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FAST FOOD RESULTS IN SIMILAR
POST-EXERCISE GLYCOGEN RECOVERY AND EXERCISE PERFORMANCE
COMPARED TO SPORT SUPPLEMENTS

By

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B.A. Colorado Mesa University, Grand Junction, CO, 2012

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of the requirements for the degree of

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CHAPTER ONE: INTRODUCTION

Introduction

Understanding of muscle glycogen and performance stems from 3 sentinel papers released in 1967. Hermansen et al. observed the depletion of muscle glycogen during prolonged exercise and that it approached zero after work to exhaustion [1]. This resulted in the awareness of muscle glycogen as a primary fuel source in exercise. Ahlborg et al. found work time to exhaustion to be directly related to initial muscle glycogen stores before exercise [2]. Therefore, muscle glycogen has a direct effect on exercise performance. Further expansion of these two concepts were confirmed when Bergstrom et al. manipulated dietary carbohydrate (CHO), thus altering concentrations of muscle glycogen pre-exercise and time to exhaustion during prolonged exercise protocols [3]. Bergstrom and Hultman discovered muscle glycogen stores to increase when CHO is ingested following a glycogen depleting bout of exercise [4]. Since then, it has been well established that regular CHO feedings after muscle glycogen depletion are required to maximize muscle glycogen re-synthesis and increase performance capabilities [5-10].

After a glycogen depleting event, glycogen synthesis has been observed to occur in two phases categorized as the rapid phase and the slow phase. The rapid phase lasts between 30 – 60 minutes and proceeds without the presence of insulin, thus being an insulin-independent phase [11-15]. The slow phase lasts up to 3.5 hours and depends on increased insulin sensitivity from exercise, thus being an insulin dependent phase [14-16]. The rapid insulin-independent phase in conjunction with the slow insulin-

dependent phase has led to the discovery of what is known as the 4-hour-window of recovery.

Many studies have looked into the macronutrient composition, timing, and quantity of these feedings and their effects on muscle glycogen re-synthesis rates [5, 6, 8-10, 17-20]. CHO composition (glucose vs. fructose vs. sucrose) and varying levels of glycemic index (GI) have been studied extensively on their effect on muscle glycogen re-synthesis [10, 15, 20, 21]. Protein (PRO) sources vary widely such as whey protein isolate, milk protein, milk protein concentrate, cream powder, and soy isolate to name a few [22-25]. Liquid vs. solid feeding strategy comparisons have shown that both are effective [26].

Both athletes and exercise enthusiasts have an interest in recovery aids as indicated by over \$4 billion dollars in sports drink sales in 2012 [<http://www.bevindustry.com/articles/85656-2012-state-of-the-industry--sports-drinks>]. Individuals prize convenience and potency when it comes to supplementation. High CHO in the presence of PRO as amino acids can further muscle glycogen synthesis. In combination with electrolytes for rehydration, a readily available and affordable fluid meeting these criteria would be ideal as a recovery drink. Many engineered supplements are available but are expensive. Because of the emphasis on maximizing glycogen re-synthesis and the importance of exact macronutrient composition, athletes and enthusiasts alike are left with the notion that these expensive engineered feedings are their only option to maximize performance.

As a cheap comparison to highly engineered supplements, chocolate milk has become a popular choice as a result of much research [22, 25, 27, 28]. Because

chocolate milk is high in CHO, PRO, and also contains essential amino acids, it is seen as easily obtainable and gained much interest as a recovery drink [28]. Results have shown that it is just as effective, if not in some cases more, in subsequent bouts of exercise after a recovery period following prolonged bouts of glycogen depleting activities when compared to high fluid CHO drinks and controls [22, 27].

Currently, no studies have looked into the viability of fast food dietary choices and their effect on glycogen re-synthesis. As a readily available, inexpensive, and convenient source of macronutrients for glycogen re-synthesis, the purpose of this study is to question if fast food can sufficiently replace glycogen stores after a depleting event in comparison to dietary supplement choices. The study hypothesizes that it doesn't matter how athletes achieve consuming the macronutrients necessary for recovery (ie McDonald's meal versus Clif Bars), what matters is the amount and timing of ingestion. This contributes to the advancement of scientific literature by affirming or disproving the aforementioned hypothesis, and can benefit research participants by finding out the ideal recovery macronutrient composition for them.

Problem

The novel use of fast food dietary choices on exercise recovery and subsequent bouts of performance has yet to be investigated. It is not known what effects these dietary choices have in these regards when compared to other novel foodstuffs such as chocolate milk and common engineered supplements such as Gatorade and Cliff Bars.

Purpose

The purpose of this study is to determine the effects of fast food dietary choices on immediate glycogen re-synthesis and subsequent performance versus traditional dietary supplement recovery products.

Null Hypotheses

1. Fast food dietary choices will have no effect on total muscle glycogen re-synthesis versus traditional engineered recovery products.
2. Fast food dietary choices will have no effect on subsequent exercise performance versus traditional engineered recovery products.
3. Fast food dietary choices will have no effect on blood glucose levels versus traditional engineered recovery products.
4. Fast food dietary choices will have no effect on blood insulin levels versus traditional engineered recovery products.

Significance and Rationale

The results of this investigation will have implications on individuals who take part in vigorous glycogen depleting activities. If fast food dietary choices are found to be effective in immediate glycogen re-synthesis and subsequent bouts of exercise, then practical guidelines may be set while increasing recovery food choice and variety. As an inexpensive and readily available option, this may prove to be convenient for many of those individuals.

Limitations

1. The lifestyle of subjects between trials cannot be controlled. To better control physical activity levels and resting muscle glycogen levels, a dietary and

physical activity recall for the day prior to exercise trials will be recorded and repeated.

2. Human error can occur with the use of instrumentation. To limit the occurrence of error, all researchers will be thoroughly trained and equipment will be carefully calibrated.
3. Participants will not be randomly sampled. They will be recruited by convenience. However, random ordering of treatments in a cross-over design will be utilized.

Delimitations

1. Subjects in this study will be males who are recreationally active. Due to the effects of the menstrual cycle and hormone fluctuations on glycogen re-synthesis components, females will be excluded from this study.

CHAPTER TWO: REVIEW OF LITERATURE

Glycogen Synthesis and Performance

Hermansen et al. [1] conducted study on muscle glycogen content during prolonged exercise looked at both 10 trained and 10 untrained individuals. Subjects performed 85 minutes for the untrained and 90 minutes for the trained of cycling to exhaustion with interval workloads at an average of 77 percent of VO_{2max} . Average values for resting muscle glycogen were 1.6 g/100g wet muscle. At the end of the exercise protocol, muscle glycogen content dropped to 0.06 g/100g wet muscle in untrained subjects and 0.12 g/100g wet muscle in trained subjects. These results showed that muscle glycogen content can be almost all but exhausted during prolonged exercise events to fatigue.

The discovery that CHO diet manipulation elevates resting muscle glycogen content and that muscle glycogen content is correlated with time to exhaustion during prolonged exhaustive exercise stem from studies by Ahlborg et al. [2] and Bergstrom et al. [3]. Bergstrom fed 9 cyclists varying diets of protein and fat (P), carbohydrate (C), and a mixture of both (M). Muscle glycogen content prior to exhaustive exercise at 75 percent of VO_{2max} was 0.63, 1.75, and 3.31 g/100g wet muscle for PMC respectively. Post muscle glycogen content was 0.13, 0.17, and 0.43g/100g wet muscle. Times to exhaustion were 59, 126, and 189 minutes for PMC diets using the same exercise protocol. CHO diet directly affects muscle glycogen content and thereby time to exhaustion during exercise.

The amount at which resting muscle glycogen could be increased by CHO diet manipulation was undertaken by Bergstrom and Hultman [4]. Two subjects exercised a single leg at a workload of 1200 kpm/min on a cycle ergometer to exhaustion while the other leg rested for the duration. Immediately after and for 3 days post protocol, the subjects consumed diets primarily composed of CHO. Muscle biopsies were taken each morning from both legs. By day 1, resting muscle glycogen increased significantly in the exercised leg above the minor increase in the rested leg. Over the course of the next 2 days, resting muscle glycogen was near double in the exercised leg than in the resting leg. These results showed that muscle glycogen re-synthesis is tissue specific to the muscle worked and that glycogen depletion followed by a CHO diet greatly increases resting muscle glycogen content.

Many novel approaches have been taken to increase and test the limits of glycogen re-synthesis rates. Macronutrient profile, timing of feeding, and amount of feedings will all be discussed as the core of this project, but it is comprehensive to note other approaches. One study sought to investigate the effects to glycogen re-synthesis by adding fenugreek extract (*Trigonella foenum-graecum*) to post exercise CHO feedings [29]. Fenugreek contains a high concentration of 4-Hydroxyisoleucine which is an amino acid that is not found within mammalian skeletal tissue. Subjects were fed $1.8\text{g}\cdot\text{kg}^{-1}$ dextrose plus a placebo capsule or $1.8\text{g}\cdot\text{kg}^{-1}$ dextrose plus a fenugreek capsule after a glycogen depleting protocol in a cross-over design. Feedings occurred approximately 15 minutes post exercise and again at 120 minutes. Analysis of muscle biopsies taken immediately post exercise and at 4 hours post exercise revealed a significant increase in re-synthesis rate for the CHO + 4-OH-Ile trials. Plasma insulin

and blood glucose levels revealed no significant difference between trials. Blood glucose and plasma insulin levels returned to pre-feeding levels at 240 minutes post exercise.

