## University of Montana ScholarWorks at University of Montana

Graduate Student Theses, Dissertations, & Professional Papers

**Graduate School** 

2019

# EFFECT OF POTATO-BASED AND PREPACKAGED SPORTS SUPPLEMENT PRODUCTS ON MUSCLE GLYCOGEN RECOVERY AND EXERCISE PERFORMANCE IN TRAINED MALES AND FEMALES

Shannon Kelly Flynn University of Montana, Missoula

## Let us know how access to this document benefits you.

Follow this and additional works at: https://scholarworks.umt.edu/etd

Part of the <u>Sports Sciences Commons</u>

## **Recommended** Citation

Flynn, Shannon Kelly, "EFFECT OF POTATO-BASED AND PREPACKAGED SPORTS SUPPLEMENT PRODUCTS ON MUSCLE GLYCOGEN RECOVERY AND EXERCISE PERFORMANCE IN TRAINED MALES AND FEMALES" (2019). *Graduate Student Theses, Dissertations, & Professional Papers.* 11431. https://scholarworks.umt.edu/etd/11431

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

## EFFECT OF POTATO-BASED AND PREPACKAGED SPORTS SUPPLEMENT PRODUCTS ON MUSCLE GLYCOGEN RECOVERY AND EXERCISE PERFORMANCE IN TRAINED MALES AND FEMALES

By

Shannon K. Flynn

## B.A. Biology for Secondary Education Carroll College Helena, MT 2011

**Thesis Paper** 

Presented in partial fulfillment of the requirements for the degree of:

Master of Science Health and Human Performance, Exercise Science The University of Montana Missoula, MT 2019

Committee Members:

Brent Ruby, Ph.D., FACSM, Chair Health and Human Performance

John Quindry, Ph.D., FACSM Health and Human Performance

Richard Willy, MPT, Ph.D. Physical Therapy and Rehabilitation Science

## **Table of Contents**

Chapters 1-3 References	
Statistical Analysis	
Tissue and Blood Sampling and Analysis	
Experimental Design	
Preliminary Testing	
Participants	
Chapter 3: Methodology	
Chapter 2: Review of Literature	
Delimitations	
Limitations	
Significance and rationale	
Null hypotheses	
Purpose	
Problem	

## **CHAPTER ONE: INTRODUCTION**

### Introduction

Muscle glycogen provides a storage mechanism for the carbohydrate monomer glucose and serves as a crucial fuel source during exercise of varying intensities (1). Endurance exercise performance can be enhanced through increased glycogen stores (2, 3). Particularly during prolonged aerobic exercise, muscle glycogen can be depleted (1). Following activity, glycogen is resynthesized from glucose during two phases: an insulin-independent fast phase and an insulin-dependent slow phase (4). While multiple reviews summarize the wealth of studies indicating that women utilize fuel sources differently than men during endurance exercise (5, 6), a relatively small amount of research shows that glycogen is restored similarly for men and women post-exercise if adequate substrate is provided (7). Regardless of sex, optimal replenishment of glycogen stores is critical for sport and occupational athletes who frequently complete glycogen-depleting activity on consecutive days or participate in multiple workouts or competitions within a single day.

Several factors influence the rates at which muscle glycogen levels are restored after depletion, including environmental conditions and nutrition. While the focus has remained on strategies for eating and drinking, glycogen re-synthesis is actually quite sensitive to other methods of enhancing recovery. For example, high skeletal muscle temperature improves re-synthesis whereas cold skeletal muscle inhibits re-synthesis (8, 9), and room temperature or cool ambient conditions increase restoration as compared to hot ambient conditions (10, 11).

1

Regarding nutrition, when a carbohydrate is provided immediately following a glycogen-depleting event, re-synthesis rates are higher than when the nutrients are delayed (12). Research shows that more carbohydrate consumption leads to a greater rate of re-synthesis, but because rates tend to level off, discussion continues regarding the exact minimum amount for maximum repletion (13-16). However, most investigations indicate that approximately 1.5 g kg<sup>-1</sup> effectively elicits re-synthesis (13-17). Some studies suggest that providing protein with the carbohydrate further enhances restoration rates (18, 19), but more recent work attributes this improvement to additional calories or finds no difference when carbohydrate levels are adequate (15, 16). While both solid and liquid carbohydrates are effective (20), glucose and sucrose are better than fructose at rapidly restoring glycogen (13). Additionally, research shows carbohydrates with a high glycemic index (GI) are more efficient for glycogen repletion than those with a low GI (21, 22), although other work indicates that the positive impact on performance is not conclusive (23).

Athletes desiring to enhance glycogen re-synthesis have two main avenues available. First, a strong sports nutrition product industry has emerged and is expected to continue to grow (24). While these commercial items can improve performance if they contain carbohydrate (25, 26), their marketing claims often are not well supported by scientific evidence (27). Still, both elite athletes and the general population report frequent consumption of sport nutrition products (28, 29), although it is uncommon for athletes to effectively choose the most appropriate and scientifically backed product for their needs (30). A second route for athletes to replenish glycogen and facilitate performance is to consume carbohydrates in traditional foods. As one illustration, research has demonstrated that raisins are a cost-effective method of maintaining blood glucose and enhancing time trial performance as compared to sports jellybeans (31). Additionally, both chocolate milk (32-35) and fast-food items (17) have been found to restore glycogen and/or impact subsequent athletic performance similarly to commercial products. The fast-food meals investigated contained a variety of carbohydrates, including simple sugars, breads, and potatoes.

The potato-based products may provide a particularly viable recovery food option for athletes. Potatoes are an important and nutritious carbohydrate source worldwide, and they are particularly abundant in fiber, Potassium, Vitamin C, and B Vitamins (36-39). For vegetables, they have the second highest nutrient value per dollar (40), and their cost is lower when compared to sport nutrition products. Furthermore, potatoes have a lack of sugar as compared to sports nutrition products (29, 41). If potatoes are as effective as commercial items in replenishing glycogen stores, both male and female athletes could utilize them as a recovery feeding strategy in a range of settings, increasing and diversifying their carbohydrate intake. These findings would continue to provide evidence for the efficacy of traditional foods in post-exercise recovery and would contribute to the growing body of literature describing the similarities and differences between men and women when exercising and recovering from exercise.

The purpose of the study was to compare glycogen recovery and exercise performance in males and females following ingestion of sport supplements and potato products. It was hypothesized that there would be no difference in glycogen resynthesis or exercise performance between sports nutrition products and potato-based meals and that subjects would be more satisfied and satiated when consuming potato products. Also, it was predicted that males and females would have equivalent rates of glycogen re-synthesis.

## Problem

Athletes often consume commercial sports nutrition items as opposed to traditional foods when attempting to optimize recovery and performance. However, chocolate milk and fast food meals have also been shown to effectively enhance recuperation and endurance function. While the fast food included several potato-based items, the specific effects of potatoes on glycogen recovery and exercise performance have not been investigated. Additionally, there is a paucity of research regarding post-exercise glycogen repletion in females.

## Purpose

The purpose of this investigation is to establish the effects of a potato-based diet as compared to commercial sports recovery items on glycogen repletion and cycling performance after glycogen depleting exercise in males and females.

#### Null Hypotheses

#### Main Measures

1. There will be no difference in post-exercise muscle glycogen re-synthesis after consuming potato-based foods as compared to commercial sports supplements.

2. There will be no difference in post-exercise glycogen re-synthesis rate between males and females.

3. There will be no difference in exercise performance after consuming potato-based foods as compared to commercial sports supplements.

## **Secondary Measures**

1. There will be no difference in post-exercise blood glucose levels after consuming potato-based foods as compared to commercial sports supplements.

2. There will be no difference in post-exercise blood insulin levels after consuming potato-based foods as compared to commercial sports supplements.

3. There will be no difference in post-exercise blood glucose levels between males and females.

4. There will be no difference in post-exercise blood insulin levels between males and females.

## Significance and Rationale

Optimal replenishment of glycogen stores is critical for sport and occupational athletes, both male and female, who frequently complete glycogen-depleting activity on consecutive days or participate in multiple workouts or competitions within a single day. Commercial recovery products have emerged in response to athletes' desire to enhance glycogen re-synthesis, but traditional foods can also be effective. Potatoes in particular are popular, cheap, nutritious, readily available, and easily prepared in an assortment of appetizing manners. If they are as effective as sports nutrition products in replenishing glycogen stores, both male and female athletes could utilize potatoes as a recovery feeding strategy in a range of settings, diversifying their carbohydrate intake. These findings would continue to provide evidence for the efficacy of traditional foods in post-exercise recovery and would contribute to the growing body of literature describing the similarities and differences between men and women when exercising and recovering from exercise.

## Limitations

1. Participants' activity, diet, and behavior cannot be controlled when not in the laboratory. Before the first trial, each subject recorded physical activity and diet. They repeated this regiment before the second trial to best control initial muscle glycogen levels.

2. Care was taken to train researchers and calibrate equipment to minimize the possible effects of human error when using instrumentation.

3. Trained subjects were recruited from convenient locations as opposed to randomly sampled. Conversely, a random cross-over design was used when ordering the two treatments.

## Delimitations

Subjects were males and females between the ages of 18 and 40. They had recently been participating in at least 30 minutes of physical activity at least 4 days each week. No subjects had a known allergy to lidocaine.

## CHAPTER TWO: REVIEW OF LITERATURE

## **Glycogen and Exercise**

## Glycogen Depletion

Muscle glycogen functions as an important fuel during activity, and it can be depleted during prolonged or intense exercise as demonstrated through several pivotal studies in the late 1960s (1-3). For example, Hermansen et al. (1) had trained (T) and untrained (UT) subjects cycle on an ergometer at an average of 77% maximum aerobic power until exhaustion. After exercise, the trained participants had a higher glycogen content than the untrained (T: 0.12, UT: 0.06 g 100 g wet muscle<sup>-1</sup>) and were able to exercise longer (T: 90, UT: 85 min). During their analysis, a relationship was found between glycogen used and carbohydrate combusted. Similarly, Ahlborg et al. (2) had participants exercise to exhaustion and found that the amount of glycogen utilized was correlated with exercise duration and energy use.

Additional studies made further manipulations to illuminate the details of depleting glycogen. While Bergstrom et al. (3) likewise found that original glycogen content and time to exhaustion when cycling were correlated, they also modified diet. Subjects performed glycogen-depleting exercise, consumed a diet of either fat+protein (P), carbohydrate (C), or a mixture of both (M), and completed additional cycling at 75% VO<sub>2Max</sub> to fatigue. When eating carbohydrate diets, individuals had higher glycogen content and exercised longer (P: 59, M: 126, C: 189 min). Gollnick et al. (42)instead controlled exercise intensity. They found that glycogen depletion was 2.7 times greater at 64% VO<sub>2Max</sub> and 7.4 times greater at 84% VO<sub>2Max</sub> as compared to 31% VO<sub>2Max</sub>. As a

7

whole, these investigations highlight the crucial role of muscle glycogen in exercise performance.

