, Run</td>
<td>FFA ↑ Glycerol ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jentjens and Jeukendrup [25]</td>
<td>2003</td>
<td>8</td>
<td>2.0 g·kg\textsuperscript{-1} CHO G, galactose, trehalose 75 g</td>
<td>45 min</td>
<td>20 min 65% W\textsubscript{max}, ~700 kJ TT, Cycle</td>
<td>N/A</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Li et al. [286]</td>
<td>2004</td>
<td>8</td>
<td>Mixed LGI vs. HGI 2.0 g·kg\textsuperscript{-1} CHO</td>
<td>3 hr</td>
<td>1.5 hr 70% VO\textsubscript{2max}, Run</td>
<td>FFA ↑</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Bennard and Doucet [272]</td>
<td>2006</td>
<td>8</td>
<td>Mixed HGI vs. LGI, 80 g CHO</td>
<td>45 min</td>
<td>400 kcal at Fat\textsubscript{max}, Run</td>
<td>N/A</td>
<td>↔</td>
<td>N/A</td>
</tr>
<tr>
<td>Stevenson et al. [63]</td>
<td>2006</td>
<td>8</td>
<td>Mixed LGI vs. HGI 2.0 g·kg\textsuperscript{-1} CHO</td>
<td>3 hr</td>
<td>1 hr 65% VO\textsubscript{2max}, Run</td>
<td>FFA ↑ Glycerol ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kern et al. [26]</td>
<td>2007</td>
<td>8</td>
<td>Raisins vs. MD gel 1 g·kg\textsuperscript{-1} CHO</td>
<td>45 min</td>
<td>45 min 70% VO\textsubscript{2max}, 15 min TT, Cycle</td>
<td>FFA ↔</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Chen et al. [27]</td>
<td>2008</td>
<td>8</td>
<td>Mixed HGI vs. LGI 1.5 g·kg\textsuperscript{-1} CHO or 0.8 g·kg\textsuperscript{-1} CHO</td>
<td>2 hr</td>
<td>1 hr 70% VO\textsubscript{2max}, 10-km TT, Run</td>
<td>FFA ↔ Glycerol ↔</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*FatOx = Fat Oxidation, Performance = Performance, HGI = High Glycemic Index, LGI = Low Glycemic Index, CHO = Carbohydrate.
Table 1 Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>n</th>
<th>Treatments</th>
<th>Timing Prior</th>
<th>Protocol</th>
<th>Lipid biomarkers</th>
<th>FatOx</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong et al. [14]</td>
<td>2008</td>
<td>8</td>
<td>Mixed HGI vs. LGI 1.5 g·kg⁻¹ CHO</td>
<td>2 hr</td>
<td>5 km 70% VO₂max, 16-km TT, Run</td>
<td>FFA ↑, Glycerol ↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Moore et al. [15]</td>
<td>2009</td>
<td>8</td>
<td>Mixed HGI vs. LGI 1 g·kg⁻¹ CHO</td>
<td>45 min</td>
<td>40-km TT, Cycle</td>
<td>N/A</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Stevenson et al. [270]</td>
<td>2009</td>
<td>8</td>
<td>Mixed HGI vs. LGI 2.0 g·kg⁻¹ CHO</td>
<td>3 hr</td>
<td>50% VO₂peak, Inclined walk</td>
<td>FFA ↔</td>
<td>↑</td>
<td>N/A</td>
</tr>
<tr>
<td>Little et al. [29]</td>
<td>2009</td>
<td>7</td>
<td>L or P 2.0 g·kg⁻¹ CHO before; 0.25 g·kg⁻¹ CHO after 45 min</td>
<td>3 hr</td>
<td>1.5 hr intermittent running</td>
<td>N/A</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Little et al. [28]</td>
<td>2010</td>
<td>16</td>
<td>L or P 1.5 g·kg⁻¹ CHO</td>
<td>2 hr</td>
<td>1.5 hr intermittent running</td>
<td>N/A</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Karamonolis et al. [16]</td>
<td>2011</td>
<td>9</td>
<td>L or P 1.5 g·kg⁻¹ CHO</td>
<td>15 min</td>
<td>5 min 60% VO₂max, 45 min 70% VO₂max, 80% VO₂max TTE</td>
<td>Glycerol ↔</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Bennett et al. [30]</td>
<td>2012</td>
<td>14</td>
<td>L, honey, berries vs. P/white bread 1.5 g·kg⁻¹ CHO</td>
<td>2 hr</td>
<td>1.5 hr intermittent running</td>
<td>FFA ↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Moore et al. [17]</td>
<td>2013</td>
<td>10</td>
<td>Mixed HGI vs. LGI 2.5 g·kg⁻¹ CHO</td>
<td>3 hr</td>
<td>TTE 60% VO₂max, Cycle</td>
<td>N/A</td>
<td>N/A</td>
<td>↑</td>
</tr>
<tr>
<td>O’Hara et al. [273]</td>
<td>2013</td>
<td>9</td>
<td>Galactose or G 75g</td>
<td>30 min</td>
<td>2 hr 60% Wmax, Cycle</td>
<td>N/A</td>
<td>↔</td>
<td>N/A</td>
</tr>
<tr>
<td>Sun et al. [62]</td>
<td>2013</td>
<td>14</td>
<td>Snack bars LGI vs. HGI 1 g·kg⁻¹ CHO</td>
<td>1.5 hr</td>
<td>45 min 65% VO₂max, Cycle</td>
<td>N/A</td>
<td>↑</td>
<td>N/A</td>
</tr>
<tr>
<td>König et al. [295]</td>
<td>2016</td>
<td>20</td>
<td>Isomaltulose or MD 75 g</td>
<td>45 min</td>
<td>90 min 60% VO₂max, 6.5 kJ·kg⁻¹ TT</td>
<td>N/A</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

Note: L, lentils; P, potato; G, glucose; TTE, time to exhaustion; F, fructose; CHO, carbohydrate; MD, maltodextrin; Fatmax, intensity that elicits maximal fat oxidation rates; FatOx, fat oxidation, TT, time trial; kJ, kilojoule; LGI, low glycemic index; HGI, high glycemic index; Wmax, watt max

* Lentils reduced CHO oxidation for first 90 min. Fat oxidation was not calculated.

** Meal consumed 4 hr following standardized breakfast (100 g glucose polymer)
Table 2. The effect of during-exercise low versus high glycemic index carbohydrate/starch on fat oxidation and performance/exercise capacity.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>n</th>
<th>Treatments</th>
<th>Ingestion rate</th>
<th>Protocol</th>
<th>Lipid biomarkers</th>
<th>GI Comfort</th>
<th>FatOx</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leijssen et al. [279]</td>
<td>1995</td>
<td>8</td>
<td>Galactose or G</td>
<td>~50 g at start, ~50 g hr⁻¹ during exercise</td>
<td>2 hr 65% W_max, Cycle</td>
<td>Glycerol ↔</td>
<td>N/A</td>
<td>↔</td>
<td>N/A</td>
</tr>
<tr>
<td>Earnest et al. [31]</td>
<td>2004</td>
<td>9</td>
<td>Honey or G</td>
<td>~30 g hr⁻¹*</td>
<td>64-km TT, Cycle</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>↔</td>
</tr>
<tr>
<td>Too et al. [32]</td>
<td>2012</td>
<td>11</td>
<td>Raisins, Sport chews (brown rice syrups and cane juice)</td>
<td>~60 g hr⁻¹*</td>
<td>80 min 75% VO₂max, 5-km TT, Run</td>
<td>FFA ↑, Glycerol ↔</td>
<td>N/A</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Oosthuyse et al. [87]</td>
<td>2015</td>
<td>9</td>
<td>Isomalutose, G/F</td>
<td>63 g hr⁻¹**</td>
<td>2 hr 60% W_max, 20-km TT, Cycle</td>
<td>FFA ↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

Note: G, glucose; G/F, glucose+fructose (1:8 ratio); CHO, carbohydrate; FatOx, fat oxidation; TT, time trial; W_max, watt max
* High CHO breakfast consumed 4 hr before TT
** High CHO meal consumed 2 hr prior to exercise

Table 3. The effect of pre- and/or during-exercise low glycemic index modified starch on fat oxidation and performance/exercise capacity.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>n</th>
<th>Treatments</th>
<th>Ingestion protocol</th>
<th>Protocol</th>
<th>Lipid biomarkers</th>
<th>GI Comfort</th>
<th>FatOx</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johannsen and Sharp [284]</td>
<td>2007</td>
<td>7</td>
<td>AAM or G</td>
<td>1 g·kg⁻¹ CHO 30 min prior</td>
<td>2 hr ~65% VO₂max, Cycle</td>
<td>N/A</td>
<td>↔</td>
<td>↔</td>
<td>N/A</td>
</tr>
<tr>
<td>Roberts et al. [7]</td>
<td>2011</td>
<td>9</td>
<td>HMW or MD</td>
<td>1 g·kg⁻¹ CHO 30 min prior</td>
<td>2.5 hr at 70% VO₂max, Cycle</td>
<td>FFA ↑, Glycerol ↑</td>
<td>N/A</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>(p = 0.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baur et al. [287]</td>
<td>2016</td>
<td>10</td>
<td>HMW or G-based sport drink</td>
<td>60 g 30 min prior; HMW and G 30 g hr⁻¹ during, or low dose HMW ~30 g hr⁻¹</td>
<td>1 hr 50% W_max, 8 x 2 min 80% W_max, 10 maximal 2-3 min sprints, Cycle</td>
<td>N/A</td>
<td>↓</td>
<td>↑</td>
<td>↔↓</td>
</tr>
</tbody>
</table>

Note: G, glucose; AAM, acid/alcohol modified starch; HMW, hydrothermally modified waxy maize starch; TTE, time to exhaustion; CHO, carbohydrate; MD, maltodextrin
CHAPTER 3
RESEARCH DESIGN AND METHODOLOGY

Study Design

This was a randomized, double-blind, counterbalanced, placebo-controlled, and crossover study to assess the impact of two pre-exercise nutritional interventions on exercise metabolism and running performance. The study design is outlined in Figure 4. Specifically, the study consisted of six total visits to the Institute of Sports Science and Medicine (ISSM) laboratory. On the first visit, participants were informed as to the details of the study before giving their oral and written informed consent (Appendix A). Thereafter, participants completed an incremental exercise test on a motorized treadmill to volitional exhaustion to determine VO$_{2\text{max}}$. The second and third visits consisted of familiarization trials of the entire exercise protocol. The final three visits were
experimental trials. Each experimental trial was separated by 7-10 d. This study was approved by the Florida State University Institutional Review Board (Appendix B).