Blood Glucose and Insulin

Insulin serves as the major regulator of blood glucose concentration by increasing uptake in adipose and muscle tissue while inhibiting hepatic glucose production [30]. Glucose uptake of the intestine and metabolically active tissues in conjunction with hepatic glucose production keep the normal resting body in a tight range of 4 – 7 mM plasma glucose concentration [30]. It is through the stimulation of lipogenesis, glycogen synthesis, and protein synthesis while inhibiting lipolysis, glycogenolysis, and protein breakdown that insulin regulates plasma glucose concentration [15, 30, 31]. Muscle glucose uptake and activation of glycogen synthase, the rate limiting enzyme of glycogen synthesis, are both stimulated by insulin [32]. The most important stimulus physiologically for the pancreatic secretion of insulin is an increase in blood glucose concentration.

Insulin stimulates GLUT 4, an intracellular protein that acts as a facilitated carrier to bring glucose into the cell through the cellular membrane [31]. Blood glucose and insulin levels peak within 60 minutes of a CHO feeding leading to an increased rate of glucose transport into tissues via increased GLUT 4 translocation to the cellular membrane. This increase adds to the idea of a 4 hour recovery window when CHO feedings can be utilized to maximize muscle glycogen re-synthesis.

It is during this time when glycogen synthesis reaches its highest rates, depending on feeding strategies, and has been seen as high as $6 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$

following the consumption of CHO immediately post-exercise [10]. During these phases an increase in glycogen synthase activity, the rate limiting enzyme of glycogen synthesis, is observed as well as GLUT 4 protein movement from intercellular storage sites to the cellular membrane which transports glucose molecules into the muscle cell [31, 33]. GLUT 4 in the rapid phase is activated through an unknown exercise response which is still under investigation at this time. During the slow phase, GLUT 4 activity is enhanced by an increase in insulin sensitivity where smaller amounts of blood insulin concentration drive a higher activation rate of GLUT 4 as a result of exercise [34].

Blood glucose levels stimulate insulin production and in turn, insulin stimulates GLUT 4 and glycogen synthase activity which increase the rate of glycogen synthesis. Therefore it is important during short term recovery studies to analyze both blood glucose and plasma insulin concentration to determine the limiting step of glycogen re-synthesis by mechanism of action.

Feeding Strategies

Macronutrient amount and composition are an important variable in maximizing muscle glycogen re-synthesis. The task of investigating the effects of CHO feeding amount on glycogen storage was undertaken by Ivy et al. [18]. Subjects depleted glycogen using an alternating protocol of cycling 15 minutes at 62 percent $\text{VO}_{2\text{max}}$ then 15 minutes at 75 percent $\text{VO}_{2\text{max}}$ which was repeated 4 consecutive times. Immediately post exercise and 2 hours post, subjects were either fed a liquid glucose drink of $1.5 \text{ g}\cdot\text{kg}^{-1}$ body weight (L), $3.0 \text{ g}\cdot\text{kg}^{-1}$ body weight (H), or $0 \text{ g}\cdot\text{kg}^{-1}$ body weight (P). Muscle biopsy of the vastus lateralis were taken immediately, 2, and 4 hours post exercise to determine rate of glycogen re-synthesis. During the first 2 hours L and H

treatments produced rates of 5.2 ± 0.9 and $5.8 \pm 0.7 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. The last 2 hours of recovery showed rates diminished by 24 and 22 percent respectively. This demonstrates similar a glycogen resynthesis rate despite a doubling of CHO feedings post exercise.

Blom et al. reported the muscle glycogen rates with immediate feeding of $0.70 \text{ g}\cdot\text{kg}^{-1}$ of glucose after depletion exercise vs. $1.4 \text{ g}\cdot\text{kg}^{-1}$ glucose, $0.35 \text{ g}\cdot\text{kg}^{-1}$ glucose, $0.70 \text{ g}\cdot\text{kg}^{-1}$ sucrose, and $0.70 \text{ g}\cdot\text{kg}^{-1}$ fructose at 2, 4, and 6 hours post exercise [10]. Results indicated an increase from $2.1\pm0.5 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ to $5.8\pm1.0 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ for low glucose and moderate glucose, respectively. The high glucose dose did not further increase resynthesis rates at $5.7\pm0.9 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$. Rates for moderate glucose were similar to moderate sucrose, $6.2\pm0.5 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$, however moderate fructose was significantly lower at $3.2\pm0.7 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$. It is suggested that $1.2 \text{ g}\cdot\text{kg}^{-1}$ of CHO as glucose and sucrose after exercise and every 4-6 hours after in a fluid source, or as multiple small meals will maximize muscle glycogen re-synthesis [15]. These longer duration recovery methods however, are beyond the scope of this study.

Moderate CHO intake in the presence of PRO increases muscle glycogen synthesis. PRO sources can vary widely such as whey protein isolate, milk protein, milk protein concentrate, cream powder, and soy isolate to name a few [22-25]. Berardi et al. conducted a study that showed CHO+PRO liquid consumption immediately post-exercise resulted in higher muscle glycogen recovery vs. CHO liquid and an isoenergetic solid meal as placebo [35]. Nuclear magnetic resonance spectroscopy (NMR) was used to measure muscle glycogen content. At one and two hours postexercise, participants ingested C+P ($4.8 \text{ kcal}\cdot\text{kg}^{-1}$; $0.8 \text{ g}\cdot\text{kg}^{-1}$ C, $0.4 \text{ g}\cdot\text{kg}^{-1}$ P), CHO

(4.8 kcal.kg; 1.2 g.kg⁻¹ C), or PLB (no energy). An absolute difference of 6.42 mmol.L⁻¹ was found in CHO+PRO vs. CHO and 10.12 mmol.L⁻¹ in CHO+PRO vs. PLB which represented an increase of 22 and 34 percent more glycogen storage respectively. Even though muscle glycogen recovery rate was higher in CHO+PRO group, performance in a repeated exercise bout was not significantly different between groups.

However, although studies have shown an increase in plasma insulin levels with the addition of PRO to less than optimal CHO feedings (approximately 0.8g.kg⁻¹ hr) [5], when CHO levels are high ($\geq 1.2\text{g.kg}^{-1}$ hr) the addition of PRO does not further increase glycogen re-synthesis rates [36]. This is important as it gives evidence to thinking that insulin is not the limiting factor in glycogen re-synthesis rates when carbohydrate delivery is saturating.

Zawadzki et al. [5] investigated the effects of CHO-whey protein, CHO only, and PRO only supplements on glycogen re-synthesis. After a two hour bout of exercise on a cycle ergometer to exhaust muscle glycogen stores, subjects were immediately fed 112g of CHO, 40.7g of PRO, or 112g and 40.7g of CHO-whey protein 2 hours after exercise. Muscle biopsies and blood analyses reported that after 4 hours of recovery, CHO produced a significantly higher blood glucose response compared to CHO-PRO but that plasma insulin was greater in both CHO and CHO-PRO than the PRO only trial. The rate of muscle glycogen storage was significantly faster in the CHO-PRO treatment versus the CHO treatment. This study has been widely criticized due to lacking a control group containing an isoenergetic amount of CHO versus the CHO-PRO treatment. It is believed that the CHO-PRO treatment exhibited a faster re-synthesis rate due to a higher amount of available substrate. However, others think that this is unlikely the

case. The high insulin levels observed in the CHO-PRO treatment would inhibit gluconeogenesis as opposed to increasing it by saving liver glycogen stores and down regulating a release of hepatic glucose [15].

Timing of feedings plays a valuable role in maximizing glycogen re-synthesis rates immediately post exercise. Ivy et al. ran a cross over design study with 12 subjects that randomly received either 2 g·kg⁻¹ body weight glucose polymer drink or placebo at one time of immediately post exercise (P-EX) or 2 hours post exercise (2P-EX) [9]. After cycling for 8 minutes at 68 percent VO₂max then 2 minutes at 88 percent VO₂max and repeating 6 segments for a total of 70 minutes, muscle biopsies were collected immediately, 2, and 4 hours. P-EX re-synthesis rate was 7.7 and only 2.5 mmol·kg⁻¹·h⁻¹ for 2P-EX treatment during the first 2 hours of recovery. The last 2 hours of recovery P-EX slowed to 4.3 while 2P-EX increased to 4.1 mmol·kg⁻¹·h⁻¹. These results demonstrated that delaying CHO feedings post exercise result in a reduced rate of muscle glycogen re-synthesis.

It is clear from these studies that muscle glycogen re-synthesis rates are enhanced with the ingestion of PRO in conjunction with only a moderate level of CHO, approximately 0.8g·kg⁻¹ hr. The mechanisms of the addition of PRO and or amino acids and its effects on re-synthesis rates are not yet fully understood. In contrast, one study found that when PRO and or amino acids are added to a high CHO supplement, 1.2 g·kg⁻¹ hr, re-synthesis rates are not increased further [36]. After a muscle glycogen depleting protocol, subjects were either fed 1.2 g·kg⁻¹ hr of CHO or 1.2 g·kg⁻¹ hr of CHO and 0.4 g·kg⁻¹ hr PRO. This occurred even in the presence of a significantly increased plasma insulin level in the CHO-PRO trial versus the CHO only trial.