## Glycogen Restoration

Following depletion, glycogen is restored via a fast insulin-independent phase and a slow insulin-dependent phase (4, 43). Muscle contractions during exercise stimulate GLUT-4 translocation, which leads to uptake of glucose during the initial rapid phase of recovery (43-45). When carbohydrate is consumed following exercise, glucose levels increase and insulin is released. Insulin release also causes GLUT-4 translocation, and exercise induced insulin sensitivity during this secondary slow phase enhances the process (44-46). Glycogen synthase, an important enzyme for muscular glycogen resynthesis, is also activated during both phases (43, 45). Because of the role of glucose and insulin in glycogen restoration, they are critical for quick resynthesis and help explain the effectiveness of particular nutritional strategies for glycogen repletion.

Research shows that glycogen restoration is connected to glycogen depletion (47, 48). Price et al. (47) had subjects perform calf raises to deplete glycogen after either normal carbohydrate consumption for one week or after carbohydrate loading to increase stored glycogen. They found that glycogen repletion rates were lower in the carbohydrate-loaded group, which they attributed to a higher glycogen concentration in that group following exercise (47). In an effective study design, Zachweija et al. (48) elicited a large amount of glycogen depletion in one leg of subjects by adding 30 minutes of single leg cycling and 10 one-minute sprints to that leg beyond 30 minutes of a small amount of glycogen depletion (48). The leg with more glycogen depletion

demonstrated a significantly higher rate of muscle glycogen re-synthesis (48). Overall, these studies indicate that more depletion of glycogen leads to quicker repletion.

## Impact of Nutritional Strategies on Glycogen Re-Synthesis

## Timing & Frequency

As early as the 1970s, research showed that carbohydrate consumption is critical for glycogen repletion (3), but additional nutritional modifications also affect resynthesis. In a study by Ivy et al. (12), cyclists exercised for 70 minutes then consumed a carbohydrate drink either immediately (I) or 2-hrs post-exercise (2P). When drinking the carbohydrate straightaway, participants had a higher rate of glycogen re-synthesis during the two hours after cycling (I: 7.7, 2P: 2.5 mM kg wet wt-<sup>1</sup> hr<sup>-1</sup>). In recovery hours 2-4, rates were similar between the two treatments, but the 2P rate was still slower than the initial rate for I (I: 4.3, 2P: 4.1 mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>). These findings indicate that quicker ingestion of nutrients after exercise enhances recovery. Studies also show that glycogen re-synthesis is more rapid when carbohydrate is provided at frequent intervals (48, 49).

#### Amount

The synthesis of results from multiple repeated measures studies indicates that optimal glycogen re-synthesis occurs when carbohydrate intake averages or exceeds  $1.2 \text{ g kg}^{-1} \text{ hr}^{-1}$  following depletion. Each investigation had participants cycle extensively to deplete glycogen, provided a variety of re-feeding strategies for the subjects in the form of carbohydrate drinks, and measured rates of glycogen restoration. Blom et al. (13) administered feedings at 0, 2, and 4 hours post-exercise and found that 0.7 g kg<sup>-1</sup> and 1.4 g kg<sup>-1</sup> of carbohydrate elicited similar re-synthesis, but both led to more

restoration than 0.35 g kg<sup>-1</sup>. Correspondingly, Ivy et al. (14) found that 1.5 g kg<sup>-1</sup> and 3.0 g kg<sup>-1</sup> provided at 0 and 2 hours of recovery resulted in comparable re-synthesis which was greater than when consuming a placebo. In a different study with the same timing of feedings, Ivy et al. (18) showed that 1.5 g kg<sup>-1</sup> hr<sup>-1</sup>carbohydrate did not lead to significantly increased glycogen restoration as compared to 1.1 g kg<sup>-1</sup> hr<sup>-1</sup>. In contrast to Blom et al., van Loon et al. (16) showed that 1.2 g kg<sup>-1</sup> hr<sup>-1</sup> carbohydrate ingestion resulted in more glycogen repletion than 0.8 g kg<sup>-1</sup> hr<sup>-1</sup>. Unlike the previous three studies, the participants imbibed drinks every 30 minutes, which may have contributed to the differing results. While discussion continues regarding ideal post-exercise carbohydrate administration, many other studies also yield high rates of re-synthesis with provision of 1.2 to 1.8 g kg<sup>-1</sup> at each feeding (17, 19, 50).

## Composition

After carbohydrates were stamped as a critical material for glycogen resynthesis, research attempted to determine whether a particular type of carbohydrate was most effective. During these investigations, subjected cycled to diminish glycogen then consumed various forms of carbohydrate during recovery. Reed et al. (20) demonstrated that solid and liquid carbohydrates are equally effective in restoring muscle glycogen. Several investigations showed that high glycemic index (GI) carbohydrates replenish glycogen more quickly than low GI (21, 22). Finally, while ingesting glucose or sucrose alone appears to enhance glycogen re-synthesis as compared to ingesting fructose alone (13), more recent research indicates that when providing iso-energetic carbohydrate feedings, glucose + sucrose or glucose + fructose are as effective as glucose on its own (51, 52). In addition, more diversified feedings may lead to less gastrointestinal discomfort (51).

Although carbohydrate is an essential macronutrient for glycogenesis, some studies show that adding protein contributes to optimal glycogen replenishment in certain scenarios. Again, these repeated measures investigations involved bouts of glycogen-depleting exercise via a cycle ergometer followed by a recovery period with multiple macronutrient beverages. When consuming iso-carbohydrate feedings of greater than or equal to 1.2 g kg<sup>-1</sup> carbohydrate at intervals less or equal to 30 minutes, added protein does not enhance repletion (50, 53). For example, van Hall et al. (53) showed no difference in glycogen re-synthesis between 1.67 g kg<sup>-1</sup> carbohydrate and 1.67 g kg<sup>-1</sup> carbohydrate + 0.5 g kg<sup>-1</sup> protein. Iso-energetic feedings provided at least every hour also resulted in similar restoration rates between carbohydrate and carbohydrate + protein treatments (7, 16). As one illustration, Tarnopolsky et al. (7) demonstrated comparable glycogen re-synthesis when delivering 1 g kg<sup>-1</sup> hr<sup>-1</sup> carbohydrate as compared to 0.75 g kg<sup>-1</sup> hr<sup>-1</sup> carbohydrate + 0.1 g kg<sup>-1</sup> hr protein + 0.02 g kg<sup>-1</sup> hr<sup>-1</sup> fat. Both this study and that of van Loon et al. (16) fed less than the recommended amount of carbohydrate in their added protein treatments. However, each demonstrated re-synthesis rates similar to their higher carbohydrate feedings, which may indicate that protein can be beneficial when carbohydrate intake is low.

When meals are consumed at a lower frequency – 0 hours and 2 hours after glycogen-depleting exercise – the addition of protein does improve repletion rates (18, 19). For instance, lvy et al. (18) provided subjects with 1.1 g kg<sup>-1</sup> carbohydrate, 1.5 g kg<sup>-1</sup> carbohydrate, or 1.1 g kg<sup>-1</sup> carbohydrate + 0.4 g kg<sup>-1</sup> protein. The protein

supplement led to greater repletion than either carbohydrate-only group even though sufficient carbohydrate was consumed. These results suggest that protein can bolster glycogen re-synthesis if carbohydrate intake cannot be frequent and regular. *Additives* 

Beyond macronutrient intake, several investigations have examined whether other additives enhance glycogen recovery. Two studies assessed the impact of fenugreek extract, which contains the unique non-muscle amino acid 4hydroxyisoleucine, on glycogen recovery following glycogen-depleting exercise (54, 55). Results were equivocal, with one investigation showing significantly improved glycogen recovery in a carbohydrate + fenugreek treatment as compared to carbohydrate only (54) and another showing no difference (55). The researchers suggested that the variance might result from differences in the duration and intensity of depletion exercise between the two studies (55). Other investigators have researched the impact of substances commonly consumed by athletes, such as caffeine and alcohol. While one study demonstrated that adding caffeine to carbohydrate exerts a positive influence on glycogen recovery after depletion exercise (56), most research shows that caffeine consumption has no impact on glycogen re-synthesis, especially when adequate carbohydrate is provided (57, 58). Regarding alcohol, research by Burke et al. (59) indicated that alcohol itself does not decrease glycogen repletion over 24 hours of recovery as long as sufficient carbohydrate is consumed. However, if alcohol replaces carbohydrate, glycogen re-synthesis is hindered at both 8 and 24 hours of recovery (59).

## Fasted vs. Fed

Most glycogen recovery investigations take place after overnight fasts and without carbohydrate supplementation before or during exercise, but some research has examined feeding scenarios more comparable to realistic athletic conditions. For example, De Bock et al. (60) showed that fasting prior to strenuous exercise resulted in greater post-exercise rates of glycogen repletion than when consuming carbohydrate before and during the exercise. Reinert et al. (61) also provided carbohydrate preexercise and throughout a 62 km outdoor cycling ride, as well as a meal two hours after the ride. They demonstrated that glycogen re-synthesis was not greater when providing additional carbohydrate as compared to a placebo immediately after the training ride (61). Taken together, these studies may indicate that sufficient and consistent overall carbohydrate consumption decreases the need for meticulous planning of carbohydrate timing and dosage when attempting to maximize glycogen recovery.

#### **Optimizing Glycogen Restoration**

#### Muscle and Ambient Temperature

Research indicates that muscle glycogen recovery is sensitive to both local and ambient temperature differences. To examine various temperature conditions, subjects performed glycogen-depleting exercise on a cycle ergometer then recovered under different ambient conditions or with local application of heat or cold. Slivka et al. (11) found that there was no difference in glycogen repletion when recovering in cold ambient conditions (7°C) as compared to room (20°C) temperature, while Naperalsky et al. (10) discovered that subjects recuperating at room temperature (22°C) increased muscle glycogen more quickly than those in hot ambient conditions (33°C). For local

application, hot or cold packs were applied intermittently to the vastus lateralis – the muscle that would later be biopsied – throughout four hours of recovery. Local heat resulted in more glycogen re-synthesis (8), and local cold resulted in less repletion (9). The time period of cold application may play a role since other research shows that ten minutes of lower body cold-water immersion does not negatively impact glycogen re-synthesis, but extensive ambient heat or local cold decrease re-synthesis.