**Inclusion Criteria**

Trained male runners were recruited to participate in this study. Inclusion criteria was as follows:

a) Gender: Male  
b) Age: 18-40 yr  
c) Running mileage of \( \geq 30 \text{ mi} \cdot \text{week}^{-1} \) for the preceding 2 months  
d) Trained runners: \( \geq 5 \text{ yr} \) running experience  
e) Personal best in the 10 km of \(< 40:00 \text{ min} \) or of equivalent in other distances  
f) \( \text{VO}_{2\text{max}} \geq 55 \text{ ml} \cdot \text{kg} \cdot \text{min}^{-1} \)

**Exclusion Criteria**

Participants were excluded from participation in this study for the following reasons:

a) Gender: female  
b) Age: \(< 18 \text{ yr} \) or \( > 40 \text{ yr} \)  
c) Family history: myocardial infarction, coronary revascularization, or sudden death before 55 yr of age in father or other male first-degree relative, or before 65 yr of age in mother or other female first-degree relative.  
d) Cigarette smoking: current cigarette smoker or those who quit within the previous 6 months.  
e) Impaired fasting glucose or diabetes: fasting glucose \( \geq 100 \text{ mg} \cdot \text{dl}^{-1} \) confirmed by measurements on at least two separate occasions.  
f) Training status: not running \( \geq 30 \text{ mi} \cdot \text{wk}^{-1} \) for the preceding 2 months.

**Participant Recruitment**

Participants were recruited from Florida State University and the surrounding Tallahassee community. The study was primarily advertised via flyers (Appendix C) posted on campus, in
local running shops, and at running trailheads. Recruitment was conducted via social media postings, email announcements, and word of mouth. Additionally, participants were recruited at races held by local running and triathlon clubs. Recruitment was also aided by incentives. Specifically, participants received a $200 honorarium for participating in the study. Participants also received performance incentives. Specifically, $100, $50, and $25 were given to the 1st, 2nd, and 3rd place finishers for mean 5-km completion times.

**Nutritional Interventions**

Participants received one of three experimental beverages 30 min prior to each experimental trial: 1) a hydrothermally modified waxy maize starch (UCAN®, The UCAN Co., Woodbridge, CT; UCAN), 2) a sucrose and glucose-based sport supplement (Gatorade®, PepsiCo®, Purchase, NY; G), or 3) a non-caloric flavor-matched and color-matched placebo (Dymatize®. Dallas, TX; PL). Beverages consisted of 600 ml of fluid. The caloric treatment beverages contained 75 g of carbohydrate. As such, the carbohydrate concentration of caloric treatment beverages was 12.5%. In addition to the pre-exercise treatment beverages, participants also received water during exercise. Specifically, participants received 250 mL of water to consume at 30 min and 60 min of running. This provided 0.6 L·hr⁻¹ during the first 60 min of exercise which falls at the midpoint of current recommendations (0.4-0.8 L·hr⁻¹) for fluid replacement during prolonged running [305].

**Physiological Measurements**

This study assessed changes in metabolism, hormones, heart rate, gastrointestinal comfort, perceived exertion, and running performance. Time points of physiological assessments are outlined in Figure 5 and described below.

*Indirect calorimetry*

Energy expenditure, the relative contribution of carbohydrate and fat to total energy expenditure, and total carbohydrate and fat oxidation rates was assessed via indirect calorimetry (Parvomedics Truemax® 2400 Metabolic Cart, Sandy, UT, USA). For these measurements,
participants were fitted with a two-way respirator valve and asked to breathe normally. These assessments were made in 5-min increments with the means of the final 3 min being utilized in the analysis. Measurements occurred at rest, -15 min (before the start of exercise; while resting), 0 min, 15 min, and 45 min. Substrate oxidation rates were calculated as previously described [306].

**Blood metabolites and hormones**

Venous blood (20 ml) was collected from an antecubital forearm vein by trained phlebotomists under sterile conditions. Blood was drawn into 6 ml vacutainer tubes coated in EDTA (Cat. No. 368661, BD Vacutainer®, Franklin Lakes, NJ) and immediately assessed for glucose and lactate (YSI 2300 Stat, YSI, Inc., Yellow Springs, OH). Thereafter, the remaining blood was centrifuged for 15 min at 3,500 rpm at 4 °C. Plasma was then separated and stored at -80 °C for later analysis. Plasma glycerol (Cat. No. MAK117, Sigma-Aldrich®, St. Louis, MO) was assessed via enzyme-linked immunosorbent assay according to manufacturer’s instructions. Measurement of plasma FFA, insulin, epinephrine, and norepinephrine was completed by the Vanderbilt University Medical Center Hormone Assay and Analytical Services Core, which is support by NIH grants DK059637 and DK020593. Plasma FFA was assessed via a Wako kit (Wako Diagnostics, Inc., Richmond, VA) adapted for use on a Packard instrument (Meriden, CT) as previously described [307]. Insulin was assessed via radioimmunoassay as previously described [308]. Lastly, norepinephrine and epinephrine was assessed via high-performance liquid chromatography as previously described [309].

**Microdialysis**

To assess adipose tissue lipolysis, the microdialysis technique was used. With this technique, interstitial glycerol is measured as an indicator of lipolysis in subcutaneous abdominal adipose tissue (SCAAT). Microdialysis was also utilized to assess changes in adipose tissue blood flow.

Microdialysis assessment requires insertion of probes into SCAAT. The microdialysis probes were inserted under sterile conditions requiring participants to lie down and have their abdominal skin cleaned with iodine and numbed with topical ethyl chloride spray to reduce discomfort. Thereafter, a small sterilized needle surrounded by a small flexible plastic Y-splitter
was inserted about 1/8 to 1/4 inch below the skin in their SCAAT, at a distance approximately 5-10 cm from their navel. Next, the needle was removed but the plastic tubing remained in the SCAAT. A sterile microdialysis probe was then inserted into the Y-splitter plastic tube. The microdialysis probe remained in the SCAAT while the Y-splitter plastic tube was removed. After the probe was inserted, it was attached to a pump that propelled a saline solution through this tubing. The pumps are external to the sterile catheter system (much like an insulin pump). The saline solution was pumped through the tubing at a rate of 2.0 µL·min⁻¹ and was collected in a vial at the exit end of the probe tubing. For the measurement of adipose tissue blood flow, a small amount of ethanol (0.1 g·L⁻¹) was added to the saline solution. By measuring (via fluorometric assay) the ratio of ethanol collected versus infused (outflow:inflow ratio), blood flow was calculated [310]. Microdialysis sampling commenced 10 min prior to ingestion of the treatment beverage. Samples were collected every 10 min thereafter and stored at -80 °C for later analysis (CMA600, CMA Microdialysis, Solna, Sweden).

As the glycerol or glucose in the dialysate is only representative of a fraction of the actual interstitial concentration, an in vitro experiment was conducted utilizing the same probes and flow rates (i.e. 2.0 uL/min) as utilized in this study. This experiment has been described elsewhere [311]. The results allow for calculation of the recovery rates of glycerol and glucose from a solution containing known concentrations. The recovery rates were utilized to adjust dialysate glycerol and glucose concentrations to accurately express the actual in vivo interstitial concentrations. This adjustment was completed by dividing the dialysate concentrations by the percentage recovery rates.

Heart rate

Heart rate was assessed via heart rate monitor (Polar® FT4M, Sempele, Finland). Heart rate was recorded at rest, every 10 min during the first 60 min of exercise, and every km of the 5 km.
Perceptual responses

Gastrointestinal comfort and perceived exertion were determined via VAS scales (Appendix D) for various symptoms and/or subjective feelings. Specifically, the following symptoms of gastrointestinal comfort were assessed: nausea, abdominal cramps, and fullness. For perceived exertion, tiredness, leg strength, and effort of running were assessed following 30 min and 60 min of running.

**Figure 5. Experimental trial procedures.**

Performance

Running performance was assessed via a 5-km TT following a 60 min pre-load (see Figure 5; described below). In prior studies [312,313], pre-loaded running TT performance was found to
have high reliability (CV 1.0-2.4%). As such, this protocol is highly sensitive to small changes in performance.

**Anthropometric Characteristics, Body Composition, and Assessment of VO$_{2\text{max}}$**

Following the signing of the informed consent document, participants were asked to change into their running clothing. Thereafter, with shoes removed, participants’ height was assessed to the nearest cm via a wall-mounted scale (Seca, Hamburg, Germany). Next, participants were weighed to the nearest 0.1 kg via an automated scale (Detecto® 750, Webb City, MO).

Following these baseline measurements, participants were asked to wear a heart rate monitor and get on a motor-driven treadmill (Woodway ELG, Woodway USA Inc., Wakesha, WI). Participants then completed a self-determined 5-min warmup prior to the commencement of an incremental exercise test to exhaustion. Following the warmup, the participant stood quietly on the treadmill and was fitted with a metabolic mask (Parvomedics, Sandy, UT). The incremental test consisted of 1-min stages in which the velocity increased 1 km·hr$^{-1}$·min$^{-1}$ from 10-16 km·hr$^{-1}$ at 0% then increased 1% every min until volitional exhaustion. A similar protocol is described elsewhere [314].