All of these studies have used a similar protocol in which subjects are fasted and not allowed to consume nutrients during experimental exercise trials. Reinert et al. investigated the effects of a recovery beverage (40g CHO and 20g PRO) or placebo immediately after exercise on glycogen resynthesis rates in response to a road cycling bout where a food bar and commercial sport drink was delivered during exercise followed by a solid meal feeding two hours later [37]. No difference in muscle glycogen was observed between trials for pre, post, and 4 hours post exercise biopsies (140 ± 9 , 56 ± 8 , and 70 ± 8 $\text{mmol} \cdot \text{kg}^{-1}$ wet wt⁻¹·hr⁻¹, respectively). These results demonstrated that supplemental feedings during exercise may diminish the fasted protocol post-exercise feeding strategies in regards to muscle glycogen recovery.

CHAPTER THREE: METHODOLOGY

Participants and Setting

Participants in this research study were 11 recreationally active males from the Missoula, MT area, between the age of 18 and 40 years, and have a $\text{VO}_2 \text{ max} \geq 45$ ml/kg/min. Participants were recruited on a volunteer basis and passed the Par-Q health and exercise questionnaire to screen for known risk factors of coronary heart disease. An informed consent form approved by the Institutional Review Board of the University of Montana in Missoula, MT was signed by participants agreeing to partake in the research study. Data collection occurred in the Montana Center for Work Physiology and Exercise Metabolism's lab at the University of Montana in Missoula, MT.

Experimental Design

In the present study, a randomized crossover experimental design was used to determine the effects of fast food dietary choices versus sport supplement recovery choices. Muscle glycogen, blood glucose, and plasma insulin were monitored during the rest period. A subsequent performance trial was utilized to assess the performance effects of both feeding regimes.

Preliminary Testing

Par – Q and Informed Consent

A brief health history and exercise questionnaire was administered as a pre-screening assessment. Participants completed the physical activity readiness questionnaire (PAR-Q) to evaluate the presence of any known risk factors for coronary

heart disease. Participants reviewed and signed a University of Montana Institutional Review Board approved informed consent form (see appendices).

Hydrodensitometry

Body composition was assessed by hydrodensitometry using estimated residual volume. Participants arrived at the lab fasted for ≥ 3 hours prior to body composition assessment. Body weight was recorded on a dry weight scale (Befour Inc., Cedarburg, WI) and height was measured. Body composition was determined using an underwater weighing tank with digitalized and calibrated weight scales (Exertech, Dresbach, MN). Participants were submerged underwater on the scale to determine underwater weight. Underwater weighing continued until consistent measurements, within 100 grams, were obtained. Underwater weight was used to calculate body density to further calculate percent body fat using estimated residual volume and the Siri equation.

Maximal Aerobic Power – $VO_2\max$

Subjects were required to be fasted 4 hours prior to max testing and refrained from exercise, alcohol, and caffeine consumption for 24 hours prior as well. The test was performed on a cycle ergometer and gas exchange was measured using a metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). The test increased in workload measured in watts by 3 minute stages. The first stage was an inherent warm up at 95w. For each stage, the workload increased by 35 watts until the subjects reached volitional fatigue. To ensure the test was representative of a true max test, a specific criterion was met. A respiratory exchange ratio (RER) of > 1.10 was achieved, a plateau in $VO_2\max$ was observed, the subjects heart rate reached within 10 beats of age predicted max,

and the volitional fatigue occurred in conjunction with a rate of perceived exertion (RPE) > 17.

Practice Time Trial

Subjects completed two practice (PTT) 20km time trials (TT) on the same cycle ergometer on two separate days to ensure TT competency prior to the completion of the experimental trials. Subjects were given instruction to complete the distance as quickly as possible and were allowed the flexibility of shifting gears electronically. Distance and time were measured using the RacerMate Inc. software. (RacerMate, Inc., Seattle, WA).

Experimental Testing

Glycogen Depletion Protocol

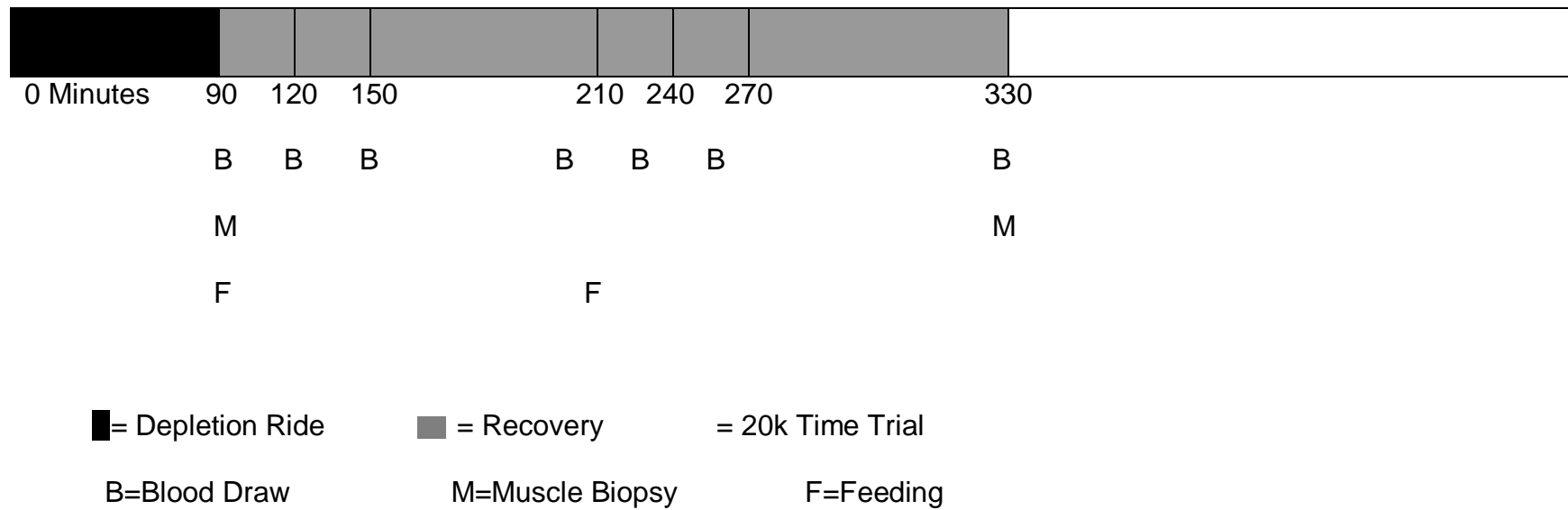
Subjects performed a glycogen depleting bout of exercise on a cycle ergometer for 90 minutes [38]. The protocol consisted of a 10 minute warm up at approximately 55% of peak VO_2 followed by a series of 10-intervals, which include two minutes at 80% peak VO_2 followed by four minutes at 50% peak VO_2 . After the series of intervals, subjects completed eight minutes at 60% peak VO_2 followed by 12 minutes at 50% peak VO_2 . Water was consumed ad libitum.

Feeding Administration

Subjects were fed in a crossover design receiving either a fast food meal or a sport supplement items of similar CHO content. The amount of CHO per feeding was between 1.2 and 1.8 $\text{g}\cdot\text{kg}^{-1}$ body weight. Feedings occurred immediately post exercise after the muscle biopsy and at 2 hours post exercise protocol. During the subjects 4 hours of recovery, they were allowed to read, study, play video games, and watch television while remaining in a rested supine position.

Proposed feeding profile for a subject of 75kg consuming $1.5 \text{ g}\cdot\text{kg}^{-1}$ CHO resulting in approximately 112.5g of CHO consumption per feeding can be seen in appendices Table 2.1 and 2.2. Following is the proposed timeline:

Proposed Timeline



Muscle Tissue and Blood Sample

Muscle tissue and blood samples were collected through muscle biopsy and intravenous puncture methods. Biopsies and blood draws were performed immediately post exercise and at 4 hours post exercise to measure glycogen re-synthesis concentration. Muscle biopsies of the vastus lateralis muscle were performed using the percutaneous biopsy needle technique with the aid of suction [39]. The 4-hour post exercise biopsies were taken from a site approximately 2 cm proximal to the previous biopsy location. Biopsies for the second trial were taken from the opposite leg from the first trial. Excess blood, fat, and connective tissue was immediately removed, and tissue samples frozen in liquid nitrogen and stored in a freezer at -80 °C for later analysis of muscle glycogen. The test subjects were asked to record their diet the day before testing and to replicate it for each testing session. This eliminated the need for a biopsy pre-exercise protocol with the diet control to assume for equal resting glycogen concentrations before each trial.

Time Trial Protocol

After the second muscle biopsy at 4 hours after the glycogen depletion protocol, subjects performed a 20k time trial on a cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). This trial was to test the practical performance effects within a laboratory setting of both prescribed feedings. Subjects were given instruction to complete the distance as quickly as possible and allowed the flexibility of shifting gears electronically. Distance and time were measured using the RacerMate Inc. software. A visible computer screen displayed distance traveled and a background of Seattle with a cycling

avatar. Subjects were separated by a partition to blind them from other participant's displays and allowed to consume water ad libitum.

Tissue Sample Analysis

Muscle and blood samples were measured using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Serum insulin was analyzed using a similar enzymatic spectrophotometric ELISA method (EIA2935, DRG International). Analysis was done in triplicate to determine muscle glycogen concentrations. Samples were weighed and placed in 0.5 ml of a 2 N HCL solution. The sample solutions were then weighed, incubated in an oven for two hours at 100 °C, and re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 M NaOH was added. A volume of 100 µl of this muscle extract was added to 1 ml of infinity glucose reagent (ThermoTrace Ltd., Middletown, VA) and read on a spectrophotometer at 340 nm. Muscle glycogen concentration was calculated using the extinction coefficient of NADH and expressed in $\text{mmol}\cdot\text{kg}^{-1}$ wet wt $\cdot\text{hr}^{-1}$ of muscle.