#### Effect of Previous Exercise

Evidence shows that certain types of previous exercise can impact rates of glycogen re-synthesis (49, 63-67). In particular, as compared to concentric exercise only, eccentric damage decreases glycogen recovery (49, 63, 67). This impact is not observed directly following depletion but instead occurs 18 hours or more after the strenuous exercise, a delay likely coinciding with delayed onset muscle soreness (DOMS) and inflammation from the eccentric activity (49, 63, 67). As one example, Doyle et al. (49) had subjects perform a 75-minute cycling protocol including intervals and leading to glycogen depletion. The cyclists then performed either extensive eccentric or concentric leg contractions, and the researchers measured their glycogen during four hours of recovery (49). Forty-eight hours after the first cycle, the subjects repeated the protocol and again had their glycogen levels analyzed (49). Although the replenishment was similar for both the eccentric and concentric group following the first ride, it was significantly lower for the eccentric group following the second ride (49).

These findings are supported by several field investigations of individuals performing extensive exercise that involves running, an activity that results in more

14

eccentric damage than cycling (64-66). In a case study, Gillum et al. (64) monitored a male participant in a half Ironman triathlon. During the four hours following the race, the subject's glycogen re-synthesis rate was lower (4.1 mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>) than rates found with individuals completing difficult cycling activity (10.6 mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>), a result that could be attributed to the eccentric damage incurred during the race (64). *Recovery Techniques* 

Several recovery techniques have been examined as methods of enhancing glycogen repletion. Research shows that manual therapies such as massage and pneumatic compression have no impact on glycogen recovery (68, 69). Although there is some evidence to the contrary (70), multiple studies have demonstrated that following both exhaustive and non-exhaustive exercise, glycogen re-synthesis is more effective with passive recovery as opposed to active recovery (71, 72). Both investigations involved low intensity active recovery with one at 20% VO<sub>2Max</sub> for four hours (71) and one at 42% VO<sub>2Max</sub> for 30 minutes (72). Considering that active recovery has been demonstrated to improve lactate clearance and is often utilized by athletes following strenuous exercise, close consideration of recovery goals may be warranted before choosing a recovery method (73).

## Commercial Sports Supplements vs. Traditional Foods

Partly due to the emphasis on the importance of glycogen re-synthesis for certain athletes, a sports nutrition product industry has emerged. The sport nutrition market is large and projected to continue growing (24). Products containing carbohydrate can indeed improve performance (25, 26). However, while marketing claims target both athletes and everyday consumers seeking an active, healthy, lifestyle (29, 74), the claims are often not well supported by scientific evidence (27).

Scientists have also examined carbohydrates found in traditional foods in exercise research. For example, raisins were demonstrated to be a cost-effective method of maintaining blood glucose and enhancing time trial performance as compared to jelly beans (31). Looking more specifically at recovery post-exercise, researchers showed that chocolate milk is as effective for glycogen repletion as sports drinks (32-35). Additionally, Cramer et al. (17) determined that fast-food items elicited the same rate of glycogen recovery after depletion exercise as sports supplements.

## Sex Differences in Glycogen Metabolism

Nutritional recommendations for glycogen recovery are often applied to both men and women despite a relatively small amount of studies including females as subjects. Multiple reviews summarize research indicating that women utilize fuel sources differently than men during endurance exercise (5, 6). Some studies indicate that these differences are attenuated when carbohydrate is provided before and during exercise (75). Other investigations show that the differences are eliminated when male and female subjects are appropriately matched for lean body mass and fitness (76, 77). Although early research showed a discrepancy in glycogen supercompensation between men and women (78), later investigations demonstrated that with adequate carbohydrate provision based on lean body mass, females' glycogen supercompensation is equivalent to males (79-82). In line with these findings, one study shows that glycogen is restored similarly for men and women post-exercise if adequate substrate is provided (7).

## CHAPTER THREE: METHODOLOGY

## **Participants**

Sixteen recreationally active athletes – eight male and eight female – completed the study (n=16). The participants were healthy, injury-free, familiar with endurance exercise, and between the ages of 18 and 40. They had recently been participating in physical activity 30 minutes per day on 4 days per week. All subjects completed a Physical Readiness Questionnaire (PAR-Q) and gave written informed consent prior to the study. The University of Montana Institutional Review Board approved all procedures.

## **Preliminary Testing**

Each subject fasted for four hours and abstained from exercise, alcohol, and caffeine for 24 hours prior to an initial visit. After measuring body mass and height, body composition was obtained via hydrodensitometry with corrections for estimated residual lung volume (83). Underwater body mass was obtained using an electronic scale (Exertech, Dreshbach, MN) and used to calculate body density. The Siri equation was utilized to convert body density to percent body fat (84).

Using a cycle ergometer (Velotron, RacerMate Inc., Seattle, WA), peak oxygen uptake (VO<sub>2 Peak</sub>) and maximal power output ( $W_{Max}$ ) were determined for each subject during a graded exercise protocol. The protocol started at 95W for men and 60W for women, and it increased 35W every three minutes until volitional fatigue and the achievement of at least two of the following specific max test criteria: respiratory exchange ratio (RER) >1.10, plateau in VO<sub>2</sub>, heart rate (HR) within 10 beats per minute (BPM) of age-predicted max, or rating of perceived exertion (RPE) >17. During the test, gas exchange was measured using a calibrated metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). VO<sub>2 Peak</sub> was calculated as the highest 15-second average oxygen uptake, and  $W_{Max}$  was calculated using the following equation:  $W_{Max}$  = (power output in W during last completed stage) + ((final stage time before fatigue in s/180s) x 35 W).

On a second and third visit, each participant completed a practice (PTT) 20km time trial (TT) on a computer-simulated course, for a total of two PTT. Subjects were directed to complete the distance as quickly as possible, and they were able to change speed and shift gears electronically while distance and time were recorded using RacerMate Inc. software (RacerMate, Inc., Seattle, WA). These sessions ensured TT competency.

## **Experimental Design**

Subjects completed two trials in a randomized crossover design with seven days between. Prior to each laboratory visit, they refrained from exercise, alcohol, and caffeine for 24 hours, and they arrived each morning following a 12-hr fast. For each trial, participants completed a glycogen depletion ride followed by a four-hour recovery period then a 20 km TT. During the recovery, they consumed either sports supplement products (SS) or potato-based foods (PB). Throughout the rest period, the subjects filled out gastrointestinal discomfort and meal satisfaction questionnaires, and muscle biopsies and blood samples were collected. Figure 1 illustrates the experimental design.

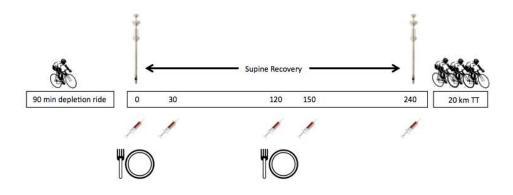


Figure 1. Experimental design

## Glycogen Depletion Protocol

Subjects completed glycogen-depleting exercise consisting of 90 minutes on the previously mentioned cycle ergometer. The protocol entailed a 10-min warm up at 55%  $W_{Max}$ , a series of 10 intervals (2-min at 80%  $W_{Max}$  followed by 4-min at 50%  $W_{Max}$ ) lasting 60-min, 8-min at 60%  $W_{Max}$ , and finally 12-min at 50%  $W_{Max}$ . Water was consumed ad libitum during the exercise. Following the protocol, participants rested in a seated or reclined position for the 4-hr recovery period.

## Feeding Strategy

At 0-hr and 2-hr of recovery, participants consumed exact amounts of macronutrients as either SS or PB. Nutritional label serving sizes were utilized, and items were weighed for accuracy. Each subject consumed 1.5 g carbohydrate kg body weight<sup>-1</sup>, and fat and protein content was also aligned between trials. Table 1 provides details regarding specific menu items and feeding schedules.

Sports Supplement 0 hours					
U HOUIS	Calories	Fat (g)	Carbohydrate (g)	Protein (g)	Sodium (mg)
Rehydrate Salt Tablet	0	0	0	0	800
Powerade Mountain Berry Blast (591 mL)	130	0	35	0	250
Lara Bar Peanut Butter Chocolate Chip	440	22	52	12	120
Gatorade Prime Energy Chews Fruit Punch (Pack=6 Chews)	83	0	20	0	58
Total 2 hours	653	22	107	12	1228
Rehydrate Salt Tablet	0	0	0	0	800
Gatorade Lemon-Lime (591 mL)	140	0	36	0	270
Cliff Mojo Bar Mountain Mix	400	18	46	20	480
Cliff Bloks Energy Chews Mountain Berry (Pack=6 Bloks)	117	0	28	0	58.5
Total	657	18	110	20	1608.5
4 Hour Total	1310	40	217	32	2836.5

Table 1. Bolus feeding protocol for 0 hr and 2 hr post-exercise recovery for body mass of 62-74 kg

#### tato Based 0 hours

0 nours					
	Calories	Fat (g)	Carbohydrate (g)	Protein (g)	Sodium (mg)
Kraft Pancake Syrup	105	0	28	0	0
Simplot Old European Potato Pancakes	240	10	34	4	620
Mott's Unsweetened Applesauce	40	0	10	0	0
Great Value Hash Brown	300	16	36	4	540
Total 2 hours	685	26	108	8	1160
Delallo Potato Gnocchi (140 g)	345	0	75	9	840
Prego Traditional Pasta Sauce (120 mL)	70	1.5	13	2	480
Great Value Seasoned Fries (84 g)	175.5	9.4	21	2.3	410
Total	590.5	10.9	109	13.3	1730
4 Hour Total	1275.5	36.9	217	21.3	2890

## Questionnaires

Subjects completed gastrointestinal discomfort questionnaires at 0, 1, 2, 3, and 4-hrs of recovery and post-meal questionnaires at 0 and 2-hrs of recovery. The gastrointestinal questionnaire gauged hunger, fullness, sickness, and stomach discomfort, and the post-meal questionnaire evaluated meal satisfaction, taste, and acceptability. Both involved a 150 mm visual analog scale (VAS) with "Not at all" on the left edge and "Extremely" on the right edge of the continuum, and participants placed an "X" along the scale for each question. The distance from "Not at all" in mm was divided by 150 mm and reported as a score (85).

## 20 km Time Trial

After the supine recovery, subjects completed a 20 km TT on the cycle ergometer described above and with the same ambient conditions each time. As with the PTT, participants were directed to complete the distance as rapidly as possible and were provided no encouragement. Distance and time were recorded similarly to the PTT. Subjects did not consume water during the TTs.