**Familiarization Trials**

For familiarization, participants completed the entire experimental trial protocol twice (described below). However, no physiological measurements were taken. Prior to and during familiarization trials, participants received only water. The familiarization trials served three purposes: 1) to familiarize participants with study procedures and equipment, 2) to attenuate learning effects in experimental trials, and 3) to establish the coefficient of variation (CV) for the 5-km TT via comparison between the second familiarization trial and the placebo trial. Familiarization trials were separated by 7 d.

**Experimental Trials**

Experimental trials are outlined in Figure 5. During each experimental trial, the participant reported to the Human Performance Laboratory at ISSM at Florida State University in the morning
following an overnight fast (7-9 hr). Upon arrival to the laboratory, the participant was asked to wear a heart rate monitor strap and lie supine for 10 min. At this time, microdialysis probes were inserted into the participant’s SCAAT. Following insertion of the probe, the participants rested for 60 min to allow the probe to equilibrate after insertion. The probes remained in place for continuous measurement for the duration of the experimental trial. During the 60 min equilibration period, blood was collected via blood draw from an antecubital vein. Following the blood draw, the participant was fitted with a metabolic mask for 5 min of resting gas exchange assessment.

Following the 60 min equilibration period, a 10-min baseline measurement of SCAAT lipolysis was collected. Thereafter, the participant consumed one of the treatment beverages. After ingesting the beverage, the participant sat quietly for 30 min. During rest, a gas exchange assessment was made immediately prior to exercise, and an additional blood draw was taken. The participant then mounted the treadmill to begin exercise. Participants ran for 30 min at 60% VO$_{2\text{max}}$ followed by an additional 30 min at 75% VO$_{2\text{max}}$. At both 30 min and 60 min, the treadmill was briefly stopped, and participants immediately completed gastrointestinal distress and perceived exertion scales. They then were seated, and another blood draw was taken. Participants then quickly (~1 min) consumed 250 ml of water and remounted the treadmill. This entire period took less than 5 minutes before resuming running. Participants were allowed to use the restroom after 30 or 60 min of running, and this pattern was kept consistent for subsequent trials.

Prior to commencing the TT, the treadmill incline was set to 1% to best replicate the energetic costs of outdoor running [315]. Additionally, the treadmill speed was set to 5 km·hr$^{-1}$, and the participant walked for 0.2 km before starting the TT. Following this, the participant ran 5 km as fast as possible. Heart rate was measured at the end of every kilometer. No other physiological measurements were taken during the TT to prevent any distraction. For the subsequent visits, the above procedures were repeated, but a different beverage before exercise was consumed in a randomized manner. To remove an effect of treatment order, the five possible variations in treatment order were determined so that each treatment order was completed by two participants. The allocation of treatment order to individual participants was randomized via Microsoft Excel.
Standardization of Diet and Training

Dietary and training standardization is outlined in Figure 4. Prior to each experimental trial, participants were asked to maintain consistent dietary and exercise habits for 4 d. Two days prior to experimental trials, participants were asked to run exactly 30 min (on their own) at a subjectively moderate intensity. For the day preceding the experimental trials, participants were asked to not exercise at all. Additionally, for both days leading up to experimental trials, participants were asked to replicate exactly their dietary intake from week to week. To ensure compliance, participants were asked to complete a 48-hr dietary intake form (Appendix E) and a 72-hr exercise log (Appendix F) for the days preceding the experimental trial. Finally, participants were asked to abstain from any alcohol and caffeine for 24 hr and 12 hr prior to the experimental trials, respectively.

Statistical Analysis

Sample size estimation

A sample size of 10 was required. Sample size was determined by estimating the sample needed to detect the smallest effect likely to result in a substantial improvement in running performance [316]. We determined a worthwhile performance enhancement to be 1.4%, calculated as 0.6 (midpoint of the range for a small effect size, 0.3-0.9 [316]) multiplied by the typical variation for endurance running (2.4%) [288,317]. For the test reliability used in the calculation, 1.7% (mean of 1% and 2.4% CV from prior studies [312,313]) was chosen.

General methods

In order to compare data with results from prior related studies, changes in performance, perceptual responses, and gas exchanges measures were analyzed via magnitude-based inferences. This approach has several advantages over traditional null hypothesis testing because the method emphasizes effect magnitudes and qualifies the probability of an important effect with interpretive descriptors [316]. Furthermore, its use has become common in many recent publications [241,318,319]. Ninety percent confidence intervals (CI) are presented to illustrate uncertainty in treatment effects because it represents an “unclear” effect having >5% chance of being positive.
and >5% chance of being negative. Threshold values were calculated as directed by Hopkins [316]. To maintain sufficient statistical power, the threshold value for the smallest likely benefit in running performance was set to 1.4%, (0.6 multiplied by 2.4%, the typical variation for endurance running [288,317]). A published spreadsheet [320] was used to classify treatment effects as beneficial/positive, harmful/negative, or trivial/negligible. Likelihoods of reaching the substantial change threshold were classified as follows: <1%, almost certainly no chance; 1%–5%, very unlikely; 5%–25%, unlikely; 25%–75%, possible; 75%–95%, likely; 95%–99%, very likely; and >99%, almost certain. If the 90% CI included values that exceeded the threshold values for both a negative and positive effect, effects were classified as “unclear.” Effect magnitudes were classified as follows: trivial, 0.0–0.3; small, 0.3–0.9; moderate, 0.9–1.6; large, 1.6–2.5; and very large 2.5–4.0 [316]. All performance and gas exchange data were log-transformed prior to analysis to account for heteroscedasticity.

Changes in other variables (blood/interstitial glucose, lactate, glycerol, insulin, FFA, and catecholamines, and heart rate) were assessed via null-hypothesis testing. Specifically, a two-way (treatment x time) repeated measures analysis of variance was used to identify interactions. A Greenhouse-Geisser correction was made when sphericity was violated. In the case of significance, a post hoc one-way ANOVA with Tukey tests was used to identify significant differences. Effect sizes (ES) were calculated by standardizing mean differences to the SD of the placebo condition. To remove small sample bias, the SD used in calculation of effect size was divided by \(1 - \frac{3(4v - 1)}{v}\), where \(v\) is equal to the degrees of freedom [316]. Effect sizes were qualified as follows: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, extremely large >4.0 [289]. For all variables, significance was set at \(p < 0.05\). Analyses was conducted using SPSS version 21.0 (SPSS, Inc., Chicago, IL). Data are presented as means (or back-transformed means) ± SD (or confidence intervals where indicated).
CHAPTER 4

RESULTS

Participant Characteristics

Trained male runners (n = 11) were recruited for this study (Table 4). One participant dropped out of the study following the informed consent process due to scheduling conflicts resulting in a total sample of 10 participants.

Table 4. Participant characteristics.

<table>
<thead>
<tr>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>VO₂max (ml·kg⁻¹·min⁻¹)</th>
<th>VO₂max (km·h⁻¹)</th>
<th>Years running</th>
<th>km·wk⁻¹</th>
<th>5-km PR (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>176.1 ± 8.2</td>
<td>67.1 ± 7.4</td>
<td>63.5 ± 5.3</td>
<td>18.4 ± 0.9</td>
<td>11.1 ± 5.3</td>
<td>68.2 ± 18.9</td>
<td>15.9 ± 3.3</td>
</tr>
</tbody>
</table>

Note: Data are presented as means ± standard deviations. VO₂max, maximal oxygen consumption; PR, personal best.

Interstitial Biomarkers

Please note, due to logistical challenges collecting dialysate from one participant, interstitial biomarker data presented below are for 9 participants.

Glycerol

Changes in interstitial glycerol concentrations over time are presented in Figure 6. There was a main effect for time (p = 0.000), but no treatment x time interaction.
Changes in interstitial glucose concentrations over time are presented in Figure 7. There were main effects for time (p = 0.000009) and treatment x time (p = 0.004). Post hoc testing revealed significant variances at -10 min (p = 0.016), 0 min (p = 0.001), 10 min (p = 0.003), and 20 min (p = 0.041). At -10 min, there was a large increase (+9.2 ± 8.1 mg·dL⁻¹; ES = 1.73) in interstitial glucose with G vs. PL (p = 0.019), and there was a large decrease (-7.6 ± 8.5 mg·dL⁻¹; ES = 1.51) with UCAN vs. G that approached significance (p = 0.055). At 0 min, there was a large increase (+11.9 ± 7.4 mg·dL⁻¹; ES = 1.86) with G vs. PL (p = 0.002) and a large decrease (-11.0 ± 10.3 mg·dL⁻¹; ES = 1.69) with UCAN vs. G (p = 0.004). At 10 min, there was a large increase (+15.5 ± 3.6 mg·dL⁻¹; ES = 1.87) with G vs. PL (p = 0.0002) and a moderate decrease (-9.7 ± 9.4 mg·dL⁻¹; ES = 1.08) with UCAN vs. G (p = 0.017). At 20 min, there was a large increase (+11.5 ± 5.0 mg·dL⁻¹; ES = 1.38) in interstitial glucose with G vs. PL (p = 0.034).