Questionnaires

Participants completed gastrointestinal discomfort questionnaires assessing feelings of hunger, fullness, sickness, and stomach discomfort at 0, 1, 2, 3, and 4-hours of recovery. A second post-meal questionnaire was administered at 0 and 2-hours of recovery assessing meal satisfaction, taste, and acceptability. Questionnaires were designed on a 150mm visual analogue scale (VAS) with "Not at all" on the left and "Extremely" on the right end points (see appendices). Subjects were asked to place an X along the continuum in response to each question. Scores were reported as the

distance from “Not at all” in mm. This technique has been previously used to evaluate dietary impacts [40].

Statistical Analysis

A two-tailed, paired t-test was used to compare rates of muscle glycogen resynthesis (Microsoft Excel, Microsoft Corp., Redmond, WA). Time trial performance was analyzed using a two-tailed paired t-test, then practice time trials and time trial performance times were analyzed using a one-way ANOVA with repeated measures (SPSS Inc., Chicago, IL). Muscle glycogen, blood glucose, serum insulin, and questionnaire data were analyzed using a two-way ANOVA (trial × time) with repeated measures (SPSS Inc., Chicago, IL). A probability of type I errors less than 5% was considered significant ($p < 0.05$).

Manuscript

***HEALTH AND HUMAN PERFORMANCE DEPARTMENT, THE UNIVERSITY OF
MONTANA**

****WPEM: MONTANA CENTER FOR WORK PHYSIOLOGY AND EXERCISE
METABOLISM**

**FAST FOOD RESULTS IN SIMILAR POST-EXERCISE GLYCOGEN RECOVERY
AND EXERCISE PERFORMANCE COMPARED TO SPORT SUPPLEMENTS**

SPORT SUPPLEMENTS VS FAST FOOD: SIMILAR RECOVERY OUTCOMES

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A variety of dietary choices are marketed to enhance glycogen recovery after physical activity. Past research provides recommendations regarding the timing, dose, and nutrient compositions to facilitate glycogen resynthesis. This study examined the effects of isocaloric sport supplements (SS) vs fast-food (FF) on glycogen resynthesis and exercise performance. Eleven male completed two experimental trials in a randomized, counterbalanced order. Each trial included a 90-minute glycogen depletion ride followed by a 4-hour recovery period. Absolute amounts of macronutrients (1.54 ± 0.27 g·kg⁻¹ carbohydrate, 0.24 ± 0.04 g·kg⁻¹ fat, and 0.18 ± 0.03 g·kg⁻¹ protein) as either SS or FF were provided at 0 and 2 hours. Muscle biopsies were collected from the vastus lateralis at 0 and 4 hours post exercise. Blood samples were obtained at 0, 30, 60, 120, 150, 180, and 240 minutes post exercise for insulin, glucose and blood lipids. A 20k time-trial (TT) was completed following the final muscle biopsy. There were no differences in the blood glucose and insulin responses. Similarly, rates of glycogen resynthesis were not different across the diets (6.9 ± 1.7 and 7.9 ± 2.4 mmol·kg⁻¹·h⁻¹ for SS and FF, respectively). There were also no differences across the diets for TT performance (34.1 ± 1.8 and 34.3 ± 1.7 minutes for SS and FF, respectively). These data indicate that short-term food options to initiate glycogen resynthesis can include a wide range of dietary options when total macronutrient composition is balanced.

KEYWORDS: glycogen recovery, fast food, sport supplements

Introduction

It has become common knowledge that muscle glycogen stores can be significantly restored when dietary carbohydrate (CHO) sources are ingested following a glycogen depleting bout of exercise [4]. The positive relationship between initial muscle glycogen stores and work time to exhaustion [2] has led to the present dogma that exercise performance necessitates an emphasis on muscle glycogen.

Research has continued to demonstrate that regular CHO feedings are required to facilitate optimal muscle glycogen resynthesis and performance capabilities [5-10]. Additional emphasis has been placed on macronutrient composition/ratios [5, 10, 17, 20], the amount of macronutrient [6, 18], and timing of ingestion [9, 19] to assist athletes, clinicians, and coaches in exercise recovery efforts.

Carbohydrate composition (glucose, fructose, and sucrose) and varying levels of glycemic index (GI) have demonstrated subtle impact on overall rates of muscle glycogen re-synthesis [10, 15, 20, 21]. Similarly, liquid versus solid feedings appear equally effective [26]. As an alternative to traditional sport supplement products, chocolate milk has gained recognition for similar benefits [22, 25, 27, 28].

Because the majority of past research has facilitated a comprehensive understanding of intake patterns, practices, and recipes to successfully restore muscle glycogen, the availability of commercialized products has rapidly increased. Interestingly, less emphasis has been placed on the glycogen recovery of traditional or mainstream foods other than chocolate milk.

While the conveniences of sport supplements provide are marketed as an asset, they often lack accessibility unless purchased in advance. In contrast, fast food dietary choices are often a widely accessible, inexpensive, and convenient source of macronutrients for potential glycogen re-synthesis. The purpose of this study was to investigate the efficacy of fast food dietary sources for glycogen resynthesis compared to common sport supplement foods/beverages. We hypothesized that when macronutrient content is held constant, the sources of dietary carbohydrate option is irrelevant to absolute glycogen restoration and subsequent exercise performance.

Methods

Subjects

Eleven recreationally active male subjects ($n = 11$) completed this randomized cross-over study design. Participants were healthy, injury-free and familiar with moderate to high intensity exercise (Table 1). Prior to data collection, each subject completed a Physical Activity Readiness Questionnaire (PAR-Q) and provided informed consent. All procedures were approved by the University Institutional Review Board. All research was conducted according to national and international laws of ethical standards in agreement with Harriss and Atkinson [41].

Preliminary Testing

All preliminary testing was completed during the same initial visit after a minimal 4-hour fast. Body composition was estimated using hydrodensitometry. Underwater weight was measured using an electronic strain-gauge scale (Exertech, Dreshbach, MN) with

estimated residual lung volume [42]. Body density was calculated using underwater weight and transposed to body composition using the Siri equation [43].

Peak oxygen uptake (VO_{2peak}) and maximal power output (W_{max}) were determined in the laboratory on a cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Subjects completed a graded exercise protocol starting at 95 watts, increasing 35 watts every 3 min until volitional fatigue. Expired gases were collected and analyzed using a calibrated metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). VO_{2peak} was determined as the highest achieved oxygen uptake during the test. Maximum power output was calculated as the last completed stage in watts plus time in the stage volitional fatigue was achieved multiplied by 35 watts. For example, each minute of each stage was assumed to be equivalent to 11.67 watts ($35 * 1 / 3 = 11.67$).

In addition to the measure of VO_{2peak} , subjects completed two practice (PTT) 20km time trials (TT) on the same cycle ergometer on two separate days to ensure TT competency prior to the completion of the experimental trials. Subjects were given instruction to complete the distance as quickly as possible and were allowed the flexibility of shifting gears electronically. Distance and time were measured using the RacerMate Inc. software. (RacerMate, Inc., Seattle, WA).

Experimental Design

Participants completed two trials with seven days between each trial in a randomized crossover design. Trials included the consumption of sport supplement products (SS) or fast food menu items (FF) during a 4-hour recovery period after a glycogen depletion ride. A 20km TT followed the recovery period to evaluate exercise performance.

Subjects were instructed to abstain from exercise and keep a dietary record of all food and drink consumed 24-hours prior to each trial. Subjects were asked to duplicate this diet for the second trial to minimize differences in resting muscle glycogen levels. The morning of each trial, participants arrived at the lab following a 12-hour fast. Each subject completed the 90-minute glycogen depleting cycle ride using the above mentioned cycle ergometer. The protocol included a 10-minute warm up at 55% W_{max} followed by a series of 10 intervals (2-minutes at 80% W_{max} followed by 4-minutes at 50% W_{max}). After the interval series, subjects completed 8-minutes at 60% W_{max} followed by a final 12-minutes at 50% W_{max} . Water consumption was ad libitum. Following the 90-minute cycling trial, subjects rested in a reclined/seated position during a 4-hour recovery period and adhered to a prescribed feeding schedule. Following the 4-hour recovery period, subjects completed the 20km TT on the same cycle ergometer as described above.

Feeding Strategy

Subjects were fed absolute amounts of macronutrients as either SS or FF at 0 and 2-hours of recovery. All food items were weighed for accuracy in conjunction with nutrition label serving sizes. Subjects consumed the same food items, which amounted to 1.54 ± 0.27 , 0.24 ± 0.04 , and 0.18 ± 0.03 $g \cdot kg^{-1}$ for carbohydrate, fat, and protein, respectively. Table 2.1 and 2.2 illustrate the detailed menu items.

Muscle Biopsies

Muscle biopsies of the vastus lateralis muscle were performed at 0 and 4-hours of recovery using the percutaneous biopsy needle technique with the aid of suction [39].

The 4-hour biopsy was taken from a site approximately 2 cm proximal to the initial 0-hour biopsy location. Second trial biopsies were taken from the opposite leg and leg order was randomized across trials. Excess blood, fat, and connective tissue were immediately removed. Tissue samples were frozen in liquid nitrogen and stored in a freezer at -80°C for later muscle glycogen analyses.