## **Tissue and Blood Sampling and Analysis**

#### Muscle Biopsies

At 0 and 4-hrs recovery post glycogen-depleting exercise, muscle biopsies of the vastus lateralis were performed using the percutaneous biopsy needle technique and suction (86, 87). Each 4-hr biopsy was performed 2 cm proximal to the first location, and each trial utilized a different leg in randomized order. Before the biopsy, 1 mL of 1% lidocaine (without adrenaline) was injected beneath the skin as a local anesthetic. This numbed an initial 2 cm<sup>2</sup> area, then another 2-3 mL of lidocaine were injected near the

fascia. After the injection, an approximately 0.5 cm incision was made through the skin and muscle fascia, and a Bergstrom biopsy needle was inserted through the opening into the belly of the vastus lateralis muscle. The process removed 30 mg of tissue, and extra blood, fat, and connective tissue were subsequently separated from the muscle sample. The remaining tissue was immediately frozen in liquid nitrogen and stored at -80°C until muscle glycogen analysis.

## Blood Sampling

Venipuncture technique was used to gather blood samples from an antecubital arm vein at 0, 30, 120, 150, and 240 min of recovery. Samples were allowed to clot then spun in a 4°C refrigerated centrifuge (Jouan Inc., MR22i) at 4000 rpm for 15 min. Aliquots of serum were prepared, and the tubes were stored at -30° until glucose and insulin analyses.

## Analysis

An enzymatic spectrophotometric method was used to analyze duplicate muscle samples (10-12 mg, collected at same time) for muscle glycogen concentration (54). After weighing, samples were added to 0.5 mL of 2N HCl solution. This solution was weighed, incubated for two hours in a 100°C oven, reweighed, reconstituted with distilled water to the original sample weight, and pH normalized using 1.5 mL of 0.67 M NaOH. Next, 100 mL muscle extract solution was added to 1 mL Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.). After reading the solution at 340 nm on a spectrophotometer, muscle glycogen concentration was calculated using a standard curve. The concentration was expressed as mM kg wet wt muscle<sup>-1</sup>.

Similarly, blood samples were analyzed for glucose in triplicate with Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read at 340 nm on a spectrophotometer. A standard curve was used to calculate blood glucose concentration. Enzymatic spectrophotometric ELISA method was used to analyze insulin from blood samples in duplicate (EIA-2935, DRG International). Mean intra-assay coefficients of variation for muscle samples, glucose, and insulin using these techniques are less than 5%.

#### **Statistical Analysis**

Multiple two-way ANOVAs (one sex x trial and one sex x trial order) with repeated measures were used to analyze PTT and TT performance times (SPSS Inc., Chicago, IL). Muscle glycogen recovery rates were compared using a two-way ANOVA (sex x trial) with repeated measures. A three-way ANOVA (sex x trial x time) with repeated measures was used to examine differences in muscle glycogen, blood glucose, and serum insulin levels between SS and PB (SPSS Inc., Chicago, IL). Gastrointestinal discomfort and meal satisfaction were compared using a three-way ANOVA (sex x trial x time) with repeated measures. Statistical significance was set at a probability of type I error less than 5% (p<0.05). All data are expressed as mean ± SD.

## References

1. Hermansen L, Hultman E, Saltin B. Muscle glycogen during prolonged severe exercise

. Acta Physiologica. 1967 October;71(2-3):129-39.

2. Ahlborg B, Bergstrom J, Edelund L, Hultman E. Muscle glycogen and muscle electrolytes during prolonged physical exercise . Acta Physiologica. 1967 June;70(2):129-42.

3. Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen, and physical performance

. Acta Physiologica. 1967 October;71(2-3):140-50.

4. Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. Journal of Applied Physiology. 1994;76(1):104-11.

5. Devries MC. Sex-based differences in endurance exercise muscle metabolism: impact on exercise and nutritional strategies to optimize health and performance in women. Experimental Physiology. 2016 Feb 1,;101(2):243-9.

6. Tarnopolsky M, Ruby B. Sex differences in carbohydrate metabolism. Current Opinion in Clinical Nutrition and Metabolic Care. 2001 Nov;4(6):521-6.

7. Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. Journal of Applied Physiology. 1997 Dec 1,;83(6):1877-83.

8. Slivka D, Tucker T, Cuddy J, Hailes W, Ruby B. Local heat application enhances glycogenesis. Applied Physiology, Nutrition, and Metabolism. 2012 Apr;37(2):247-51.

9. Tucker TJ, Slivka DR, Cuddy JS, Hailes WS, Ruby BC. Effect of local cold application on glycogen recovery

. Journal of Sports Medicine and Physical Fitness. 2012 April;52(2):158-64.

10. Naperalsky M, Ruby B, Slivka D. Environmental temperature and glycogen resynthesis

. International Journal of Sports Medicine. 2010;31:561-6.

11. Slivka D, Heesch M, Dumke C, Cuddy J, Hailes W, Ruby B. Effects of post-exercise recovery in a cold environment on muscle glycogen, PGC-1 $\alpha$ , and downstream transcription factors. Cryobiology. 2013 Jun;66(3):250-5.

12. Ivy JL, Katz AL, Cutler CL, Sherman WM, Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. Journal of Applied Physiology. 1988 Apr 1,;64(4):1480-5.

13. Blom P, Hostmark AT, Vaage O, Kardel KR, Maehlum S. Effect of different postexercise sugar diets on the rate of muscle glycogen synthesis. Medicine and Science in Sports and Exercise. 1987;19(5):491-6.

14. Ivy JL, Lee MC, Brozinick JJ, Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. Journal of Applied Physiology. 1988 Nov 1,;65(5):2018-23.

15. Roy L. P. G. Jentjens, Luc J. C. van Loon, Christopher H. Mann, Anton J. M. Wagenmakers, Asker E. Jeukendrup. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. Journal of Applied Physiology. 2001 Aug 1,;91(2):839-46.

16. van Loon, L. J. C, Saris WHM, Kruijshoop M, Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. American Journal of Clinical Nutrition. 2000 Jan 1,;72(1):106-11.

17. Cramer MJ, Dumke CL, Hailes WS, Cuddy JS, Ruby BC. Postexercise glycogen recovery and exercise performance is not significantly different between fast food and sport supplements. International Journal of Sport Nutrition and Exercise Metabolism. 2015 Oct 1,;25(5):448.

18. Ivy JL, Goforth HJ, Damon BM, McCauley TR, Parson EC, Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. Journal of Applied Physiology. 2002 Oct 1,;93(4):1337-44.

19. Zawadski KM, Yaspelkis BB, Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. Journal of Applied Physiology. 1992 May 1,;72(5):1854-9.

20. Reed MJ, Brozinick JJ, Lee MC, Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. Journal of Applied Physiology. 1989 Feb 1,;66(2):720-6.

21. Burke LM, Collier GR, Hargreaves M. Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. Journal of Applied Physiology. 1993 Aug 1,;75(2):1019-23.

22. Kions B, Raben AB, Valeur AK. Benefit of dietary simple carbohydrates on the early post-exercise muscle glycogen repletion in male athletes . Medicine and Science in Sports and Exercise. 1990;22(4):S88.

23. Brown, Laura J.S.|Midgley, Adrian W.|Vince, Rebecca V.|Madden, Leigh A.|McNaughton, Lars R. High versus low glycemic index 3-h recovery diets following glycogen-depleting exercise has no effect on subsequent 5-km cycling time trial performance. Journal of Science and Medicine in Sport. 2012;16(5):450-4.

24. Technavio Releases Report on the Global Sports Nutrition Products Market. Professional Services Close - Up. 2016 Jun 21,.

25. Campbell C, Prince D, Braun M, Applegate E, Casazza GA. Carbohydratesupplement form and exercise performance . International Journal of Sport Nutrition and Exercise Metabolism. 2008;18:179-90.

26. Coombes JS, Hamilton KL. The Effectiveness of Commercially Available Sports Drinks. Sports Med. 2000 Mar 1,;29(3):181-209.

27. Heneghan C, Howick J, O'Neill B, Gill PJ, Lasserson DS, Cohen D, et al. The evidence underpinning sports performance products: a systematic assessment. BMJ open. 2012;2(4):e001702.

28. Lun V, Erdman KA, Fung TS, Reimer RA. Dietary supplementation practices in Canadian high-performance athletes. International journal of sport nutrition and exercise metabolism. 2012 Feb;22(1):31-7.

29. Zytnick D, Park S, Onufrak SJ, Kingsley BS, Sherry B. Knowledge of Sugar Content of Sports Drinks is Not Associated with Sports Drink Consumption. American Journal of Health Promotion. 2015 Nov;30(2):101-8.

30. Newland BL, Chalip L, Ivy JL. Coping with a Cluttered Marketplace: Athlete Choice of Products to Support Training. Journal of Sport Management. 2013 Jan;27(1):59-72.

31. Rietschier H, Henagan T, Earnest C, Baker B, Cortez C, Stewart L. Sun-Dried Raisins are a Cost-Effective Alternative to Sports Jelly Beans in Prolonged Cycling. Journal of Strength and Conditioning Research. 2011 Nov;25(11):3150-6.

32. Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, Stager JM. Chocolate milk as a post-exercise recovery aid. International journal of sport nutrition and exercise metabolism. 2006 Feb;16(1):78.

33. Lunn WR, Pasiakos SM, Colletto MR, Karfonta KE, Carbone JW, Anderson JM, et al. Chocolate milk and endurance exercise recovery: protein balance, glycogen, and performance. Medicine and science in sports and exercise. 2012 Apr;44(4):682-91.

34. Pritchett K, Bishop P, Pritchett R, Green M, Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Applied Physiology, Nutrition, and Metabolism. 2009 Dec 1,;34(6):1017-22.

35. Thomas K, Morris P, Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Applied Physiology, Nutrition, and Metabolism. 2009 Feb 1,;34(1):78-82.

36. Camire ME, Kubow S, Donnelly DJ. Potatoes and Human Health. Critical Reviews in Food Science and Nutrition. 2009 Dec 10,;49(10):823-40.

37. Carlson JJ, Eisenmann JC, Norman GJ, Ortiz KA, Young PC. Dietary Fiber and Nutrient Density Are Inversely Associated with the Metabolic Syndrome in US Adolescents. Journal of the American Dietetic Association. 2011;111(11):1688-95.

38. Kanter M. High-Quality Carbohydrates and Physical Performance: Expert Panel Report. Nutrition Today. 2018 Jan;53(1):35-9.

39. Zaheer K, Akhtar MH. Potato Production, Usage, and Nutrition-A Review. Critical Reviews in Food Science and Nutrition. 2016 Apr 3,;56(5):711-21.

40. Drewnowski A, PhD. New Metrics of Affordable Nutrition: Which Vegetables Provide Most Nutrients for Least Cost? Journal of the Academy of Nutrition and Dietetics. 2013;113(9):1182-7.