Glucose

Figure 6. Interstitial glycerol concentrations. Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch.
Plasma Biomarkers

Blood glucose

The treatment effect on blood glucose over time is presented in Figure 8. There were main effects for time ($p = 0.000$) and treatment x time ($p = 0.000$) for blood glucose. Post hoc testing revealed a significant variance at 0 min ($p = 0.000$). Specifically, there was a very large increase ($+53.0 \pm 21.3 \text{ mg} \cdot \text{dL}^{-1}; \text{ES} = 3.91$) with G vs. PL ($p = 0.000$) and a very large decrease ($-36.6 \pm 24.9 \text{ mg} \cdot \text{dL}^{-1}; \text{ES} = 2.44$) with UCAN vs. G ($p = 0.00007$). There was also a slight trend ($p = 0.078$) for a large increase ($+16.4 \pm 13.6 \text{ mg} \cdot \text{dL}^{-1}; \text{ES} = 1.47$) in blood glucose with UCAN vs. PL. There were no differences in blood glucose during exercise.
Changes in plasma insulin concentrations are presented in Figure 9. There were main effects for time ($p = 0.000$) and treatment x time (0.000). Post hoc testing revealed significant variances at 0 min ($p = 0.000$) and 30 min ($p = 0.007$). At 0 min, there was an extremely large increase ($+33.9 \pm 11.0 \mu U \cdot mL^{-1}; ES = 4.16$) and large increase ($+8.7 \pm 4.4 \mu U \cdot mL^{-1}; ES = 1.72$) with G vs. PL ($p = 0.000$) and UCAN vs. PL ($p = 0.039$), respectively. Additionally, there was a very large decrease ($-25.2 \pm 11.0 \mu U \cdot mL^{-1}; ES = 2.44$) with UCAN vs. G ($p = 0.000$). At 30 min, there was a large increase ($+5.9 \pm 4.9 \mu U \cdot mL^{-1}; ES = 1.79$) in insulin with G vs. PL ($p = 0.005$).
Glycerol

The effect of treatment on plasma glycerol concentrations over time is presented in Figure 10. While there was a time effect ($p = 0.000$), there was no main effect for treatment x time.

Free fatty acids

The effect of treatment on plasma FFA concentrations over time is presented in Figure 10. There were main effects for time ($p = 0.00001$) and treatment x time ($p = 0.002$). However, post hoc testing revealed no significant variances for any time point.

Epinephrine and norepinephrine

Changes in plasma concentrations of epinephrine and norepinephrine are presented in Figure 11. For epinephrine, there was a main effect for time ($p = 0.000003$), but no treatment x
time interaction. Similarly, there was a time effect for norepinephrine \( (p = 0.000) \), but no main effect for treatment x time.

**Adipose Tissue Blood Flow**

There was a main effect for time as blood flow increased from rest to exercise \( (p = 0.001) \), but there was no treatment x time interaction.
Oxygen Consumption and Substrate Utilization Patterns

Treatment effects on gas exchange measures are presented in Table 5. At 0 min, there were very likely (98/1/0 likelihoods [%] increase/trivial/decrease) moderate (19.6% ± 12.5; ES = 0.93) and likely (86/11/2) small (10.9 ± 12.2%; ES = 0.54) increases in VO$_2$ with G vs. PL and UCAN vs. PL, respectively. All differences in VO$_2$ among conditions during exercise were trivial.

For carbohydrate oxidation at 0 min, there was a most likely (100/0/0) large increase (200.1 ± 89.9%; ES = 2.33) with G vs. PL and a very likely (0/0/99) very large decrease (-75.5 ± 20.0%; ES = 2.98) with UCAN vs. G. At 15 min, there were likely (95/5/0 and 75/24/1) small increases (22.9 ± 17.5% and 11.2 ± 11.7%; ES = 0.56 and 0.27) in carbohydrate oxidation with G vs. PL and UCAN vs. PL, respectively. Additionally, at 15 min of running, there was a possible (1/26/73) small decrease (-9.5 ± 9.0%; ES = 0.27) in carbohydrate oxidation with UCAN vs. G.

![Figure 11. Plasma free fatty acid concentrations. Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch; FFA, free fatty acid. * denotes treatment x time interaction (p < 0.01).](image)
Prior to exercise (0 min), there was a very likely (1/2/97) moderate decrease (-50.1 ± 26.4%; ES = 1.02) with G vs. PL and a most likely (100/0/0) moderate increase (121 ± 74.2%; ES = 1.16) in fat oxidation with UCAN vs. G. At 15 min, there were likely (1/5/94 and 0/10/90) small decreases (-14.6 ± 9.9% and -9.9 ± 6.8%; ES = 0.53 and 0.35) in fat oxidation with G vs. PL and UCAN vs. PL, respectively. There were no clear differences at 45 min among conditions.

![Figure 12](image_url). Plasma norepinephrine (A) and epinephrine (B) concentrations. Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch.

**Performance**

Pairwise comparisons in running performance are presented in Figure 13. Mean 5-km completion times were 18.7 ± 1.8 min, 18.4 ± 2.0 min, and 18.3 ± 1.9 min for PL, G, and UCAN, respectively. All relative (%) differences among conditions (G – PL: -1.4 ± 2.5 [90% confidence interval], ES = 0.15; UCAN – PL: -1.7 ± 1.9, ES = 0.18; UCAN – G: -0.3 ± 2.7, ES = 0.03) were unclear and trivial.
Perceptual Responses

Gastrointestinal comfort

The effect of treatment on mean gastrointestinal comfort responses is presented in Figure 14. There were no clear differences at any point for nausea and fullness. However, there was a small (-3.2 ± 4.3 scale unit; ES = 0.24) possible decrease (likelihoods [%] increase/trivial/decrease: 2/36/62) in feelings of abdominal cramping with G vs. PL at 30 min of running. Additionally, at 60 min, there were likely (1/6/94) moderate (-18.2 ± 14.3; ES = 0.66) and likely (1/19/80) small reductions (-10.0 ± 10.4; ES = 0.36) in feelings of abdominal cramping with UCAN vs. PL and

Figure 13. Treatment effects on 5-km running performance. Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch. Bars represent the 90% confidence interval.
UCAN vs. G, respectively. Following the time trial, there were no clear differences for ratings of abdominal cramping.

Figure 14. Ratings of nausea (A), fullness (B), and abdominal cramp (C). Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch. * denotes likely reduced vs. G and PL.
The effect of treatment on mean perceived effort is presented in Figure 15. At 30 min, the effort of running was possibly (62/36/2) higher (+3.4 ± 4.3 scale units; ES = 0.24 [small]) with G vs. PL. At 60 min, there was also a likely (1/14/85) small reduction (-6.2 ± 5.7; ES = 0.41) in the effort of running (A), tiredness (B), and leg strength (C). Note: PL, placebo; G, glucose-based supplement; UCAN, modified starch. ** denotes likely reduced vs. G and PL. * denotes likely reduced vs. PL. † denotes likely increased vs. PL.

**Perceived exertion**

The effect of treatment on mean perceived effort is presented in Figure 15. At 30 min, the effort of running was possibly (62/36/2) higher (+3.4 ± 4.3 scale units; ES = 0.24 [small]) with G vs. PL. At 60 min, there was also a likely (1/14/85) small reduction (-6.2 ± 5.7; ES = 0.41) in the
effort of running with UCAN vs. G. Following the time trial, the effort of running was likely (78/22/0) higher (+3.3 ± 2.8; ES = 0.29 [small]) with G vs. PL. For tiredness, differences were unclear and/or trivial at 30 min and following the time trial. However, at 60 min, tiredness was likely (84/15/1) reduced (-6.5 ± 6.6; ES = 0.42 [small]) with UCAN vs. PL.

**Body Mass**

There were no treatment effects on body mass losses from pre- to post-exercise.

**Heart Rate**

There was a main effect for time for steady state (i.e. collected at rest and during exercise at 60% and 75% VO$_{2\text{max}}$; $p = 0.000$) and time trial ($p = 0.000$) heart rate, but no interactions were present.

**Laboratory Conditions**

There were no differences in laboratory conditions. Temperature and humidity were maintained at 22.1 ± 0.8 °C and 53.0 ± 4.1%, respectively.

**Analytical Results**

Intra-assay CV for plasma glycerol, insulin, and FFA were 8.7%, 2.3%, and 1.0%, respectively. Inter-assay CV for plasma glycerol, insulin and FFA were 10.7%, 15.0% and 5.0%, respectively. Inter-assay CV for epinephrine and norepinephrine were 8.0% and 6.8%, respectively. Intra-assay CV for interstitial glycerol and glucose were 2.1% and 2.9%, respectively. Inter-assay CV for interstitial glycerol and glucose were 2.1% and 2.8%, respectively. Finally, intra-assay CV for blood flow analysis was 7.2%. The *in vitro* recovery rates for interstitial glycerol and glucose at a perfusion of 2.0 µL·min$^{-1}$ were 73.2% and 58.6%, respectively.
Table 5. Oxygen consumption and substrate oxidation.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Pre-Exercise</th>
<th>60% VO_{2\text{max}}</th>
<th>75% VO_{2\text{max}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO_{2} (L \cdot \text{min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.26 ± 0.04</td>
<td>0.31 ± 0.05</td>
<td>2.52 ± 0.34</td>
<td>3.12 ± 0.39</td>
</tr>
<tr>
<td>G</td>
<td>0.28 ± 0.03</td>
<td>0.37 ± 0.05**</td>
<td>2.52 ± 0.30</td>
<td>3.11 ± 0.35</td>
</tr>
<tr>
<td>UCAN</td>
<td>0.28 ± 0.04</td>
<td>0.34 ± 0.06*</td>
<td>2.51 ± 0.32</td>
<td>3.14 ± 0.38</td>
</tr>
<tr>
<td>CHO oxidation (g \text{min}^{-1})</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.10 ± 0.07</td>
<td>0.10 ± 0.05</td>
<td>1.43 ± 0.48</td>
<td>2.22 ± 0.57</td>
</tr>
<tr>
<td>G</td>
<td>0.08 ± 0.06</td>
<td>0.30 ± 0.07***††</td>
<td>1.69 ± 0.28*†</td>
<td>2.22 ± 0.32</td>
</tr>
<tr>
<td>UCAN</td>
<td>0.10 ± 0.06</td>
<td>0.11 ± 0.09</td>
<td>1.56 ± 0.45*</td>
<td>2.21 ± 0.51</td>
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<tr>
<td>Fat oxidation (g \text{min}^{-1})</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.09 ± 0.03</td>
<td>0.12 ± 0.05</td>
<td>0.67 ± 0.16</td>
<td>0.66 ± 0.18</td>
</tr>
<tr>
<td>G</td>
<td>0.11 ± 0.03</td>
<td>0.06 ± 0.03**†††</td>
<td>0.57 ± 0.13*</td>
<td>0.65 ± 0.16</td>
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<tr>
<td>UCAN</td>
<td>0.10 ± 0.04</td>
<td>0.13 ± 0.03</td>
<td>0.62 ± 0.17*</td>
<td>0.67 ± 0.22</td>
</tr>
</tbody>
</table>

Note: Data are presented as means ± standard deviations. Data were analyzed utilizing magnitude-based inferences as described in the methods. VO_{2\text{max}}, maximal oxygen consumption; VO_{2}, oxygen consumption; CHO, carbohydrate; PL, placebo; G, glucose- and sucrose-based sport drink; UCAN, modified starch.