Blood Sampling

Blood samples were obtained from an antecubital arm vein using a venipuncture technique at scheduled intervals of 0, 30, 60, 120, 150, 180, and 240 min of recovery ($n=10$). Samples were allowed to clot then spun at 4000 rpm for 15 minutes in a refrigerated centrifuge (4°C) (Jouan Inc., MR22i). Serum was aliquoted into tubes and stored at -30°C for later glucose and insulin analyses. Whole blood samples were collected at 0 and 4 h of recovery and sent to Providence St. Patrick Hospital in Missoula, MT for lipid analyses.

Questionnaire

Participants completed gastrointestinal discomfort questionnaires assessing feelings of hunger, fullness, sickness, and stomach discomfort at 0, 1, 2, 3, and 4-hours of recovery. A second post-meal questionnaire was administered at 0 and 2-hours of recovery assessing meal satisfaction, taste, and acceptability. Questionnaires were designed on a 150mm visual analogue scale (VAS) with “Not at all” on the left and “Extremely” on the right end points. Subjects placed an X along the continuum in response to each question. Scores were reported as the distance from “Not at all” in

mm divided by 150mm. This technique has been previously used to evaluate dietary impacts [40].

20km Time Trial

After recovery, participants performed a 20km TT on the same cycle ergometer as described above (Velotron, RacerMate Inc., Seattle, WA). Subjects were given the same instructions to complete the distance as quickly as possible and were allowed the flexibility of shifting gears electronically. Subjects were separated by partitions to blind them from other participants and their displays.

Tissue and Blood Analysis

Two separate muscle samples (obtained at the same time point) were each analyzed in duplicate to determine muscle glycogen concentrations using an enzymatic spectrophotometric method [29]. Samples were weighed and placed in 0.5 ml of 2N HCl solution. Sample solutions were weighed, incubated in an oven for two hours at 100 °C, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 M NaOH was added. Then 100 μ l of the muscle extract solution was added to 1 ml of infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340 nm. Muscle glycogen was calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ wet weight of muscle.

Blood samples were analyzed for glucose in triplicate using Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340 nm. Blood

glucose concentration was calculated using the extinction co-efficient of NADH. Samples were analyzed for insulin in duplicate using an enzymatic spectrophotometric ELISA method (EIA-2935, DRG International). Serum lipid analyses were performed by the laboratory at Providence St. Patrick Hospital (Missoula, MT). Samples were allowed to clot for 30 minutes in serum separating tubes then spun at 2500 x gravity in a refrigerated centrifuge (Beckman Coulter INC). Samples were then placed in a chemistry analyzer for reading (Dimension Vista 500, Siemens). Average intra-assay coefficient of variation for muscle samples, glucose, and insulin was < 5%.

Statistical Analysis

A two-tailed, paired t-test was used to compare rates of muscle glycogen resynthesis (Microsoft Excel, Microsoft Corp., Redmond, WA). Time trial performance was analyzed using a two-tailed paired t-test then practice time trials and time trial performance times were analyzed using a one-way ANOVA with repeated measures (SPSS Inc., Chicago, IL). Muscle glycogen, blood glucose, serum insulin, and questionnaire data were analyzed using a two-way ANOVA (trial x time) with repeated measures (SPSS Inc., Chicago, IL). A probability of type I errors less than 5% was considered significant ($p < 0.05$). All data are reported as mean \pm SD.

Results

Muscle Glycogen

Muscle glycogen concentrations post-exercise were not significantly different for SS and FF trials at 0 and 4-hours of recovery ($p > 0.05$). There was a significant main effect for

time, demonstrating an overall increase in muscle glycogen concentrations following the 4-hour recovery period ($p < 0.05$, $n = 11$) (Figure 1). Similarly, the calculated rates of muscle glycogen resynthesis were not significantly different between the diets (6.9 ± 1.7 and 7.9 ± 2.4 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the SS and FF trials, respectively ($p > 0.05$, $n = 11$). As feedings were in absolute macronutrient amount, correlations for subject body weight versus muscle glycogen resynthesis rates were $r = -0.27$ for SS and $r = -0.32$ for FF. Correlations for $\text{CHOg} \cdot \text{kg}^{-1}$ versus muscle glycogen resynthesis rates were $r = 0.24$ and $r = 0.30$ for SS and FF, respectively.

Serum Glucose

There was not a statistically significant difference for serum glucose concentrations between SS and FF trials at 0, 30, 60, 120, 150, 180, and 240 minutes of recovery ($p > 0.05$, $n = 10$) (Figure 2). The main effect for time was significant and demonstrated that serum glucose was elevated at 30 and 150 minutes compared to time 0 ($p < 0.05$, $n = 10$). One subject had difficulty providing blood samples and was omitted for serum glucose analyses due to an inability to obtain samples.

Serum Insulin

There was not a statistically significant difference for serum insulin concentrations between SS and FF trials at 0, 30, 60, 120, 150, 180, and 240 minutes of recovery ($p > 0.05$, $n = 10$) (Figure 3). The main effect for time was significant and demonstrated that serum insulin was elevated at 30, 60, 150, and 180 minutes compared to time 0 ($p < 0.05$, $n = 10$). One subject had difficulty providing blood samples and was omitted for serum insulin analyses due to an inability to obtain samples.

Blood Lipids

No significant statistical difference was observed between SS and FF trials for total cholesterol, high-density, low-density lipoproteins, and triglycerides at 0 hours and 4 hours post-exercise (Table 3). The main effect for time was significant and demonstrated that CHOL, HDL, and LDL were lower 4 hours post-exercise compared to time 0 ($p < 0.05$, $n = 11$)

20k Time Trial

A paired-samples t-test was conducted to compare SS and FF 20km TT results. There was not a significant difference in the scores for SS (34.1 ± 1.8 min) and FF (34.3 ± 1.7 min) conditions; $t(10) = 1.27$, $p = 0.234$. There were no significant differences in TT performance between PTT and the experimental trials (34.3 ± 2.1 , 34.5 ± 1.9 , 34.1 ± 1.8 , and 34.3 ± 1.7 minutes for PTT1, PTT2, SS, and FF trials, respectively, $p > 0.05$, $n = 11$).

Questionnaire

There were no significant differences for feelings of sickness and discomfort between the trials observed at 0, 1, 2, 3, and 4 hours of recovery ($p > 0.05$, $n = 11$). Hunger displayed a main effect for time with scores of 42 ± 8 , 64 ± 6 , 28 ± 6 , 53 ± 7 , and 72 ± 6 millimeters at 0, 1, 2, 3, and 4 hours of recovery, respectively. ($p < 0.05$, $n = 11$). Hunger was significantly higher at 4 hours compared to time 0 hours of recovery. The trial x time interaction was significant for the measure of fullness and demonstrated that SS was greater than FF immediately after the 2-hour feeding (108 ± 33 vs 75 ± 42 mm,

respectively, $p < 0.05$, $n = 11$). No significant differences were observed for perceived meal taste and acceptability after 0 and 2-hour feedings ($p > 0.05$, $n = 11$). The trial x time interaction was significant for the measure of satiety. Although there were no significant differences between the diets for feelings of satiety after 0 and 2 hour feedings, the FF meal was more satisfying at 2 hours compared to the initial 0 hour FF meal (78 ± 32 vs 52 ± 27 mm, respectively, $p < 0.05$, $n = 11$).

Discussion

The protocol used for this investigation was designed to evaluate the impacts of varied nutritional choices on recovery, specifically glycogen resynthesis and subsequent exercise performance. This was accomplished by matching macronutrient composition from fast food menu items versus commercially available sport nutrition products used for the 0 and 2 hour post-exercise feedings. Primary findings demonstrate that in the 4-hour recovery period following glycogen-depleting exercise, the source of macronutrients is irrelevant when the feeding intervals are held constant. Moreover, rates of glycogen recovery and subsequent 20K cycling performance were not significantly different under both recovery diets. These data are novel in demonstrating effective glycogen recovery benefits from fast food menu items comparable to products most often advertised to enhance recovery. In addition, these data suggest that a wide range of appropriate nutritional strategies can be implemented to initiate exercise recovery and prepare for subsequent bouts of performance.

A wide range of feeding strategies have been implemented (macronutrient composition, amount, and timing of ingestion) so as to develop specific suggested guidelines to

enhance immediate glycogen resynthesis [8, 9, 15, 19, 32]. Administration of CHO immediately after exercise has been shown to improve glycogen resynthesis by 45% versus delayed feedings and is further enhanced with the addition of a 2-hour feedings [9]. However, if feeding is provided prior and during extended exercise, the inclusion of a carbohydrate/protein recovery product immediately post-exercise did not enhance rates of glycogen recovery compared to a 2-hour delayed feeding [37]. Carbohydrate amount used in the present study of $1.54 \pm 0.27 \text{ g} \cdot \text{kg}^{-1}$ was in accordance with recommendations of $\geq 1.2 \text{ g} \cdot \text{kg}^{-1}$ every two hours to enhance glycogen resynthesis [10, 18, 36, 37]. In addition, muscle glycogen resynthesis rates of 6.9 ± 1.7 and $7.9 \pm 2.4 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for SS and FF, respectively, are comparable to previous research of 4.1-10.6 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ given a variety of modalities and environments [29, 44, 45].

While the presence of protein in the form of essential amino acids (EAA) enhances muscle glycogen resynthesis in conjunction with a moderate amount of CHO (approximately $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{hr}$), protein added to a high CHO supplement ($\geq 1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{hr}$) does not further increase glycogen resynthesis rates [36]. Although, the inclusion of additional protein and/or novel amino acids may alter short-term rates of glycogen recovery [19, 29], our present data demonstrate that the sources of carbohydrate and protein (1.54 ± 0.27 and $0.18 \pm 0.03 \text{ g} \cdot \text{kg}^{-1}$ respectively) from fast food result in comparable rates of glycogen synthesis despite the inclusion of additional fat ($0.24 \pm 0.04 \text{ g} \cdot \text{kg}^{-1}$).