41. Ng, Shu Wen, PhD|Slining, Meghan M., PhD|Popkin, Barry M., PhD. Use of Caloric and Noncaloric Sweeteners in US Consumer Packaged Foods, 2005-2009. Journal of the Academy of Nutrition and Dietetics. 2012;112(11):1834.e6.

42. Gollnick PD, Piehl K, Saltin B. Selective glycogen depletion in human muscle fibres after exercise of varying intensity and at varying pedalling rates . Journal of Physiology. 1974;241:45-57.

43. Murray B, Rosenbloom C. Fundamentals of glycogen metabolism for coaches and athletes. Nutrition Reviews. 2018 Apr 1,;76(4):243-59.

44. Borghouts L, Keizer H. Exercise and Insulin Sensitivity: A Review. Int J Sports Med. 2000 Jan;21(1):1-12.

45. Burke LM, van Loon L, Hawley JA. Post-exercise muscle glycogen resynthesis in humans. Journal of Applied Physiology. 2017;122:1055-67.

46. Saltiel A, Kahn R. Insulin signalling and the regulation of glucose and lipid metabolism

. Nature. 2001;414:799-806.

47. Price TB. Glycogen loading alters muscle glycogen resynthesis after exercise. Journal of Applied Physiology. 2000 Feb 1,;88(2):698-704.

48. Zachwieja JJ, Costill DL, Pascoe DD, Robergs RA, Fink WJ. Influence of muscle glycogen depletion on the rate of resynthesis. Medicine and Science in Sports and Exercise. 1991;23(1):44-8.

49. Doyle JA. Effects of eccentric and concentric exercise on muscle glycogen replenishment. Journal of Applied Physiology. 1993 Apr 1,;74(4):1848-55.

50. Jentjens R, Jeukendrup AE. Determinants of Post-Exercise Glycogen Synthesis During Short-Term Recovery. Sports Med. 2003;33(2):117-44.

51. Trommelen J, Beelen M, Pinckaers P, Senden JMG, Cermak NM, van Loon, Luc J. C. Fructose Coingestion Does Not Accelerate Postexercise Muscle Glycogen Repletion. Medicine and Science in Sports and Exercise. 2016;48(5):907-12.

52. Wallis GA, Hulston CJ, Mann CH, Roper HP, Tipton KD, Jeukendrup AE. Postexercise Muscle Glycogen Synthesis with Combined Glucose and Fructose Ingestion. Medicine and science in sports and exercise. 2008 Oct;40(10):1789-94.

53. van Hall G, Shirrefs SM, Calbet J. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. Journal of Applied Physiology. 2000 May 1,;88(5):1631-6.

54. Ruby BC, Gaskill SE, Slivka D, Harger SG. Addition of a fenugreek extract to glucose feedings increases muscle glycogen resynthesis after exercise . Amino Acids. 2005;28(Supplement):71-6.

55. Slivka D, Cuddy J, Hailes W, Harger S, Ruby B. Glycogen resynthesis and exercise performance with the addition of fenugreek extract (4-hydroxyisoleucine) to post-exercise carbohydrate feeding. Amino Acids. 2008 Aug;35(2):439-44.

56. Pederson DJ. High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine. Journal of Applied Physiology. 2008 Jul 1,;105(1):7-13.

57. Battram DS. Caffeine ingestion does not impede the resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate supplementation in humans. Journal of Applied Physiology. 2004 Mar 1,;96(3):943-50.

58. Beelen M, van Kranenburg J, Senden JM, Kuipers H, van Loon, L. J. C. Impact of caffeine and protein on postexercise muscle glycogen synthesis. Medicine and Science in Sports and Exercise. 2012 Jan 1,;44(4):692-700.

59. Burke L, Collier G, Broad E, Davis P, Martin D, Sanigorski A, et al. Effect of alcohol intake on muscle glycogen storage after prolonged exercise. . 2003.

60. De Bock K. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. The Journal of Physiology. 2005 Apr 15,;564(2):649-60.

61. Reinert A, Slivka D, Cuddy J, Ruby B. Glycogen synthesis after road cycling in the fed state

. . 2009;30:545-9.

62. Gregson W, Allan R, Holden S, Phibbs P, Doran D, Campbell I, et al. Postexercise cold-water immersion does not attenuate muscle glycogen resynthesis. Medicine & Science in Sports & Exercise. 2013 Jun;45(6):1174-81.

63. Costill DL. Impaired muscle glycogen resynthesis after eccentric exercise. Journal of Applied Physiology. 1990 Jul 1,;69(1):46-50.

64. Gillum TL, Dumke CL, Ruby BC. Muscle Glycogenolysis and Resynthesis in Response to a Half Ironman Triathlon: A Case Study. International journal of sports physiology and performance. 2006 Dec;1(4):408-13.

65. O'Reilly KP. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. Journal of Applied Physiology. 1987 Jul 1,;63(1):252-6.

66. Sherman WM. Effect of a 42.2-km footrace and subsequent rest or exercise on muscle glycogen and enzymes. Journal of Applied Physiology. 1983 Oct 1,;55(4):1219-24.

67. Widrick JJ. Time course of glycogen accumulation after eccentric exercise. Journal of Applied Physiology. 1992 May 1,;72(5):1999-2004.

68. Crane JD, Ogborn DI, Cupido C, Melov S, Hubbard A, Bourgeois JM, et al. Massage therapy attenuates inflammatory signaling after exercise-induced muscle damage

. Science Translational Medicine. 2012;4(119).

69. Keck N, Cuddy J, Hailes W, Dumke C, Ruby B. Effects of Commercially Available Pneumatic Compression on Muscle Glycogen Recovery After Exercise. Journal of Strength and Conditioning Research. 2015 Feb;29(2):379-85.

70. Peters Futre EM, Noakes TD, Raine RI, Terblanche SE. Muscle glycogen repletion during active postexercise recovery . American Journal of Physiology. 1987;253.

71. Bonen A. Mild exercise impedes glycogen repletion in muscle. Journal of Applied Physiology. 1985 May 1,;58(5):1622-9.

72. Choi 1994 Effect of passive and active recovery on resynthesis of muscle glycogen.

73. Menzies P, Menzies C, McIntyre L, Paterson P, Wilson J, Kemi OJ. Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. Journal of Sports Sciences. 2010 Jul 1,;28(9):975-82.

74. Lane J. The next chapter in sports nutrition: the category continues to wield enormous power in the dietary supplement market, so where will it go from here? The sky's the limit. Nutraceuticals World. 2012 May 1,;15(4):34.

75. Riddell MC, Partington SL, Stupka N, Armstrong D, Rennie C, Tarnopolsky MA. Substrate utilization during exercise performed with and without glucose ingestion in female and male endurance-trained athletes

. International Journal of Sport Nutrition and Exercise Metabolism. 2003;13:407-21.

76. Roepstorff C, Steffensen CH, Stallknecht B, Kanstrup I, Richter EA, Kiens B. Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. The American Journal of Physiology. 2002 Feb 1,;282(2):E435.

77. Ruby BC, Coggan AR, Zderic TW. Gender differences in glucose kinetics and substrate oxidation during exercise near the lactate threshold. Journal of Applied Physiology. 2002 Mar 1,;92(3):1125-32.

78. Tarnopolsky MA, Atkinson SA, Phillips SM, MacDougall JD. Carbohydrate loading and metabolism during exercise in men and women. Journal of Applied Physiology. 1995 Apr 1,;78(4):1360-8.

79. James AP, Lorraine M, Cullen D, Goodman C, Dawson B, Palmer TN, et al. Muscle glycogen supercompensation: absence of a gender-related difference . European Journal of Applied Physiology. 2001;86:533-8.

80. Paul DR, Mulroy SM, Horner JA, Jacobs KA, Lamb DR. Carbohydrate-loading during the follicular phase of the menstrual cycle: effects on muscle glycogen and exercise performance

. International Journal of Sports Nutrition and Exercise Metabolism. 2001;22:430-41.

81. Tarnopolsky MA, Zawada C, Richmond LB, Carter S, Shearer J, Graham T, et al. Gender differences in carbohydrate loading are related to energy intake. Journal of Applied Physiology. 2001 Jul 1,;91(1):225-30.

82. Walker JL, Heigenhauser JF, Hultman E, Spriet LL. Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. Journal of Applied Physiology. 2000 Jun 1,;88(6):2151-8.

83. Goldman HI, Becklake MR. Respiratory function tests; normal values at median altitudes and the prediction of normal results

. American Journal of Tuberculosis. 1959;79(4):457-67.

84. Siri WE. Body composition from fluid spaces and density: analysis of methods . Nutrition. 1993;9(5):480-91.

85. Kissileff HR, Carretta JC, Geliebter A, Pi-Sunyer FX. Cholecystokinin and stomach distension combine to reduce food intake in humans

. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2003;285(5):R998.

86. Bergstrom J. Percutaneous needle biopsy in physiological and clinical research . Scandinavian Journal of Clinical Lab Investigations. 1975;35(7):609-16.

87. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size

. Medicine and Science in Sports and Exercise. 1982;14(1):101-2.

## Manuscript

Males and females exhibit similar muscle glycogen recovery across varied diets

Shannon Flynn\*, \*\*

Alejandro Rosales\*, \*\*

Walter Hailes\*\*

Brent Ruby, FACSM\*, \*\* 32 Campus Drive, McGill Hall – HHP Missoula, MT 59812 <u>brent.ruby@mso.umt.edu</u> 406-243-2117

\*Health and Human Performance Department The University of Montana, Missoula, MT

\*\*WPEM: Montana Center for Work Physiology and Exercise Metabolism The University of Montana, Missoula, MT

#### Abstract

#### Purpose

Research has elucidated the impact of post exercise carbohydrate nutrition and environmental conditions on muscle glycogen re-synthesis. However, research has minimally considered the implications of glycogen recovery in females and has focused on commercial sport nutrition products. The purpose of this study was to determine the effects of varied mixed macronutrient feedings on glycogen recovery and subsequent exercise performance in both sexes.

#### Methods

8 males and 8 females participated in a crossover study. Subjects completed a 90-minute cycling glycogen depletion trial then rested for 4 hours. Two carbohydrate feedings  $(1.6 \text{ g} \text{ kg}^{-1})$  of either sport supplements or potato-based products were delivered at 0 and 2 hours post exercise. Muscle biopsies and blood samples (glucose, insulin) were collected during the recovery. Afterwards, subjects completed a 20km cycling time trial. *Results* 

There was no difference between sexes or trials for glycogen recovery rates (male:  $7.9 \pm 2.7$ , female:  $8.2 \pm 2.7$ , potato-based:  $8.0 \pm 2.5$ , sport supplement:  $8.1 \pm 3.1$  mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>, p > 0.05). Time trial performance was not different between diets ( $38.3 \pm 4.4$  and  $37.8 \pm 3.9$  minutes for potato and sport supplement, respectively, p > 0.05). *Conclusions* 

These results indicate that food items, such as potato-based products, can be as effective as commercially marketed sports supplements when developing glycogen recovery oriented menus and that carbohydrate dose feedings ( $g^{+}kg^{-1}$ ) can be applied to both males and females.