*** almost certain vs. PL
** very likely vs. PL
* likely vs. PL
††† most likely vs. UCAN
†† very likely vs. UCAN
† possibly vs. UCAN
CHAPTER 5

DISCUSSION

This is the first study to investigate the effect of ingesting a low glycemic index modified starch on SCAAT lipolysis during exercise. Primary findings of the present study included: 1) no differences in SCAAT lipolysis at rest or during exercise regardless of treatment or exercise intensity, 2) interstitial glucose was increased following ingestion of carbohydrate, and the magnitude of elevation was greater with the high (G) versus low (UCAN) glycemic index carbohydrate, 3) blood glucose was elevated immediately prior to exercise with high glycemic index carbohydrate (G), 4) carbohydrate ingestion elevated insulin prior to exercise, and the increase was larger with high (G) versus low (UCAN) glycemic index carbohydrate, 5) fat oxidation was reduced at rest and at 60% VO$_{2\text{max}}$ with pre-exercise carbohydrate relative to PL; however, there were no clear differences among high (G) and low (UCAN) glycemic index carbohydrate treatments nor among any treatment at 75% VO$_{2\text{max}}$, and 6) performance was unaltered regardless of treatment.

Interstitial Glycerol and Glucose

Interstitial glycerol was unaffected by carbohydrate ingestion at rest or during exercise regardless of its glycemic index. When controlled for changes in blood flow, interstitial glycerol can be utilized to quantify SCAAT lipolysis [321]. As blood flow was not different among treatments (discussed below), it follows that SCAAT lipolysis was unaffected by carbohydrate ingestion. Worth noting, the majority of FFA mobilized to skeletal muscle during exercise are derived from upper body subcutaneous adipose tissue [184,322]. Thus, the SCAAT depot is of primary interest during exercise.

Prior studies examining the effect of pre-exercise carbohydrate on resting and exercise lipolysis are conflicting. Using stable isotopes, Horowitz et al. [3] reported attenuated lipolysis at rest and during exercise (44% VO$_{2\text{max}}$ cycling) with ingestion of either glucose or fructose (0.8 g·kg$^{-1}$) versus a placebo 60 min prior to exercise. In a similarly designed study but using arteriovenous difference methods, Enevoldsen et al. [10] reported reduced lipolysis at rest, but no change during exercise (55% VO$_{2\text{max}}$ cycling) with ingestion of a high carbohydrate meal (121 ± 8 g
carbohydrate, 20 ± 1 g fat, and 21 ± 3 g protein) 60 min before exercise. The present finding of no differences in SCAAT lipolysis at rest contrasts with these prior studies. One possible explanation for this difference is the timing of carbohydrate intake. Carbohydrate was consumed 30 min prior to exercise in the current study compared to 60 min pre-exercise in these other studies [3,10]. Importantly, resting lipolysis was not different relative to fasting 30 min following ingestion of carbohydrate in both prior studies [3,10], which is in agreement with the current study. In fact, there was no change in resting lipolysis in either study until between 40 min [3] and 60 min [10] after carbohydrate ingestion. This suggests that differences in resting lipolysis are only observable given a sufficient postprandial duration (>30 min). Indeed, insulin levels peak ~30 min post-ingestion of carbohydrate [45]. While insulin is potently antilipolytic [252], it is possible that its effects on lipolysis are not observable until after reaching a sufficiently high threshold level and/or following a necessary duration of stimulation (30+ min).

There were no differences in SCAAT lipolysis during exercise. This finding agrees with those of Enevoldsen and colleagues [10], but not Horowitz et al. [3]. While it is possible that the lack of agreement between studies is the result of different carbohydrate treatments utilized or the timing of ingestion, one likely contributing factor is differences in exercise intensity. Exercise intensity was substantially higher in the present and Enevoldsen et al. [10] studies (60-75% and 55% VO$_{2\text{max}}$, respectively) relative to the Horowitz et al. [3] study (44% VO$_{2\text{max}}$). This has important potential implications for the catecholamine response to exercise. Norepinephrine and epinephrine respond nearly exponentially to increases in exercise intensity [40]. As such, it is likely that epinephrine and norepinephrine levels were higher in the present and Enevoldsen et al. [10] studies relative to the Horowitz et al. [3]. Also worth noting, the participants in the present study had higher aerobic fitness levels relative to the two prior studies (63.5 vs. ~52 ml·kg·min$^{-1}$). As such, catecholamine responsiveness would have been altered, and the absolute intensity of exercise would be elevated further relative to the prior studies [323]. Given that the antilipolytic effects of insulin can be overridden with sufficient sympathetic stimulation [253], it seems likely that high levels of catecholamines may have a similar effect. If so, catecholamine levels in the present and Enevoldsen et al. [10] studies may have been sufficient to override any effects of insulin on lipolysis. Regardless of the mechanism, the present and prior results [10] taken together suggest that pre-exercise carbohydrate has no impact on exercise lipolysis given a moderate or higher exercise intensity.
This is the first study to investigate the impact of the glycemic index of pre-exercise carbohydrate on interstitial glycerol concentrations. There were no differences in resting or exercise SCAAT lipolysis between G or UCAN. This contrasts with our hypothesis that UCAN would enhance SCAAT lipolysis relative to G. Indeed, UCAN has substantially attenuated effects on blood glucose and insulin as evidenced by this study and prior studies [7,287]. Moreover, these effects have been repeatedly associated with increases in plasma markers of lipolysis (e.g. FFA and/or glycerol) and whole body fat oxidation during exercise following low glycemic index meals [14,21,23,63,268] or modified starches [7,287]. While these effects are clearly suggestive of changes in subcutaneous adipose tissue lipolysis, the present study found no changes in interstitial glycerol concentration. However, it is worth noting that there were minimal to null effects on plasma markers of lipolysis and fat oxidation (despite substantial changes in blood glucose and insulin in the present study), which disagrees with prior studies [7,14,21,23,63,268,287]. Clearly, future research is warranted to elucidate these contrasting findings between studies.

This is also the first study (to the author’s knowledge) to report changes in interstitial glucose concentrations with pre-exercise carbohydrates of different glycemic indices. Interstitial glucose concentrations were elevated post-ingestion of carbohydrate, and the magnitude of the elevation was greater with high glycemic index (G) relative to low glycemic index (UCAN) carbohydrates. This finding is predictable based on the effects of UCAN and G on blood glucose (discussed below). However, one interesting finding was that the timing and magnitude of glucose excursions were later and attenuated, respectively, in the interstitial space relative to the blood. This has been reported elsewhere [324,325], and a physiological “push-pull” model for these effects has been proposed. According to Aussedat and colleagues [325], the ~5 min lag in the elevation of interstitial glucose relative to blood glucose is due to the time necessary for blood glucose to be “pushed” from the vascular compartment into the interstitial space. Furthermore, the rise in interstitial glucose is blunted relative to blood glucose because of the subsequent counterbalancing insulin-stimulated “pull” of interstitial glucose into cells [325].

Blood flow within SCAAT increased during exercise, but was not affected by treatment. Prior studies have reported adipose tissue blood flow to increase [326,327] and decrease [254] during exercise. Exercise results in a sympathetic response that redistributes blood flow to the active muscles [328]. As such, it could be predicted that blood flow to adipose tissue would be attenuated during exercise. However, reductions in adipose tissue blood flow seem to primarily
occur in adipose regions that are not adjacent to active muscle [326]. In the current study, the exercise mode was running, which is characterized by activation of a large portion of the body’s muscle mass including the abdominal region [329]. Therefore, the finding of increased adipose tissue blood flow over time in the present study was expected.

**Plasma Metabolites and Hormones**

Pre-exercise ingestion of carbohydrate elevated blood glucose immediately prior to exercise, and the magnitude of the increase was larger with G relative to UCAN. This was predicted based on the glycemic indices of the treatments (G: ~100 vs. UCAN: 32). G is primarily sucrose- and glucose-based. Following ingestion and emptying from the stomach, these carbohydrate moieties require no processing prior to being absorbed from the small intestine via SGLT1 and GLUT5 transporters into the blood [330]. Conversely, UCAN is a waxy maize starch derivative, which upon reaching the small intestine requires the action of various amolytic enzymes to liberate glucose from the heavily branched and crystalline starch structure [102,103]. Consequently, the rate of appearance of glucose in the blood is faster and higher following ingestion of high glycemic index carbohydrates (ex. G) versus low (ex. UCAN), and this has been consistently reported in the literature for both mixed meals [11–14,16,19,21–24,27–29,63,268,270,273,280,295,331] and modified starches [7,287].

Insulin concentrations were elevated immediately prior to exercise following carbohydrate ingestion relative to PL mirroring the elevations in blood glucose. Additionally, insulin concentrations were higher with G relative to UCAN. These findings were expected because insulin is released from the pancreas in response to elevations in blood glucose [332], and the magnitude of insulin release is highly sensitive to the magnitude of blood glucose elevations. As evidence, numerous studies have reported relatively higher insulin levels post-ingestion of high versus low glycemic index carbohydrate [7,11,12,14,16,19–23,63,268,270,273,280]. Thus, our findings align with prior work on the impact of the glycemic index on insulinemia.