The blood response in the current study demonstrates a rapid rise in blood glucose and insulin 30-minutes following each feeding with a concomitant return to normal by 60-

minutes post feeding. This is comparable to prior research using varied strategies in the carbohydrate dose [18], feeding intervals [9], and type of feedings [19]. The near identical response patterns for glucose and insulin with the two diets highlight the similarities between diets in terms of digestion, absorption and ultimately CHO delivery to the muscle. The similar glucose and insulin response provides the rationale for the near identical rates of glycogen recovery noted during both trials.

While it is commonly hypothesized that the chronic consumption of fast food choices have a negative effect on dyslipemia, cardiovascular risk, and obesity [46], the acute consumption has received little attention in the literature when applied to young, active individuals. However, the effects of fast food consumption after acute periods of glycogen depletion exercise have not been evaluated. Our data shows that lipid source in acute recovery feedings does not notably result in elevated fatty acid levels after glycogen depletion. Triglyceride concentrations dropped as an expected result of acute exercise. The observed lowering of total cholesterol during four hours of recovery following ingestion of a fatty meal was expected and supported by previous research. [47, 48]. Neither dietary approach with the presence of dietary fat blunted muscle glycogen recovery while supporting further exercise performance. Previous research has shown that the presence of fat significantly slows gastric emptying rates [49] and is further slowed with solid fats vs liquid [50]. However, exercise has been shown to increase gastric emptying rates of solid foods [51]. Therefore it is hypothesized with these results that the influence of exercise on gastric emptying is greater than the slowing effect by the presence of solid fats. It has been established that serum lipid

oxidation is increased after glycogen depleting exercise while intramuscular triacylglycerol have been observed to remain constant up to 18 hours of recovery [52]. This leads to thought that plasma fatty acids and low density lipoproteins are important fuels in aiding recovery from aerobic energy demands. Although increasing systemic fatty acids have been associated with increasing insulin resistance, research has shown that dietary fat in the presence of saturating CHO recovery feedings does not alter glucose tolerance or muscle glycogen concentrations immediately after exercise and up to 24 hours post [53].

Despite glycogen depletion, both SS and FF diets allowed participants to return to a recovered state that did not reduce TT performance capability. Both dietary options resulted in substantial glycogen recovery amounting to a 60 and 62% increase in muscle glycogen for SS and FF, respectively. It is also interesting to note that TT performance post-recovery were not different or impaired compared to two previously held practice 20K TT events completed in a fully rested state. A recent study was conducted that measured 5k TT performance after recovering on diets of varying glycemic index and found no difference [54]. However, little research investigated the effects of meals on short recovery periods between multiple bouts of exercise.

While rates of muscle glycogen recovery appear altered in response to varied approaches to post-exercise re-feeding (dose, timing, frequency of feeding), altering the sources of macronutrients while maintaining eucaloric feedings does not affect muscle recovery and subsequent exercise performance. Food sources that are

marketed differently have similar potential for providing basic recovery needs of the muscle and may offer a convenient and economical approach to glycogen recovery under some circumstances.

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Authors' Contributions

MJC participated in conception, design, data acquisition, assisted in muscle glycogen, blood parameter, questionnaire, and TT analysis and interpretation of data, and wrote the manuscript. CLD participated in conception, design, assisted in muscle glycogen, blood parameter, questionnaire, and TT analysis and interpretation of data, and aided in the drafting and revising of the manuscript. JSC participated in conception, design, data acquisition, analysis and interpretation of data, and aided in the drafting and revising of the manuscript. WSH participated in conception, design, data acquisition, analysis and interpretation of data, and aided in the drafting and revising of the manuscript. BCR participated in conception, design, and data acquisition, assisted in analysis and interpretation of data, and aided in the drafting and revising of the manuscript. All authors have read and given final approval of this version of the manuscript for publication.

Funding and Conflicts of Interest

The authors declare that they have no competing interests in access to these data or associations with companies involved with products used in this research.

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Table 1 Subject Descriptive Data (n = 11)

	Mean \pm SD
Age (years)	27.7 \pm 6.3
Height (cm)	180.3 \pm 7.7
Weight (kg)	76.8 \pm 10.2
Body Composition (%fat)	10.0 \pm 5.1
VO _{2peak} (L/min)	4.2 \pm 0.39
W _{max} (watts)	309 \pm 32
PTT 1 (min)	34.3 \pm 2.1
PTT 2 (min)	34.5 \pm 1.9

VO_{2peak}: Peak oxygen uptake

W_{max}: Maximal power output on cycle ergometer

Table 2.1 McDonalds fast food feeding

FF						
<u>0 hr</u>	<u>Calories</u>	<u>Fat</u>	<u>Cho</u>	<u>Pro</u>	<u>Qty</u>	<u>Sodium (mg)</u>
Hotcakes	350	9	60	8	1	590
Hashbrown	150	9	15	1	1	310
Orange Juice (small)	150	0	34	2	1	0
Total	650	18	109	11		900
<u>2 hr</u>						
Hamburger	250	9	31	12	1	480
Coke (medium)	200	0	54	0	1	45
Fries (small)	230	11	29	3	1	160
Total	680	20	114	15		685
4 Hour Total	1330	38	223	26		1585

Table 2.2 Sport supplement feeding

SS						
<u>0 hr</u>	<u>Calories</u>	<u>Fat</u>	<u>Cho</u>	<u>Pro</u>	<u>Qty</u>	<u>Sodium (mg)</u>
Gatorade (20 oz)	130	0	34	0	1	270
Kit's Organic PB	200	11	25	6	2	95
Cliff Shot Bloks (1 blok)	33.3	0	8	0	4	16.7
Total	663.2	22	116	12		526.8
<u>2 hr</u>						
Cytomax (1 scoop, 10 oz)	90	0	22	0	2	120
Power Bar Recovery PBCC	260	10	30	12	1	180
Power Bar Energy Chews	200	0	46	3	1	30
Total	640	10	120	15		450
4 Hour Total	1303.2	32	236	27		976.8

Table 3 Blood lipid profile by trial and time point

	SS		FF	
	<u>0 hour</u>	<u>4 hour</u>	<u>0 hour</u>	<u>4 hour</u>
CHOL(mg•dL ⁻¹)	177 ± 28	164 ± 29*	173 ± 32	160 ± 34*
TRIG(mg•dL ⁻¹)	112 ± 50	130 ± 102	106 ± 31	108 ± 53
HDL(mg•dL ⁻¹)	62 ± 13	54 ± 12*	62 ± 15	56 ± 16*
LDLc(mg•dL ⁻¹)	93 ± 25	84 ± 29*	89 ± 27	83 ± 28*

*p<0.05 (n=11) main effect for time vs 0 hours.

Figure Legends

Figure 1 Muscle glycogen during recovery.

* $p < 0.05$ (n=11) main effect for time vs 0 hours.

Figure 2 Serum glucose during recovery.

* $p < 0.05$ (n=10) main effect for time vs 0 hours.

Figure 3 Serum insulin during recovery.

* $p < 0.05$ (n=10) main effect for time vs 0 hours.