Key Words: glycogen re-synthesis, post-exercise recovery, sex differences, sports supplements

#### Introduction

Endurance exercise performance and time to exhaustion has historically been linked to initial levels of glycogen storage within the skeletal muscle (1, 2). Additionally, given the expected depletion rates of glycogen and the finite storage capacity within the muscle, sport nutrition guidelines have developed evidence-based strategies to optimize glycogen recovery after fuel-depleting training or competition. Most commonly, these recommendations suggest a systematic increase in dietary carbohydrate (3-5) or mixed diet sources (6-9) in a timely fashion post glycogen compromising or depleting exercise. Collectively, the underlying rates of glycogen re-synthesis are influenced by the extent of glycogen depletion (10, 11) the timing of ingestion (5), dose and amount (3, 4, 6, 8), and the composition as either varied carbohydrate source (3-5, 12-14) or in combination with amino acid blends or protein (6-9, 15).

When compared to sport nutrition products, food items have demonstrated success in promoting 4-hour glycogen re-synthesis rates. Both chocolate milk (21-24) and fast-food items (25) have been shown to either restore glycogen or impact subsequent exercise performance similarly to common commercial sport nutrition products.

Overall, these data demonstrate that a variety of dietary strategies can be effective when appropriate macronutrients are provided.

The overall recommendations for optimal re-synthesis appear to be uniformly applied to both men and women despite a relatively small amount of research including female participants. When comparing women to men, research provides conflicting results regarding both the impacts of glycogen supercompensation (or pre-loading) (38-41) and substrate use during endurance exercise (33-37). While some of these investigations illustrate similarities and differences between males and females, few directly report post exercise glycogen resynthesis rates for both sexes (7). Glycogen is restored similarly for men and women post-exercise if adequate substrate is provided, but only liquid supplements have been examined and subsequent exercise performance has not been evaluated after post-exercise carbohydrate dosing (7).

The purpose of the present investigation was to compare glycogen recovery and subsequent exercise performance in males and females following ingestion of either a potato-derived mixed macronutrient diet or commercial sport nutrition oriented products. Key dependent measures included rates of muscle glycogen resynthesis and a 20 km cycling time trial. We hypothesized that there would be minimal differences in glycogen resynthesis or exercise performance between the two recovery diets with a similar distribution of macronutrients. We further hypothesized that males and females would demonstrate similar rates of glycogen re-synthesis when total exogenous carbohydrate was provided proportionate to total body weight (1.6 g kg<sup>-1</sup> for each feeding).

## Methods

## **Participants**

Recreationally active athletes (n=16; n=8 males and n=8 females) completed the study (n=16). The participants were healthy, injury-free, and familiar with endurance exercise (refer to Table 2 for descriptive statistics). All subjects completed a Physical Readiness Questionnaire (PAR-Q) and gave written informed consent prior to the study. All procedures were approved by the university's review board.

## **Preliminary Testing**

Each subject fasted for four hours and abstained from exercise, alcohol, and caffeine for 24 hours prior to an initial visit. After measuring body mass and height, body composition was obtained via hydrodensitometry with corrections for estimated residual lung volume (42). Underwater body mass was obtained using an electronic scale (Exertech, Dreshbach, MN) and used to calculate body density. Sex-specific equations were utilized to convert body density to percent body fat (43).

Using a cycle ergometer (Velotron, RacerMate Inc., Seattle, WA), peak oxygen uptake (VO<sub>2 Peak</sub>) and maximal power output ( $W_{Max}$ ) were determined for each subject during a graded exercise protocol. The protocol started at 95W for men and 60W for women, and it increased 35W every three minutes until volitional fatigue and the achievement of at least two of the following specific max test criteria: respiratory exchange ratio (RER) >1.10, plateau in VO<sub>2</sub>, heart rate (HR) within 10 beats per minute (BPM) of age-predicted max, or rating of perceived exertion (RPE) >17. During the test, gas exchange was measured using a calibrated metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). VO<sub>2 Peak</sub> was calculated as the highest 15-second average oxygen uptake, and  $W_{Max}$  was calculated using the following equation:  $W_{Max} =$  (power output in W during last completed stage) + ((final stage time before fatigue in s/180s) x 35 W).

On a second and third visit, each participant completed a practice (PTT) 20km time trial (TT) on a computer-simulated course, for a total of two PTT. Subjects were directed to complete the distance as quickly as possible, and they were able to change speed and shift gears electronically while distance and time were recorded using RacerMate Inc. software (RacerMate, Inc., Seattle, WA). These sessions ensured TT competency.

#### **Experimental Design**

Subjects completed two trials in a crossover design with seven days between, and they were randomly assigned one of two specific diets for each trial. Prior to each laboratory visit, participants refrained from exercise, alcohol, and caffeine for 24 hours, and they arrived each morning following a 12-hr fast. For each trial, participants completed a glycogen depletion ride followed by a four-hour recovery period then a 20 km TT. During the recovery, they consumed one of the two mixed macronutrient diets (sports supplement products = SS, potato-based foods = PB, refer to Table 1). To maintain a similar carbohydrate dose for all subjects (approximately 1.6 g kg<sup>-1</sup> each feeding), one of four unique serving sizes was assigned to each participant based on body mass ranges from preliminary testing. Throughout the rest period, the subjects filled out gastrointestinal discomfort and meal satisfaction questionnaires, and muscle biopsies and blood samples were collected. Figure 1 illustrates the experimental design.

Sports Supplement					
0 hours	Calorie s	Fat (g)	Carbohydrate (g)	Protein (g)	Sodium (mg)
Rehydrate Salt Tablet	0	0	0	0	800
Powerade Mountain Berry Blast (591 mL)	130	0	35	0	250
Lara Bar Peanut Butter Chocolate Chip	440	22	52	12	120
Gatorade Prime Energy Chews Fruit Punch (Pack=6 Chews)	83	0	20	0	58
Total 2 hours	653	22	107	12	1228
Rehydrate Salt Tablet	0	0	0	0	800
Gatorade Lemon-Lime (591 mL)	140	0	36	0	270
Cliff Mojo Bar Mountain Mix	400	18	46	20	480
Cliff Bloks Energy Chews Mountain Berry (Pack=6 Bloks)	117	0	28	0	58.5
Total	657	18	110	20	1608.5
4 Hour Total	1310	40	217	32	2836.5
Potato Based					
0 hours	Calorie s	Fat (g)	Carbohydrate (g)	Protein (g)	Sodium (mg
Kraft Pancake Syrup	105	0	28	0	0
Simplot Old European Potato Pancakes	240	10	34	4	620
Mott's Unsweetened Applesauce	40	0	10	0	0
Great Value Hash Brown	300	16	36	4	540
Total 2 hours	685	26	108	8	1160
Delallo Potato Gnocchi (140 g)	345	0	75	9	840
Prego Traditional Pasta Sauce (120 mL)	70	1.5	13	2	480
Great Value Seasoned Fries (84 g)	175.5	9.4	21	2.3	410
Total	590.5	10.9	109	13.3	1730
4 Hour Total	1275.5	36.9	217	21.3	2890

# Table 1. Bolus feeding protocol for 0 hr and 2 hr post-exercise recovery for body mass of 62-74 kg

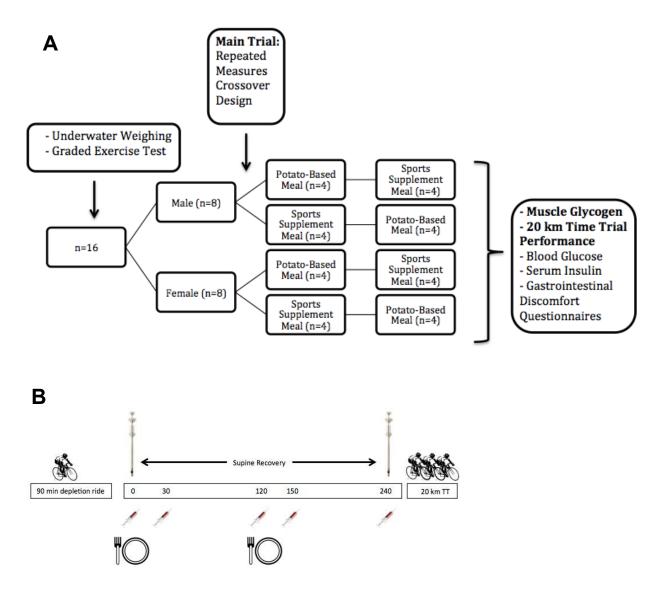


Figure 1. A Study design with dependent measures included (primary measures in bold) B Main experimental trial design

Glycogen Depletion Protocol during Main Experimental Trials

Subjects completed a glycogen-depleting exercise via cycle ergometer (90 minutes). The protocol entailed a 10-min warm up at 55%  $W_{Max}$ , a series of 10 intervals (2-min at 80%  $W_{Max}$  followed by 4-min at 50%  $W_{Max}$ ) lasting 60-min, 8-min at 60%  $W_{Max}$ , and finally 12-min at 50%  $W_{Max}$ . Water was consumed ad libitum during the exercise. Following the protocol, participants rested in a seated or reclined position for the 4-hr recovery period. *Feeding Strategy* 

At 0-hr and 2-hr of recovery, participants consumed one of two mixed macronutrient diets with equivalent macronutrient distribution (SS or PB). Nutritional label serving sizes were utilized, and items were weighed for

accuracy. Each subject consumed 1.6 g carbohydrate kg body weight <sup>-1</sup>, and fat and protein content was also aligned between trials. Table 2 provides details regarding specific menu items and feeding schedules.

## Questionnaires

Subjects completed gastrointestinal discomfort questionnaires at 0, 1, 2, 3, and 4-hrs of recovery and postmeal questionnaires at 0 and 2-hrs of recovery. The gastrointestinal questionnaire gauged hunger, fullness, sickness, and stomach discomfort, and the post-meal questionnaire evaluated meal satisfaction, taste, and acceptability. Both involved a 150 mm visual analog scale (VAS) with "Not at all" on the left edge and "Extremely" on the right edge of the continuum, and participants placed an "X" along the scale for each question. The distance from "Not at all" in mm was divided by 150 mm and reported as a score (44).

## 20 km Time Trial

After the supine recovery, subjects completed a 20 km TT on the cycle ergometer described above and with the same ambient conditions each time. As with the PTT, participants were directed to complete the distance as rapidly as possible and were provided no encouragement or entertainment. They were blinded to time and intensity. Distance and time were recorded similarly to the PTT. Subjects did not consume water during the TTs.