Despite the large magnitude changes in blood glucose and insulin with carbohydrate versus PL and with G versus UCAN, catecholamine levels were unaffected by any treatment. Norepinephrine and epinephrine are primarily responsible for the mobilization of energy during exercise as they have been shown to be highly stimulatory of both lipolysis [152,153] and
glycogenolysis [333,334]. As energy availability would presumably be enhanced by pre-exercise feeding of carbohydrate, it is plausible that the exercise-induced elevations in catecholamines would be attenuated. Indeed, there is some evidence that the catecholamine response to exercise is blunted with feeding versus an extended fast (59 hr) or when glucose is infused or consumed during prolonged exercise [335–337]. However, most studies report no differences at rest or during exercise in catecholamine levels with pre-exercise carbohydrate vs. placebo (e.g. 8-12 hr fast) [13,238,338,339]. Few studies have examined the catecholamine response to exercise following ingestion of carbohydrate of different glycemic indices with most showing no differences [10,13,28]. Our finding confirms these prior studies and provides a plausible mechanism for a lack of change in SCAAT lipolysis.

The changes in plasma markers of lipolysis in response to consuming carbohydrate before exercise were suggestive of differences in FFA reesterification between treatments. Specifically, the rise in plasma glycerol levels was not different between treatments. This would be expected based on our finding of no change in SCAAT lipolysis and the fact that plasma glycerol is indicative of adipose (rather than skeletal muscle) lipolysis [223]. For plasma FFA, there were subtle indications that FFA release trends were different. Specifically, there was a main effect for a treatment x time interaction. The hydrolysis of a triglyceride results in the liberation of one glycerol and three FFA molecules. As such, the ratio of FFA to glycerol rate of appearance in the blood (3:1) can be assessed when arterio-venous or isotope tracer methods are utilized. Importantly, a change in this ratio (i.e. the ratio becomes < 3), can be indicative of FFA reesterification [10]. While the present study did not utilize either method, our finding of unchanged glycerol concentrations despite subtle differences in FFA concentrations over time (i.e. treatment x time interaction) indicates differences in FFA reesterification. This finding agrees with the study by Enevoldsen et al. [10] who reported no differences in blood glycerol or plasma FFA appearance during exercise but did find a reduced ratio of FFA to glycerol with a pre-exercise high carbohydrate meal (121 ± 8 g carbohydrate, 20 ± 1 g fat, and 21 ± 3 g protein) relative to fasting. Any change in FFA reesterification in the current or prior studies [10] was likely a result of elevations in insulin, which has been shown to stimulate reesterification via a mechanism involving phosphodiesterase 3 [340].

Interestingly, there were no differences in plasma glycerol or FFA between UCAN and G. A number of prior studies have reported elevated concentrations of FFA and/or glycerol during
exercise following ingestion of low versus high glycemic index carbohydrate [14,19,20,22,23,63,268,280,286]. Moreover, Roberts et al. [7] found increased serum glycerol and FFA during 2.5 hr cycling at 70% VO\(_{2\text{max}}\) 30 min following ingestion of the same modified starch supplement utilized in the current study (UCAN). It is possible that these seemingly contradictory findings can be explained by differences in exercise mode and the timing of carbohydrate ingestion. For example, several studies have reported increases in FFA and/or glycerol when low glycemic index carbohydrate is ingested within 45 min of the start of exercise [19,20,22]. However, all of these studies involved cycling exercise. As cycling and running have unique fuel demands [275–277], it is possible that the metabolic effects of low glycemic index carbohydrate are more apparent in cycling versus running. Additionally, the studies involving running that show elevations in FFA or glycerol with low glycemic index carbohydrate versus high are all characterized by a longer (2-3 hr) pre-exercise postprandial period [14,23,63,268,286]. With this in mind, perhaps observing changes in fat metabolism during running stemming from pre-exercise ingestion of low glycemic index carbohydrate is primarily the result of differences in pre-exercise glycogen levels. Due to the slower intestinal absorption rate of low glycemic index carbohydrate, it is conceivable that attaining a physiologically relevant difference in glycogen storage between high and low glycemic index carbohydrate treatments requires a longer postprandial period. Future research is needed to determine precisely how timing and exercise mode impact the metabolic responses to low glycemic index carbohydrate.

**Oxygen Consumption and Substrate Utilization**

Oxygen consumption was elevated with carbohydrate versus PL during the postprandial period immediately prior to exercise. This increase in oxygen consumption could potentially indicate nutrient-induced thermogenesis or a non-metabolic orally stimulated increase in sympathetic activity. Indeed, Acheson et al. [341] utilized a beta adrenergic receptor blockade combined with glucose infusion to determine the role of the sympathetic nervous system in nutrient thermogenesis. This study revealed that carbohydrate thermogenesis exceeds that required simply for processing and storing, and that this elevation in oxygen consumption is likely due to insulin-induced upregulation of sympathetic activity [341,342]. As carbohydrate elevated insulin versus PL, it seems likely that insulin contributed to the pre-exercise increases in oxygen consumption.
Additionally, it is also possible that stimulation of oropharyngeal carbohydrate receptors in the mouth enhanced oxygen consumption via sympathetic stimulation. Indeed, a recent study reported elevated exercise heart rates following a carbohydrate mouth rinse, (i.e. no carbohydrate was ingested), versus a placebo rinse [343]. However, we did not find differences in heart rate among treatments; thus, this requires further study for confirmation.

Ingestion of carbohydrate enhanced carbohydrate oxidation immediately prior to exercise and during running at 60% \( \text{VO}_{2 \text{max}} \); however, carbohydrate glycemic index did not appear to influence substrate usage during exercise. The increase in carbohydrate oxidation at rest and during exercise following carbohydrate ingestion was predicted based on evidence from numerous prior studies [11,13,19,20,22,229,235,238,344,345]. As described above, carbohydrate ingestion resulted in elevated concentrations of insulin, which increases skeletal muscle glucose uptake and glycolysis [256,260,346] and seems to inhibit fat oxidation by impairing FFA transport into the mitochondria [34]. Based on these same mechanisms, it was also predictable that UCAN would attenuate fat oxidation, but to a lesser extent relative to G due to its distinct effects on blood glucose and insulin. While this did occur at rest, it was not apparent during exercise, which contrasts with prior studies examining low glycemic index meals and/or modified starches [12,14,20,22,23,62,63,268–270,287,295]. The reasons for this discrepancy are not immediately clear and may be due to a number of possible methodological considerations that were described above (e.g. carbohydrate timing, exercise mode, etc.). Regardless, the present study indicates that changes in substrate utilization at rest and during exercise following ingestion of carbohydrate are primarily the result of differences in carbohydrate availability and/or mitochondrial FFA transport rather than differences in adipose tissue metabolism (based on little to no effect on SCAAT lipolysis, plasma glycerol, and plasma FFA between treatments).

**Performance and Perceptual Responses**

There was no clear effect of treatment on 5-km running performance. Generally, pre-exercise carbohydrate has been shown to enhance endurance performance [for review see ref# 45]. It is not immediately clear why carbohydrate was not ergogenic in the present study. This finding could be related to the duration of exercise. Indeed, the exercise protocol in the present study was chosen to address an important limitation in previous studies—overly long (2+ hr) exercise
protocols for a pre-exercise-only nutritional intervention when it is well-documented that performance during prolonged (2+ hr) endurance exercise is optimized with both pre- and during-exercise carbohydrate [233]. Thus, for a more valid simulation of exercise for which one might conceivably consume carbohydrate before but not during exercise, the exercise protocol was designed to be relatively short (~1.5 hr). As such, performance with this protocol was likely not limited by endogenous carbohydrate stores (by design), which may have negated any metabolic benefits from pre-exercise carbohydrate [1]. Worth noting, prior studies have indicated that exercise performance in ~60 min intense cycling is enhanced with pre-exercise carbohydrate; but, this effect seems to be non-metabolic in nature (i.e. mouth rinse effect) [343,347,348]. It is possible that the duration of exercise in the present study was overly long to be impacted by seemingly transient mouth rinse effects, but simultaneously short enough to negate the metabolic benefits of pre-exercise carbohydrate. However, this is purely speculation, and more research is needed.

Performance was also unaffected by carbohydrate glycemic index. This finding is in line with prior studies examining the effect of the same modified waxy maize starch supplement (UCAN) utilized in the present study on cycling performance [7,287], and a number of studies examining the impact of low versus high glycemic index carbohydrate meals [18–30]. However, enhanced performance with pre-exercise ingestion of low versus high glycemic index carbohydrate has also been reported in several studies [11–17,295]. Importantly, metabolic effects in these studies vary widely. Due to these conflicts, it is challenging to draw firm conclusions regarding the performance effects of modified starch, or low glycemic index carbohydrate generally, as observing a clear and consistent presumably metabolically-derived performance benefit would require reliable metabolic effects. More research is necessary to elucidate the factors (i.e. methodological or otherwise) contributing to clear metabolic and performance effects in some studies, but not others.

Perceptual responses suggested that UCAN likely attenuated abdominal cramping and tiredness following 60 min of running. An explanation for these findings is not immediately clear. While UCAN has a significantly lower osmolality (~50 vs. ~350 mOsm·kg\(^{-1}\)) relative to G, which would presumably enhance gastric emptying, Baur et al. [287] reported that gastrointestinal comfort is actually impaired with pre-exercise UCAN ingestion. It is possible that this discrepancy is due to intensity/mode/etc.; but, more research is needed to confirm this. In terms of tiredness, previous work examining the impact of UCAN on metabolism indicates enhanced FFA availability
during exercise [7,287]. This would seemingly enhance overall energy availability, particularly late in exercise, which might attenuate tiredness. However, we found little evidence of altered FFA availability with UCAN in the present study. Regardless, differences in perceptual responses had no clear impact on running performance. Thus, these effects seem to have little practical relevance.