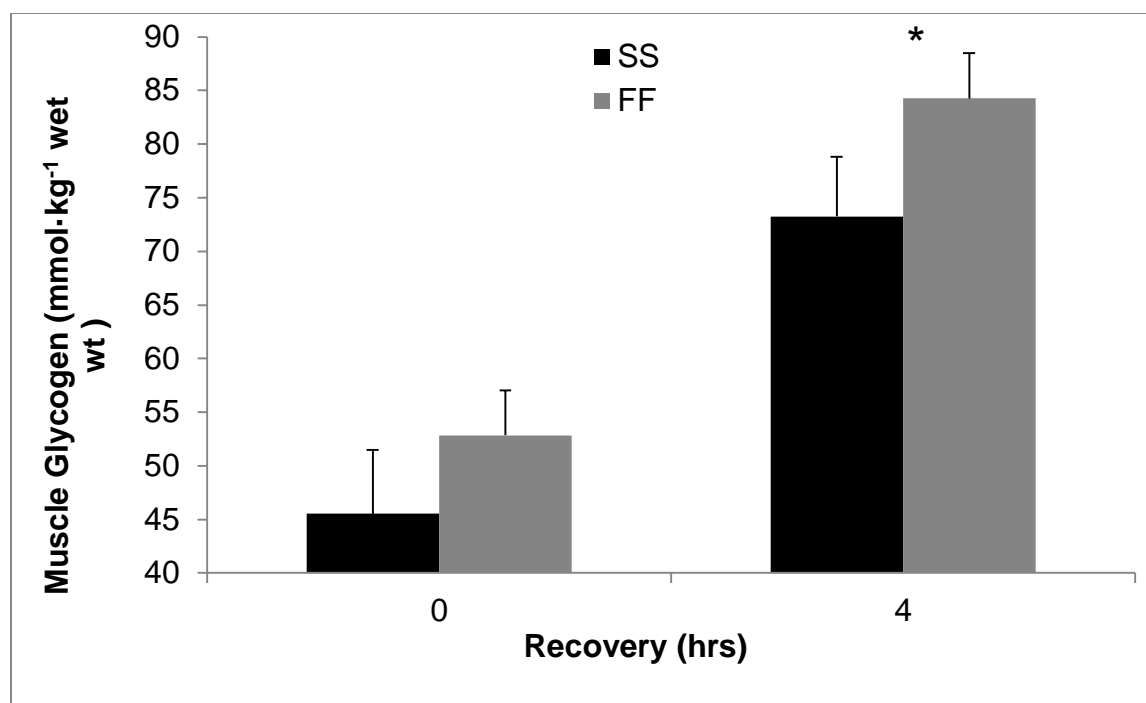
Figure 1 Muscle glycogen concentrations

Figure 2 Serum glucose concentrations during 4 hours of recovery

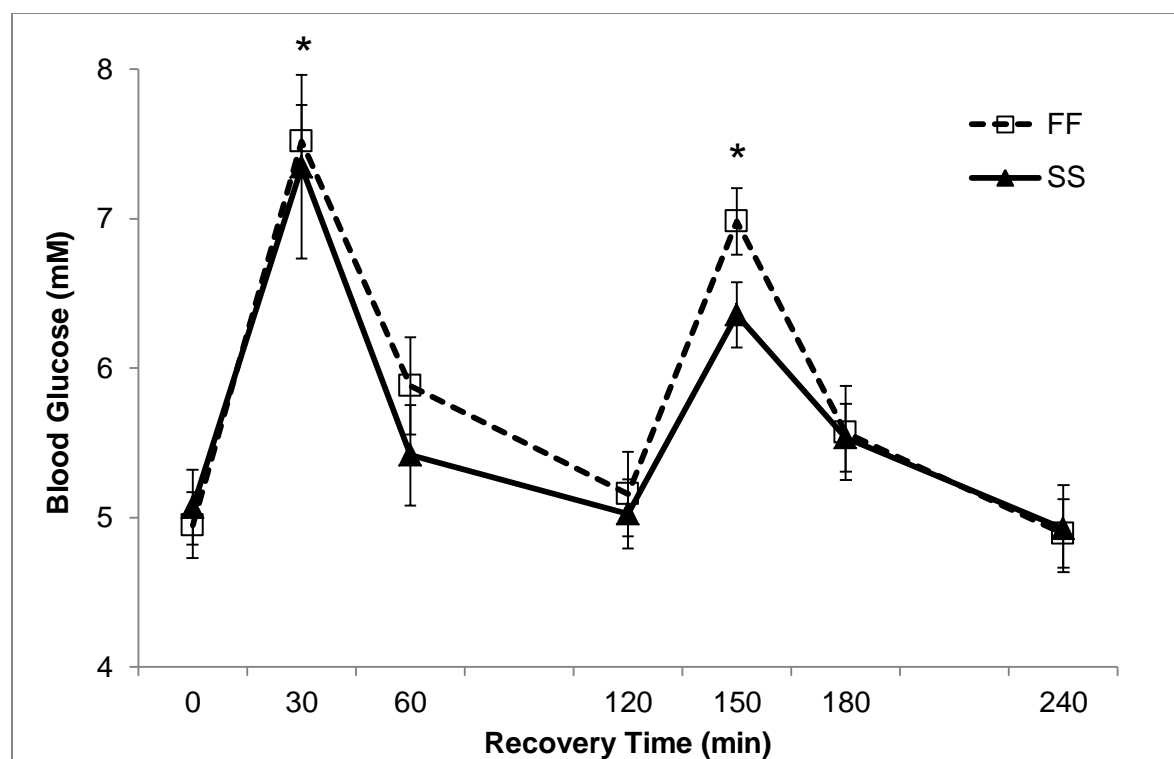
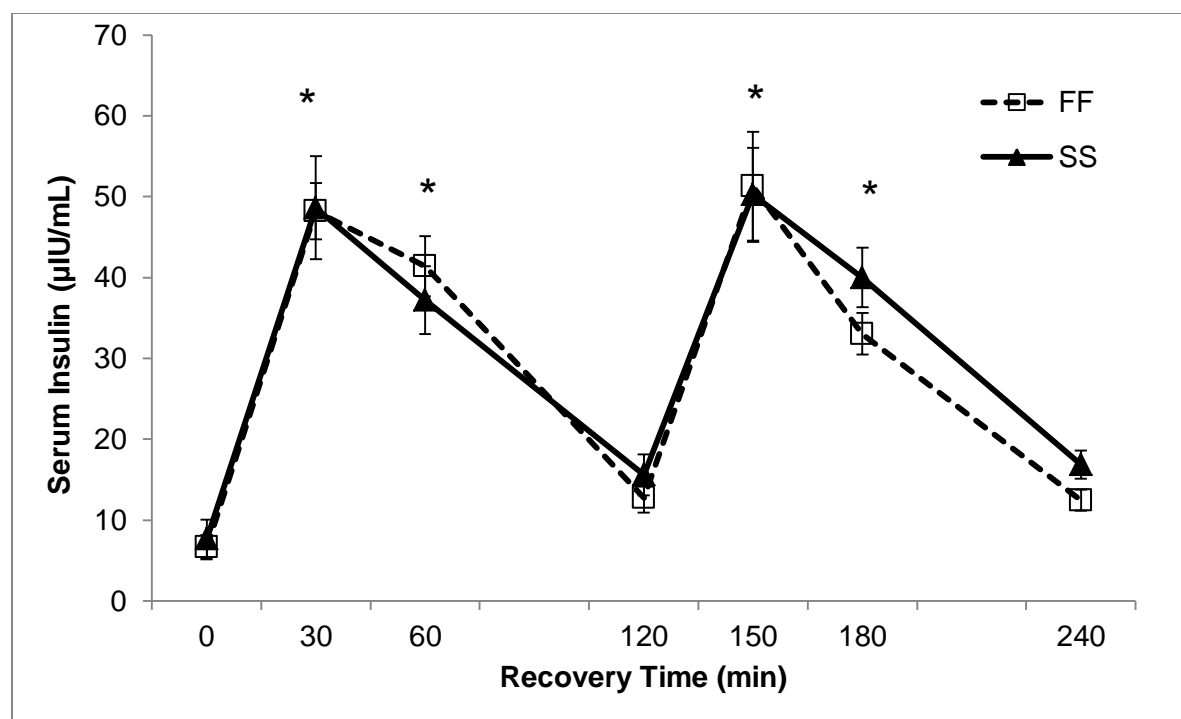


Figure 3 Serum insulin concentrations during 4 hours of recovery



Appendix



Montana Center for Work Physiology and Exercise Metabolism

SUBJECT INFORMATION AND CONSENT FORM

PROJECT IN BRIEF: The effects of fast food versus commercial recovery product dietary choices on immediate post-exercise glycogen re-synthesis and exercise performance

SPONSOR: Montana Center for Work Physiology and Exercise Metabolism (WPEM)

RESEARCHERS: Dr. Brent Ruby, PhD (406) 243-2117
Dr. Charles Dumke, PhD
John Cuddy
Walter Hailes
Michael Cramer

The University of Montana
Montana Center for Work Physiology and Exercise Metabolism
32 Campus Drive
McGill Hall – HHP
Missoula, MT 59812
(406) 243 – 2117 (Dr. Brent Ruby, PhD)

Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.

REQUIREMENTS

This research study requires that you meet the following criteria:

- Participants must be males between the ages of 18 and 40.
- VO₂ max greater than or equal to 40 mL/kg/min.
- Participants must not have a known allergy to lidocaine.

PURPOSE OF THE STUDY

The study is designed to evaluate the effects of fast food dietary choices on recovery after exercise. This study will address a lack of research literature specific to these dietary choices in regards to exercise recovery.

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TEST PROCEDURES

5 VISITS TO THE LABORATORY WILL BE REQUIRED (20 HOURS), AS SUMMARIZED BELOW

PRE TESTING (Visit 1)

1. A pre-screening assessment which involves a health/exercise questionnaire (Par-Q)
 - a. Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease.
2. If you successfully complete the PAR-Q (do not answer 'yes' to any known coronary heart disease risk factors), you will then provide written informed consent following the reading of this informed consent form.
3. A measure of percent body fat obtained using underwater weighing
 - a. This test session will require that you do not eat for a minimum of 3 hours or exercise 12 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighing tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 20 minutes.
4. A maximal cycle ergometer test to measure aerobic fitness
 - a. This test will consist of cycling on a cycle ergometer to volitional fatigue. The workload of the cycle ergometer will begin at 90 watts and increase every three minutes by 35 watts progressing to fatigue. You will be encouraged to continue until volitional fatigue, the point at which you can no longer continue cycling. During this test you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise so researchers can determine the appropriate exercise intensities for your experimental trial rides. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 30 minutes. You will be asked to fast for approximately 3 hours prior to this test.

PRE TESTING (Visits 2 and 3)

You will perform two 20k time trials before your experimental trials in order to become familiar with the 20k time trial protocol. You will be asked to work on a cycle ergometer as quickly as possible to a completed distance of 20 kilometers. Intensity and pacing is entirely up to you as the time to completion will be the only measured outcome. You will have a visual representation of your position on the continuum of start to finish displayed by a computer screen that is not associated with power output or time. This will help eliminate ordering bias in the experimental trials through inherent training effects of repeated exercise.