## **Tissue and Blood Sampling and Analysis**

## Muscle Biopsies

At 0 and 4-hrs recovery post glycogen-depleting exercise, muscle biopsies of the vastus lateralis were performed using the percutaneous biopsy needle technique and suction (45, 46). Each 4-hr biopsy was performed 2 cm proximal to the first location, and each trial utilized a different leg in randomized order. Before the biopsy, 1 mL of 1% lidocaine (without adrenaline) was injected beneath the skin as a local anesthetic. This numbed an initial 2 cm<sup>2</sup> area, then another 2-3 mL of lidocaine were injected near the fascia. After the injection, an approximately 0.5 cm incision was made through the skin and muscle fascia, and a Bergstrom biopsy needle was inserted through the opening into the belly of the vastus lateralis muscle. The process removed 30 mg of tissue, and extra blood, fat, and connective tissue were subsequently separated from the muscle sample. The remaining tissue was immediately frozen in liquid nitrogen and stored at -80°C until muscle glycogen analysis.

## Blood Sampling

Venipuncture technique was used to collect blood samples from an antecubital arm vein at 0, 30, 120, 150, and 240 min of recovery. Samples were allowed to clot then spun in a 4°C refrigerated centrifuge (Jouan Inc.,

MR22i) at 4000 rpm for 15 min. Aliquots of serum were prepared, and the tubes were stored at -30°C until glucose and insulin analyses.

## Analysis

An enzymatic spectrophotometric method was used to analyze duplicate muscle samples (10-12 mg, collected at same time) for muscle glycogen concentration (47). After weighing, samples were added to 0.5 mL of 2N HCl solution. This solution was weighed, incubated for two hours in a 100°C oven, reweighed, reconstituted with distilled water to the original sample weight, and pH normalized using 1.5 mL of 0.67 M NaOH. Next, 100 mL muscle extract solution was added to 1 mL Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.). After reading the solution at 340 nm on a spectrophotometer, muscle glycogen concentration was calculated using a standard curve. The concentration was expressed as mM kg wet wt<sup>-1</sup> hr<sup>-1</sup> of muscle.

Similarly, blood samples were analyzed for glucose in triplicate with Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read at 340 nm on a spectrophotometer. A standard curve was used to calculate blood glucose concentration. Enzymatic spectrophotometric ELISA method was used to analyze insulin from blood samples in duplicate (EIA-2935, DRG International). Mean intra-assay coefficients of variation for muscle samples, glucose, and insulin using these techniques are less than 5%.

## **Statistical Analysis**

Sex-specific demographics were analyzed via two-tailed independent *t* tests (Microsoft Excel, Microsoft Corp., Redmond, WA). Multiple two-way ANOVAs (sex x trial order and sex x trial) with repeated measures were used to analyze PTT and TT performance times (SPSS Inc., Chicago, IL). Muscle glycogen recovery rates were compared using a two-way ANOVA (sex x trial) with repeated measures. A three-way ANOVA (sex x trial x time) with repeated measures was used to examine differences in muscle glycogen, blood glucose, and serum insulin levels between males and females (SPSS Inc., Chicago, IL). Gastrointestinal discomfort and meal satisfaction were compared using a three-way ANOVA (sex x trial x time) with repeated measures. Statistical significance for all analyses was set at a probability of type I error less than 5% (p<0.05). Data in text are expressed as mean  $\pm$  SD. Graphical data are expressed as mean  $\pm/$ - SEM.

## Results

# Subject Descriptive Data

Sixteen subjects completed the study (8 males, 8 females). Descriptive data is presented in Table 2. There was a significant difference between men and women for weight, fat free mass (FFM), and  $VO_{2 Peak}$ . However, there was no difference between males and females for age, body fat percentage, or  $VO_{2 Peak}$  when calculated using FFM.

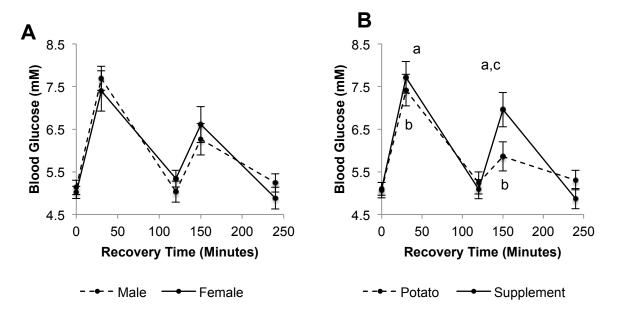
## **Table 2. Descriptive statistics**

Characteristic	Men (n=8)	Women (n=8)	Significance (p value)
Age (yr)	$27.1 \pm 6.8$	$25.1 \pm 4.8$	<i>p</i> >0.05
Weight (kg)	$70.9 \pm 8.3$	$59.2 \pm 4.8$	p < 0.05
Fat Free Mass (kg)	$62.9 \pm 5.7$	$48.4 \pm 4.9$	p < 0.05
Body Fat (%)	$14.6 \pm 4.2$	$18.3 \pm 5.8$	<i>p</i> >0.05
VO <sub>2 Peak</sub> (mL kg <sup>-1</sup> min <sup>-1</sup> )	$56.7 \pm 4.2$	$46.5 \pm 6.6$	<i>p</i> <0.05
VO <sub>2 Peak</sub> (mL kg FFM <sup>-1</sup> min <sup>-1</sup> )	$63.8 \pm 5.0$	$57.2 \pm 6.3$	<i>p</i> >0.05

Values are means  $\pm$  SD, NS=not significant, p values by independent t-test

## Blood Glucose

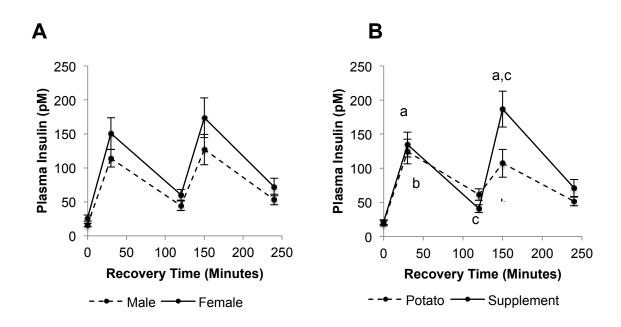
There was a time x trial interaction for blood glucose (Figure 2, p < 0.05). It was elevated at 30 and 150 min (30 minutes post each feeding) for both PB and SS trials (p < 0.05). At 150 min, blood glucose was significantly higher during the SS trial than the PB trial (p < 0.05). There was no sex-difference in blood glucose at 0, 30, 120, 150, or 240 min post-exercise (p > 0.05) and no difference in blood glucose between the diets at 0, 30, 120, or 240 min post-exercise (p > 0.05).



**Figure 2.** Blood glucose concentration during recovery. **A** - Between sexes (n=8 males, n=8 females). **B** - Time x Trial interaction (p<0.05, n=16 potato, n=16 supplement). **a** p<0.05 from 0-minutes recovery (supplement) **b** p<0.05 from 0-minutes recovery (potato) **c** p<0.05 supplement from potato. Values are mean ± *SEM*.

# Serum Insulin

There was a time x trial interaction for serum insulin (Figure 3, p<0.05). It was elevated at 30 and 150 min due to meal consumption for both PB and SS trials (p<0.05). The PB treatment demonstrated higher insulin at 120 min (p<0.05), while the SS treatment noted higher insulin at 150 min (p<0.05). There was no sex-difference in serum insulin at 0, 30, 120, 150, or 240 min post-exercise (p>0.05) and no difference in insulin between the diets at 0, 30, or 240 min post-exercise (p>0.05).



**Figure 3.** Plasma insulin concentration during recovery. **A** - Between sexes (n=8 males, n=8 females). **B** - Time x Trial interaction (p<0.05, n=16 potato, n=16 supplement). **a** p<0.05 from 0-minutes recovery (supplement) **b** p<0.05 from 0-minutes recovery (potato) **c** p<0.05 supplement from potato. Values are mean ± *SEM*.

# Muscle Glycogen

There was a main effect for time with muscle glycogen increasing over the recovery period (Figure 4, p < 0.05). There was no difference in muscle glycogen between males and females at 0 hours of recovery or 4 hours of recovery (p > 0.05). Correspondingly, there was no difference between males and females in the rate of glycogen recovery (Male:  $7.9 \pm 2.7$ , Female:  $8.2 \pm 2.7$  mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>, p > 0.05). There was also no difference between SS and PB at 0 hours of recovery or 4 hours of recovery (p > 0.05) and no difference between SS and PB in the rate of glycogen recovery (PB:  $8.0 \pm 2.5$ , SS:  $8.1 \pm 3.1$  mM kg wet wt<sup>-1</sup> hr<sup>-1</sup>, p > 0.05).

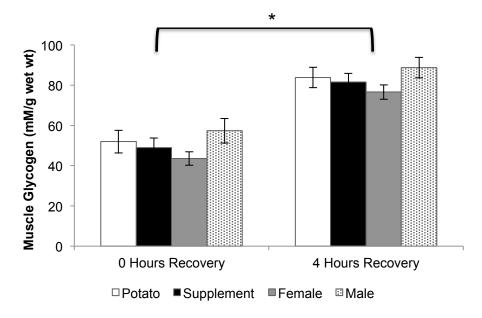


Figure 4. Muscle glycogen concentration during recovery. \*p<0.05 main effect for time from 0 hour recovery. Values are mean  $\pm$  *SEM*.

# Questionnaire

For hunger, there was a main effect for time (p < 0.05) and sex (p < 0.05). Participants rated hunger higher at 0 and 2 hours of recovery, which was before each meal (0 hr:  $0.62 \pm 0.05$ , 1 hr:  $0.45 \pm 0.04$ , 2 hr:  $0.55 \pm 0.04$ , 3 hr:  $0.32 \pm 0.04$ , 4 hr:  $0.31 \pm 0.04$  VAS Scores). Males rated hunger higher than females (Male:  $0.53 \pm 0.05$ , Female:  $0.36 \pm 0.05$ ). There was a trial x sex interaction for stomach discomfort (p < 0.05). Males had less stomach discomfort during the potato trial than the supplement trial (PB:  $0.12 \pm 0.04$ , SS:  $0.20 \pm 0.06$ ). Regarding meal satisfaction, there was a main effect for trial for how tasty (p < 0.05), how satisfying (p < 0.05), and how acceptable (p < 0.05) the meals were. The potato based meals were rated more tasty (PB:  $0.65 \pm 0.04$ , SS:  $0.43 \pm 0.04$ ), satisfying (PB:  $0.65 \pm 0.05$ , SS:  $0.45 \pm 0.05$ ), and acceptable (PB:  $0.59 \pm 0.04$ , SS:  $0.47 \pm 0.05$ ) than the sports supplement meals. 20 km Time Trial

There was no interaction or main effect of trial when time trial performance was analyzed for order (Trial 1:  $37.9 \pm 3.8$ , Trial 2:  $37.6 \pm 4.1$ , Trial 3:  $38.2 \pm 3.8$ , Trial 4:  $37.9 \pm 4.4$  minutes, p>0.05) or for practice and experimental trials (PTT1:  $37.9 \pm 3.8$ , PTT2:  $37.6 \pm 4.1$ , PBTT:  $38.3 \pm 4.4$ , SSTT:  $37.8 \pm 3.9$  minutes, p>0.05). This indicates there was no learning effect and no difference between SS and PB. However, there was a main effect for sex, with males completing the time trials significantly faster than females (Male:  $34.7 \pm 1.7$ , Female:  $41.0 \pm 3.0$  minutes, p<0.05).