Conclusions

This study investigated the impact of consuming carbohydrate supplements differing in glycemic index on fat metabolism and running performance. Our data reveal that while carbohydrate consumption did attenuate fat oxidation at rest and during moderate intensity exercise compared to a non-caloric placebo, this effect did not appear to be the result of alterations in adipose tissue metabolism. Additionally, the glycemic index of carbohydrate did not influence metabolic responses. Thus, our first and second hypotheses must be rejected. These findings suggest that adipose tissue metabolism is resilient to nutritional stimuli (i.e. carbohydrate) given that nutrients are consumed within relatively close proximity to exercise (i.e. 30 min) and that exercise intensity is adequate to elicit a catecholamine response capable of overriding the antilipolytic effects of insulin (~55% VO2max or more). However, more research is needed to confirm these hypotheses.

Pre-exercise carbohydrate ingestion, regardless of glycemic index, also had no impact on running performance following exercise of a duration that is not glycogen-limited (i.e. ~90 min). Thus, our third hypothesis must also be rejected. As longer duration and/or glycogen-limited exercise is maximized by both pre- and during-exercise intake, our finding suggests that carbohydrate intake prior to exercise of a duration in which an athlete might feasibly only consume carbohydrate before, but not during exercise, has no clear benefit. However, more research is warranted as this finding contrasts with prior studies that showed enhanced performance in moderate duration (1-2 hr) exercise following carbohydrate ingestion [235,239,240,344,349]. In addition, while the present study indicates that acute low glycemic index carbohydrate ingestion does not benefit endurance performance, some prior research indicates that low glycemic index carbohydrate can be ergogenic when consumed chronically [350]. Thus, more research is necessary to determine under what conditions low glycemic carbohydrates are ergogenic.
This study had several limitations. First, the exercise mode utilized in the present study presented logistical challenges for data collection. In order to draw blood and collect microdialysis samples, participants were required to stop running and either straddle the treadmill or dismount and sit. While all samples were collected as rapidly as possible, the intermittent nature of the resultant exercise may have increased the variability of plasma and/or interstitial variables. In addition, lipolysis measurements in the present study were confined to the SCAAT. While this region has been reported to be the primary source of adipose-derived FFA during exercise [184,322], it is possible that alterations in adipose tissue metabolism occurred in other regions (e.g. subcutaneous adipose tissue of the exercising legs, intra/extramuscular triglyceride, etc.), which influenced whole body fat oxidation.

Future research should investigate if exercise mode mediates nutritionally-derived alterations in adipose tissue metabolism. The present study assessed the impact of pre-exercise carbohydrate on metabolic responses during running. While pre-exercise carbohydrate attenuated fat oxidation during exercise, there was no impact on adipose tissue lipolysis. Moreover, the glycemic index of carbohydrate did not alter substrate selection during exercise. Both findings contrast with prior studies utilizing cycling as the exercise stimulus [3,287]. Cycling and running have different fuel demands [275]. It has been theorized that this could be due to different muscle and/or fiber-type contributions, hormonal responses, or diet-induced adaptations between cyclists and runners. Future research should elucidate what primarily contributes to these different fuel selection patterns and whether these differences alter the adipose tissue responses to nutritional stimuli.
APPENDIX A

IRB APPROVED INFORMED CONSENT

The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance

Informed Consent Form

1. I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled “The effects of pre-exercise energy beverages on adipose tissue lipolysis, metabolism, insulin, and performance.” This study is being conducted by Dr. Mike Ormsbee and Daniel Baur of the Department of Nutrition, Food, & Exercise Sciences and the Institute of Sports Sciences & Medicine at Florida State University.

2. The purpose of the proposed study is to determine the impact of various pre-exercise supplements on metabolism and performance. Twelve trained men (18-40 years of age) will be recruited for this study.

3. My participation in this study will require coming to the Human Performance Laboratory at the Institute of Sport Sciences and Medicine at Florida State University for testing on four occasions over 4 weeks to complete the measurements and assessments as described below.

On my first visit, I will be given an informed consent document to sign and a medical history form to complete before I can participate in the study.

On my first visit, I will be given an informed consent document to sign and a medical history form to complete before I can participate in the study. In order to participate in this study I must meet the following conditions:

a) Gender: Male
b) Age: 18-40
c) Running mileage of >30 miles/week for the preceding 2 months
d) Trained runners: > 5 years running experience
e) Personal best in the 10km of <40:00 minutes of equivalent in other distances
f) VO2max > 55 ml/kg/min or 4.5 L/min

I cannot participate in this study if I meet more than one of the conditions below:

a) Age: Males > 40 yrs
b) Family History: Myocardial infarction, coronary revascularization, or sudden death before 55 years of age in father or other male first-degree relative, or before 65 years of age in mother or other female first-degree relative.

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c) Cigarette Smoking: Current cigarette smoker or those who quit within the previous 6 months.
d) Hypertension: Systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg, confirmed by measurements on at least two separate occasions, or on antihypertensive medication.
e) Dyslipidemia: Low-density lipoprotein (LDL) cholesterol > 130 mg/dl or high-density lipoprotein (HDL) < 40 mg/dl, or on lipid-lowering medication. If total serum cholesterol is all that is available use > 200 mg/dl rather than LDL > 130 mg/dl.
f) Impaired fasting glucose: Fasting glucose ≥ 100 mg/dl confirmed by measurements on at least two separate occasions.
g) Obesity: Body mass index > 30 kg/m^2
h) Sedentary Lifestyle: Persons not participating in a regular exercise program or not meeting the minimal physical activity recommendations from the U.S. Surgeon General’s Report.
i) High-serum HDL Cholesterol: This is considered a “negative risk factor”, so 1 of the above risk factors can be subtracted if HDL > 60 mg/dl.

During the study I must discontinue dietary or ergogenic supplementation. If I am taking a supplement I will be asked to complete a washout period of one to four weeks depending on the supplement being taken at that time prior to participation in the study with the exception of a multivitamin.

Following completion of the consent form, I will be asked to change into clothing that is free of metal and/or hard plastic (buttons, zippers, snaps, etc.) and asked to remove all metal from my body (jewelry, eyeglasses, hair accessories, etc.). Height and weight will be measured via the use of a wall-mounted SECA 216 stadiometer and a digital scale (SECA, Hamburg, Germany), respectively. All measurements will be taken without shoes wearing minimal clothing (e.g. running shorts). Body composition will be measured non-invasively via the use of the Hologic DXA® Scanner (Discovery QDR Series, Hologic Inc., Bedford, MA), with one scan while lying supine on a padded table. Very low doses of radiation are used; however, this test is non-invasive. Testing will be completed according to the manufacturer’s instructions and specifications by a certified X-ray technician.

Following completion of anthropometric and body composition testing, I will complete a graded running exercise test on a treadmill to volitional fatigue to measure aerobic fitness. After completion of this test I will receive a 24-hr dietary record and a 72-hr exercise record. I will be informed on how to complete these forms prior to each experimental trial. Following my first experimental trial, a copy of the completed 24-hr dietary record will be given to me so that I can replicate my diet in the day before each trial. I will be asked to maintain consistent exercise habits between each trial. I will be asked to abstain

The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance from alcohol and caffeine for 24-hrs and 12-hrs prior to the experimental trials, respectively. Total time commitment for this visit will be 90 min.

The second visit to the laboratory will occur 1-3 days following baseline testing. For this visit, I will report to the laboratory fasted 7-9 hours. Upon arrival, I will complete a familiarization trial consisting of the entire exercise and performance protocol with no data being collected. Prior to the familiarization trial, I will receive only water in the same amount that will be given prior to experimental trials. Total time commitment for this visit will be 2 hr.

During each experimental trial, I will report to the Human Performance Laboratory and the Institute of Sports Sciences and Medicine at Florida State University in the morning following an overnight fast (7-9 hours). Upon arrival at the laboratory, I will put on a heart rate monitor strap and be seated. At this time, microdialysis probes will be inserted into my subcutaneous adipose tissue (SCAAT) and will remain there for the duration of the trial. Insertion of the probes will require me to lie down and have my abdominal skin cleaned with iodine and numbed with topical ethyl chloride spray to reduce discomfort. To measure the blood flow in the abdominal fat tissue, a small sterilized needle surrounded by a small flexible plastic tube will be inserted about 1/8 to 1/4 inch below my skin in my abdominal fat, at a distance approximately 5-10 cm from my belly button. Thereafter, the needle will be removed but the plastic tubing will remain in the SCAAT. This tubing will not be located in any blood vessels; it will be located between the fat cells in my abdominal area. A sterile microdialysis probe is then inserted into the small flexible plastic tube. The microdialysis probe will remain under the SCAAT while the flexible plastic tube is removed. All insertion pieces are sterile, medical grade, one-time use items. After the probe is inserted, it will be attached to a pump that will propel a salt solution containing a small amount of alcohol through this tubing to monitor blood flow in fat tissue. The pumps are external to the sterile catheter system (much like an insulin pump). The amount of alcohol in this solution is very small and the total amount of alcohol going through the tubing over the entire duration of the experiment is less than that in one small drop of alcoholic beverage (1/4 the size of the eraser on a pencil). Because this amount is very small, I will not feel the presence or effects of any of these substances. Moreover, since the pumped fluid is similar to the fluid already present in the body, this fluid is harmless to them. The solution will be pumped through the tubing at a rate of approximately one small drop per hour and will be collected in a vial at the exit end of the PROBE tubing. Following insertion of the PROBE, the participants will rest for 1 hour to allow the probes to equilibrate after insertion. The probes will remain in place for continuous measurement for the duration of the experimental trial.