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EXPERIMENTAL TRIALS (Visits 4 and 5)

Trial 1) cycling for 90 minutes at varying intensities in laboratory followed by 4 hours of recovery followed by a 20k cycle ergometer time trial

Trial 2) cycling for 90 minutes at varying intensities in laboratory followed by 4 hours of recovery followed by a 20k cycle ergometer time trial

Experimental Protocol

Following a controlled diet (with NO alcohol consumption) and exercise plan the day before and after an overnight fast, you will arrive to the laboratory in the early morning following a 12 hour fast. Trials will be completed in a randomized order, but you will complete each of the two trials (7-14 days in between trials). The exercise protocol will be completed on a cycle ergometer in the laboratory. You will complete a 10-minute warm up at approximately 55% peak VO_2 . Thereafter, you will complete a series of ten intervals, which includes two minutes at approximately 80% peak VO_2 followed by four minutes at approximately 50% peak VO_2 . After the series of 10 intervals, you will complete 8 minutes at 60% peak VO_2 followed by 12 minutes at 50% peak VO_2 . Total cycle time will be 90 minutes. You will be provided water ad libitum during the ride. Immediately upon cessation of exercise, a blood sample will be drawn and muscle biopsy will be taken from the *vastus lateralis* (quad muscle) from an incision 1/4 inch in length. You will then void if necessary, have body weight measured, and change clothing into a standardized shorts and t-shirt. You will then recover for 4 hours (sitting or lying) in the laboratory. During this 4 hour recovery you will be supervised at all times by research staff. The two trials differ by the provision of a meal feeding of McDonalds breakfast and lunch items or commercially available recovery products at 0 hours and 2 hours into the recovery phase. Each feeding will be at a carbohydrate amount between 1.3 and 1.8 g/kg of body weight. During the recovery, blood samples will be taken at 0, 30, 60, 120, 150, 180, and 240 minutes; muscle biopsies will be collected during 0 and 4 hours of recovery, the second taken from a separate incision ~2 cm above the first muscle biopsy sample. Water will be provided ad-libitum during the 4 hour recovery. Following the 4 hour recovery, you will perform a 20k cycle ergometer time trial.

Biopsies

A total of 4 (2 per trial x 2 trials) muscle biopsies (2 from each leg) will be obtained from the front of your thigh muscle (*vastus lateralis*, approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the site be sterilized. After the site is cleaned, a small amount of local anesthesia (lidocaine) will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the site on the leg. After the area is treated with the lidocaine (approximately 5 mL, 1% lidocaine), a small incision (approximately 1/4 inch long) will be made through the skin and the outer covering (fascia) of your muscle to a depth of approximately 3/4-1.5 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips and/or a single stitch and bandaid and wrapped with a compression bandage. The biopsy samples will be obtained a) after the exercise session, and b) following the 4 hours of recovery (biopsies for each trial will be on the same leg, above the initial or previous sample). This will be repeated for the second trial using the opposite leg. The muscle biopsies will be used to evaluate alterations in muscle carbohydrate utilization kinetics in response to physical activity. Latex free

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bandages will be provided if subjects have a known allergy to latex. All of the muscle biopsies will be conducted by Dr. Brent Ruby or Dr. Charles Dumke.

Blood Samples

A total of 14 blood samples (7 per trial) will be collected using a venipuncture technique from the arm. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. These samples will be collected to measure blood glucose and insulin. All of the blood samples will be obtained under the direction of Dr. Brent Ruby or Dr. Charles Dumke. Blood samples will be taken at intervals 0, 30, 60, 120, 150, 180, and 240 minutes into the recovery. ~10 mL will be drawn each time for a total of ~70 mL per trial. Blood samples will be used to evaluate alterations in glucose, insulin and blood lipids.

Feedings

Immediately following exercise and at 2 hours of recovery, you will be provided in random order a standardized iso-energetic and iso-CHO meal of McDonald's breakfast at 0 hrs and lunch items at 2 hrs consisting of hotcakes, hash browns, and orange juice for breakfast and hamburgers, Sprite, and french fries for lunch or commercially available recovery products consisting of Gatorade, Gatorade Endurance Chews, and Kit's Organic peanut butter bars for 0 hr feeding and Cytomax powdered drink mix, Power Bar Recovery Peanut Butter Caramel Crunch bar, and Power Bar Energy Chews for 2 hr feeding. You will be asked to consume this food in a 10 to 15 minute time period. The amount of carbohydrate consumed at each feeding time point (post ride and 2 hours into recovery) will be between 1.3 and 1.8 g/kg.

Questionnaires

You will fill out a gastrointestinal discomfort questionnaire at 0, 1, 2, 3, and 4 hours of recovery. The questionnaire is composed of 4 questions assessing feelings of hunger, fullness, sickness, and stomach discomfort. A second post-meal questionnaire will be administered immediately post-feeding at 0 and 2 hours of recovery assessing the satisfaction, taste, and acceptability of the meal.

Dietary and Activity Recall

For 24-hours before your first exercise trial you will be asked to record the foods and quantity that you consume. You are not allowed to consume any alcohol during this time period. For the second trial, you will consume the same foods and quantity of those foods that you consumed for the first trial. 2 days before your first trial day you can exercise as you wish, but this must be repeated at the same time of day and the same exercise prior to the second trial. For the 24-hours before each trial you cannot participate in any physical exercise.

Body Weight

Nude body weight will be measured in private on a calibrated scale. Weights will be taken before, during recovery, and after each trial.

RISKS AND DISCOMFORTS

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.

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3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. The laboratory has standard emergency procedures should any potential problems arise.
4. Symptoms of dehydration such as headache and general fatigue may result during and after the exercise.
5. You will be informed of any new findings that may affect your decision to remain in the study.
6. The muscle biopsy and blood sampling techniques may cause some local and temporary discomfort. It is normal to have the sensation of a deep tissue bruise around the site of the muscle biopsy. This pain should be manageable and not above the pain associated from a "charlie horse" type bruise. Risks involved with muscle biopsies include: nerve damage, moderate stiffness, hematoma, minimal scarring, bleeding, fainting, and seizure.
7. There is a minor risk of infection associated with blood sampling and the muscle biopsy. Should you notice unusual redness, swelling or drainage at the biopsy incision site or at the sites of the blood sampling sites you should seek medical attention and then notify Brent Ruby, study director.
8. There are minimal risks associated with the use of lidocaine (the local anesthetic). Risks include: pain at the injection site, dizziness, confusion, shakiness, visual changes, nausea, and unusually slow heart rate. The risk of a reaction to the lidocaine is extremely low (approximately 1/1,000,000). However to minimize this risk, no more than 9 mL of a 1% lidocaine solution will be used per biopsy. You will be excluded from participation if you have a known history of allergic reactions to local anesthetics.
9. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.

PAYMENT FOR PARTICIPATION

Payment will be according to the following scale:

Experimental trial #1: \$200

Experimental trial #2: \$200

There will be no payment for the pre-testing. Upon completion of the entire study, you will be paid a total of \$400. If you decide to withdraw at any time, you will be compensated for the test sessions you have completed or initiated.

BENEFITS OF PARTICIPATION

1. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to you in the study.
2. There is no promise that you will receive any benefit as a result of taking part in this study.

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3. The scientific benefit includes elucidating the effects of timed carbohydrate feedings from fast food and engineered supplement sources on recovery, muscle glycogen stores, and subsequent bouts of performance.

CONFIDENTIALITY

1. Your records will be kept private and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data.

COMPENSATION FOR INJURY

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by the negligence of the University of Montana or any of its employees, you may be entitled to reimbursement or compensation pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University's Risk Manager (406-243-2700; kathy.krebsbach@umontana.edu) or the Office of Legal Counsel (406-243-4742; legalcounsel@umontana.edu). (Reviewed by University Legal Counsel, May 9, 2013)

VOLUNTARY PARTICIPATION AND WITHDRAWAL

It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive full compensation for all the test sessions you complete or initiate. A copy of this consent form will be provided for you at your request. In addition, the data collected during this study will be done at no cost to you.

QUESTIONS

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Dr. Brent C. Ruby, PhD at (406) 243-2117 (office) or (406) 396-4382. If you have any questions regarding your rights as a subject, you may contact the Chair of the IRB through the University of Montana Research Office at (406) 243-6672.

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STATEMENT OF CONSENT

I have read the above statements and understand the risks involved with this study. I authorize Dr. Brent C. Ruby, PhD and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Dr. Brent C. Ruby, PhD at home (406) 542-2513, cell (406) 396-4382 or at the Human Performance Laboratory (406) 243-2117.

Participant (print) _____

Signature _____

Date _____

Disclosure of Personal Health Information

My individual health information that may be used to conduct this research includes:

Age, height, weight, %body fat, VO₂ max, gene expression in response to exercise/hypoxia, muscle glycogen levels, and markers of oxidative stress.

I authorize Dr. Brent C. Ruby, PhD and the researcher's staff to use my individual health information for the purpose of conducting the research project entitled "The Effects of Fast Food Dietary Choices on Immediate Glycogen Re-Synthesis."

Since I receive compensation for participating in this study, identifying information about me may be used as necessary to provide compensation.

Signature: _____ Date: _____

STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names and physiological data will not be associated with them.

Signature _____ Date _____

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Gastrointestinal Discomfort Questionnaire

Subject:

Trial:

Time:

Date:


Place an "X" on the line in response to the following questions:

1. How hungry do you feel right now?




Not at all Extremely

2. How full do you feel right now?




Not at all Extremely

3. How sick do you feel right now?



Not at all Extremely

4. How much stomach discomfort do you feel right now?




Not at all Extremely

5. How satisfying do you feel this meal is?




Not at all Extremely

6. How tasty do you feel this meal is?



Not at all Extremely

7. How acceptable do you feel this meal is?



Not at all Extremely

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of any other reason why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

- If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
 - take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT
or GUARDIAN (for participants under the age of majority) _____

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.



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