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My blood will then be collected via venous blood draw. My blood will be collected under sterile conditions in the amount of 20 milliliters from a forearm vein and stored for later analysis. The blood samples will not be used for any other research or testing purposes other than those specified in the research proposal. I will also be fitted with a metabolic mask for five minutes of assessment.

I will consume one of two beverages. After ingesting the beverage, I will sit quietly for 30 minutes. During rest, metabolic measurements via mask will be taken every 15 minutes until the start of exercise. After 30 minutes, I will mount the treadmill. I will run for 30 min at 60% \( VO_2 \text{max} \) followed by 30 minutes at 75% \( VO_2 \text{max} \). Following this, I will run 5km as fast as possible. During running, metabolic measurements will be taken at 15 minutes of exercise and 45 minutes of exercise. A venous blood sample will be taken prior to the start of exercise, at minute 30, and minute 60. My heart rate and rating of perceived exertion will be measured every 15 minutes. For the subsequent visit, I will repeat the above, but consume a different beverage before exercise. Each experimental visit will require a 3-hr time commitment.

4. I understand there is a minimal level of risk involved if I agree to participate in this study. I may experience some muscle soreness from the maximal oxygen uptake test and other exercise testing. The risks associated with the maximal oxygen uptake test and the exercise protocol are minimal and the selected protocols have been previously used in other studies. There is a possibility of muscle fatigue and soreness related with cycle exercise.

The risks when drawing blood are small and there may be some local discomfort at the site of needle placement with possible bruising or swelling. The risk of local infection is also small. These risks will be minimized by the use of a registered nurse using sterile techniques and equipment.

Body composition will be evaluated by DXA. This involves some radiation of approximately 0.5 millirem (mREM) per total body scan, which is very low compared to a full dental X-ray (300 mREM). The measurement of body composition using DXA is non-invasive.

The risk of adverse events from these commercially available supplements is also small. Ingestion of supplements prior to exercising can potentially cause gastrointestinal distress. However, the risk of gastrointestinal distress with moderate intensity exercise is quite minimal. I am aware that the facility that produces the supplements for this study also manufacture products made from soy, wheat, and grain.

The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance at the facility. It is possible that cross-contamination could occur, but is unlikely. If I have an allergy to soy, what, or grain I must make this known to the research team.

5. The possible benefits of my participation in this research project include knowledge about my body composition and aerobic fitness. I will also receive $100 for completion of the study.

6. The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with the participant’s name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his office.

7. In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense. The researchers involved in this study, the Department of Nutrition, Food, and Exercise Sciences, Florida State University disclaims any and all liability from and in connection with this exercise testing program undertaken in the Human Performance Laboratory at The Florida State University and in no way will they be held responsible for any injuries that may occur as a result of the exercise testing completed for this study.

8. Any question I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Dr. Michael Ormsbee at or Daniel Baur at for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

9. In case of an injury, or if I have questions about my rights as a subject/participant in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at

10. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance

11. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

Print Name

Signature Date

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8673 - FAX (850) 644-4392

APPLICATION B
IRB APPROVAL LETTER AND RENEWAL LETTER

APPROVAL MEMORANDUM

Date: 02/15/2016
To: Daniel Bauer
Address:
Dept.: NUTRITION FOOD AND EXERCISE SCIENCES
From: Thomas L. Jacobson, Chair
Re: Use of Human Subjects in Research
The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 02/10/2016. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 02/08/2017 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.

Cc: Michael Ormsbee  Advisor
HSC No. 2015.17148
RE-APPROVAL MEMORANDUM

Date: 01/13/2017
To: Daniel Bur
Address:
Dept.: NUTRITION FOOD AND EXERCISE SCIENCES
From: Thomas L. Jacobson, Chair
Re: Re-approval of Use of Human subjects in Research:

The effects of pre-exercise performance supplements on adipose tissue lipolysis, metabolism, insulin, and performance

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 01/10/2018, you are must request renewed approval by the Committee.

If you submitted a proposed consent form with your renewal request, the approved stamped consent form is attached to this re-approval notice. Only the stamped version of the consent form may be used in recruiting of research subjects. You are reminded that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chairman of your department and/or your major professor are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols at least as necessary to ensure that the project is being conducted in compliance with our institution and with DHHS regulations.

Co: HSC No. 2015-199958
APPENDIX C

STUDY FLYER

TRAINED RUNNERS WANTED FOR RUNNING PERFORMANCE STUDY

The Human Performance Laboratory at the Institute of Sport Sciences and Medicine at FSU will be conducting a study examining the effects of ingesting various pre-exercise meals on pre-, during-, and post-exercise metabolism and performance.

Who Are We Looking For?

- Males
- 18-40 years old
- Trained runners
  - >30 miles/week for preceding 2 months
  - > 5 years running experience
  - < 40:00 minute personal best in 10km or equivalent in other distances

What Will You Be Asked to Do?

- Complete preliminary fitness and body composition testing/screening
- Participate in three exercise protocols, each of which will consist of 1 hour of running on a treadmill at a moderate intensity followed by a 5km time trial. You will receive a meal prior to exercising.
- Receive laboratory assessments (including venous blood sampling and microdialysis assessment of abdominal fat utilization) during each session
- Each of the 3 exercise protocols above will be separated by 7 days

What are the benefits of participation?

- Free evaluation of aerobic capacity ($\text{VO}_{2\text{peak}}$) and body composition
- $100 for study completion

For more information, please contact Dan Baur at
APPENDIX D

GASTROINTESTINAL DISTRESS AND PERCEIVED EXERTION SCALES

<table>
<thead>
<tr>
<th>Nausea</th>
<th>Fullness</th>
<th>Abdominal Cramps</th>
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<td>Extremely Strong</td>
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<td>Extremely Weak</td>
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Subject #__________

Time point __________

Trial #__________

<table>
<thead>
<tr>
<th>Effort of Cycling</th>
<th>Tiredness</th>
<th>Leg Strength</th>
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<tr>
<td></td>
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<td>Absolute Maximum</td>
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<td>Extremely Weak</td>
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Subject #__________

Time point __________

Trial #__________
APPENDIX E

24-HR DIETARY LOG

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<tr>
<th>Time</th>
<th>Food and/or Drink</th>
<th>Method of Preparation</th>
<th>Quantity Consumed</th>
<th>Brand Name</th>
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</table>

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment*. 2nd ed. United States of America: Mosby; 1996
APPENDIX F

72-HR EXERCISE LOG

3-Day Physical Activity Records

<table>
<thead>
<tr>
<th>Date</th>
<th>Type of Exercise Performed</th>
<th>Duration of Exercise (minutes)</th>
<th>Intensity of Exercise (use scale below)</th>
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</table>

Intensity Scale

6  Very, very light
7  Very light
8  Fairly light
9  Somewhat hard
10  Hard
11  Very hard
12  Very, very hard
13  
14  
15  
16  
17  
18  
19  
20  

Subject #__________  Trial #__________  Date:__________
REFERENCES


BIOGRAPHICAL SKETCH

DANIEL ALAN BAUR

Education

PhD Exercise Physiology
Florida State University, Tallahassee, FL
Dissertation: “The effect of pre-exercise modified starch ingestion on adipose tissue lipolysis and running performance”
Mentor: Dr. Michael J. Ormsbee

MS Exercise Physiology
James Madison University, Harrisonburg, VA
Thesis: “The effect of a mixed carbohydrate beverage versus a moderate dose of glucose on cycling performance”
Mentor: Dr. Michael J. Saunders

BA Political Science and History, Summa cum Laude
University of Pittsburgh, Pittsburgh, PA
Richmond University, London, UK

Experience

Research Assistant
Florida State University
Institute of Sports Sciences and Medicine
August 2014 – May 2017

Teaching/Research Assistant
Florida State University
August 2013 – August 2014

Teaching Assistant
James Madison University
August 2012 – May 2013

Research Assistant
James Madison University
August 2011 – May 2013

Awards and Honors

Dissertation Award, FSU College of Human Sciences’ Dissertation Award Program, 2016
Runner-up Oral Presentation, FSU College of Human Sciences’ Annual Research and Creativity Day, 2016
Jean A. Reutlinger and Lillian H. Munn Scholarship, 2015
Hortense Glenn Society, 2015
Florence Smith McAllister Endowed Fellowship, 2014
Marilyn Crawford Kinesiology Graduate Scholarship, 2012

Manuscripts


**Conference Presentations**


**Baur, DA**, de Carvalho Silva Vargas, F, Garvey, JA, Bach, CW, and Ormsbee, MJ. A modified starch supplement ingested before and during cycling enhances fat oxidation but not performance. Thematic poster presentation at annual meeting, American College of Sports Medicine, Boston, MA (National). June 2016.

**Baur, DA**, de Carvalho Silva Vargas, F, Garvey, JA, Bach, CW, and Ormsbee, MJ. Accuracy of a continuous glucose monitoring device for the assessment of carbohydrate glycemic effects during exercise. Poster presentation at annual meeting, Southeast Chapter of American College of Sports Medicine, Greenville, SC. February 2016.


Kramer, SJ, **Baur, DA**, Panton, LP, Spicer, MT, and Ormsbee, MJ. The effects of six days of dietary nitrate supplementation on strength, power, and endurance in male CrossFit athletes. Poster presentation at annual meeting, Southeast Chapter of American College of Sports Medicine, Greenville, SC. February 2016.


**Baur, DA**, Bach, CW, Hyder, WS, and Ormsbee, MJ. Competing in a multistage ultra-endurance triathlon reduces body mass and fat mass and may increase fluid retention and insulin resistance. Podium presentation at annual meeting, National Strength and Conditioning Association, Orlando, FL (National). July 2015.


Schroer, AB, Saunders, MJ, Baur, DA, Womack, CJ, Becker, M, and Luden, ND. Cycling time trial performance may be impaired by whey protein and l-alanine intake during prolonged exercise. Poster presentation at annual meeting, American College of Sports Medicine, Orlando, FL (National). April 2014.