#### Discussion

This study examined the impact of varied mixed macronutrient diets on glycogen recovery and performance in both males and females. The results demonstrated that glycogen re-synthesis and subsequent exercise performance were not different for potato-based and sport supplement recovery meals. Additionally, our data parallel the reported findings of Tarnopolsky et al. (7) and demonstrate similar rates of post-exercise glycogen recovery in men and women. Collectively, these results suggest that a wide range of dietary choices can elicit effective glycogen recovery for both men and women.

Prior research has recommended that carbohydrate is provided immediately following exercise (5) and that at least 1.0 g kg<sup>-1</sup> hr<sup>-1</sup> CHO is provided each hour during recovery at intervals of 30 minutes or less (8, 48, 49). The current study did provide immediate carbohydrate after exercise, but the dosage was 1.6 g kg<sup>-1</sup> CHO at 0 and 2 hours of recovery. Similar feedings schedules have been demonstrated to effectively stimulate glycogen recovery and lead to a plateau in re-synthesis rates (3, 4, 16, 49). The timing and amount of carbohydrate provided in this study simulate the reality of recovery in many sport and tactical situations where the ideal sources and quantities of nutrients may not be available at all times. Nevertheless, muscle glycogen recovery rates of  $8.0 \pm 2.5$  and  $8.1 \pm 3.1$  mM kg wet wt<sup>-1</sup> hr<sup>-1</sup> for PB and SS respectively were comparable to the rates of 6.5 to 11.7 mM kg wet wt<sup>-1</sup> hr<sup>-1</sup> obtained through prior research using an assortment of exercise, recovery, and feeding conditions (17, 20, 25, 47, 50).

No significant difference was observed between PB and SS feedings for rates of glycogen re-synthesis or exercise performance, demonstrating that diverse diets can similarly enhance glycogen recovery. This finding extends upon a body of research supporting the use of foods as an alternate or additional form of sport nutrition as compared to commercial products. Cramer et al. (25) similarly showed that fast food menu items stimulated glycogen recovery and contributed to exercise performance as effectively as sports supplements. Other research has demonstrated that golden raisins and chocolate milk are as effective as sports supplements for providing carbohydrate during (51) and after (21-24) exercise respectively. While one of these studies did measure muscle glycogen and another looked at time trial performance, most of the investigations only examined time to exhaustion (TTE). This methodology does provide a significant limitation of the previous research on foods. Importantly, during the current study, the PB meals were rated as more tasty and satisfying than the SS meals, and the PB meals led to less sickness and stomach discomfort for males. These findings indicate that it could be easier and more

agreeable for athletes to consume foods than commercial products. Additionally, potatoes are high in nutrients (26-29), low in cost (30), and low in simple sugar (31, 32).

Similar to other studies using a variety of feeding strategies (4, 5, 9), blood glucose and insulin rose quickly following the feedings at 0 hours and 2 hours of recovery then returned to baseline. While there was no difference in response between PB and SS at 30 minutes after the 0 hour feeding, there was a difference at 30 minutes after the 2 hour feeding with PB eliciting a significantly lower glucose and insulin response. This disparity could result from differences in gastric emptying between the SS and PB meals for the 2-hour feeding, since quicker gastric emptying can lead to quicker glucose absorption in the small intestine (52). Gastric emptying is enhanced with decreased fat (53) and fiber (54) content and with liquids as compared to solids (55). Although the SS and PB meals at both feedings were matched for fat and protein content, both PB meals had higher fiber content and both SS meals had a higher portion of calories in liquid form. The lack of difference in glucose and insulin response following the 0 hour feeding could indicate that gastric emptying has varying levels of influence based on timing. For example, directly following exercise, muscle glycogen is most depleted so gastric emptying may have less impact than after the 2 hour feeding when glycogen re-synthesis has already commenced. Regardless, the differences in glucose and insulin in the current study did not impact the overall rate of glycogen re-synthesis, showing that variances in carbohydrate digestion and absorption had limited effect on delivery of carbohydrate to the muscle.

The similarity in re-synthesis rates between men and women demonstrates that a variety of diets can be utilized for glycogen recovery in both males and females. This corroborates the findings of Tarnopolsky et. al (7) who observed comparable rates of post exercise glycogen re-synthesis in similarly trained males and females (25.5 and 23.5 mM kg wet wt<sup>-1</sup> hr<sup>-1</sup> respectively) when providing a solution of carbohydrate, protein, and fat. Like Tarnopolsky et al. (7) , the current study found no significant difference between men and women in VO<sub>2 Peak</sub> when calculated using FFM. However, fitness in this study was lower for both sexes, supporting the application of recovery recommendations across varied training statuses. The lack of sex differences in the study also align with findings that when men and women are matched for FFM and fitness, they use substrate similarly during exercise (36, 37) and that females can supercompensate (or pre-load) their glycogen stores as effectively as males (34, 38-40). These results are logical when considering that GLUT-4, hexokinase, and glycogen synthase levels and

activities are similar between men and women (56), which would contribute to limited differences in muscular glucose delivery and storage across sex.

These findings regarding muscle glycogen re-synthesis and exercise performance can be applied to both male and female sport and tactical athletes who require glycogen restoration after extended periods of work or between multiple bouts of work in a single day. As long as over 1.6 g kg<sup>-1</sup> of carbohydrate is provided at 0 and 2 hours of recovery, the source of macronutrients may be diverse and could come from foods, including potatoes. It is important to note that feeding during exercise may alter these requirements, as carbohydrate provision while exercising can impact glycogen depletion (57) and re-synthesis (16). Additionally, consideration of other factors during recovery is critical. Recovering in room temperature ambient conditions (19, 20) and with warm muscles (17, 18) will enhance glycogen re-synthesis as demonstrated by prior research on males. These elements have not yet been investigated in women so the question of environmental impacts on female glycogen recovery could provide a compelling avenue of future research.

## Acknowledgements

The authors are grateful to the participants for their time and investment in the investigation. They also thank Tiffany Quindry for her assistance and the sharing of her lab and equipment.

## **Authors' Contributions**

SKF participated in design, organization, data acquisition, and data interpretation. SKF also participated in muscle glycogen, blood, questionnaire, TT, and statistical analysis and wrote the manuscript. AMR participated in design, organization, data acquisition, and data interpretation. AMR also participated in muscle glycogen, blood, questionnaire, TT, and statistical analysis. WSH participated in conception, design, organization, data acquisition, and data interpretation. WSH also participated in muscle glycogen, blood, and statistical analysis. BCR participated in conception, design, data acquisition, data analysis, and data interpretation 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**

This study was funded by the Alliance for Potato Research and Education. The authors declare no competing interests in access to these data. They also declare no associations with companies involved with the products used in the investigation.

#### References

1. Ahlborg B, Bergstrom J, Edelund L, Hultman E. Muscle glycogen and muscle electrolytes during prolonged physical exercise

. Acta Physiologica. 1967 June;70(2):129-42.

2. Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen, and physical performance . Acta Physiologica. 1967 October;71(2-3):140-50.

3. Blom P, Hostmark AT, Vaage O, Kardel KR, Maehlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. Medicine and Science in Sports and Exercise. 1987;19(5):491-6.

4. Ivy JL, Lee MC, Brozinick JJ, Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. Journal of Applied Physiology. 1988 Nov 1,;65(5):2018-23.

5. Ivy JL, Katz AL, Cutler CL, Sherman WM, Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. Journal of Applied Physiology. 1988 Apr 1,;64(4):1480-5.

6. Roy L. P. G. Jentjens, Luc J. C. van Loon, Christopher H. Mann, Anton J. M. Wagenmakers, Asker E. Jeukendrup. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. Journal of Applied Physiology. 2001 Aug 1,;91(2):839-46.

7. Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, Roy BD. Postexercise proteincarbohydrate and carbohydrate supplements increase muscle glycogen in men and women. Journal of Applied Physiology. 1997 Dec 1,;83(6):1877-83.

8. van Loon, L. J. C, Saris WHM, Kruijshoop M, Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. American Journal of Clinical Nutrition. 2000 Jan 1,;72(1):106-11.

9. Ivy JL, Goforth HJ, Damon BM, McCauley TR, Parson EC, Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. Journal of Applied Physiology. 2002 Oct 1,;93(4):1337-44.

10. Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. Journal of Applied Physiology. 1994;76(1):104-11.

11. Zachwieja JJ, Costill DL, Pascoe DD, Robergs RA, Fink WJ. Influence of muscle glycogen depletion on the rate of resynthesis. Medicine and Science in Sports and Exercise. 1991;23(1):44-8.

12. Reed MJ, Brozinick JJ, Lee MC, Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. Journal of Applied Physiology. 1989 Feb 1,;66(2):720-6.

13. Burke LM, Collier GR, Hargreaves M. Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. Journal of Applied Physiology. 1993 Aug 1,;75(2):1019-23.

14. Kions B, Raben AB, Valeur AK. Benefit of dietary simple carbohydrates on the early post-exercise muscle glycogen repletion in male athletes. Medicine and Science in Sports and Exercise. 1990;22(4):S88.

15. Zawadski KM, Yaspelkis BB, Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. Journal of Applied Physiology. 1992 May 1,;72(5):1854-9.

16. Reinert A, Slivka D, Cuddy J, Ruby B. Glycogen synthesis after road cycling in the fed state . . 2009;30:545-9.

17. Slivka D, Tucker T, Cuddy J, Hailes W, Ruby B. Local heat application enhances glycogenesis. Applied Physiology, Nutrition, and Metabolism. 2012 Apr;37(2):247-51.

18. Tucker TJ, Slivka DR, Cuddy JS, Hailes WS, Ruby BC. Effect of local cold application on glycogen recovery . Journal of Sports Medicine and Physical Fitness. 2012 April;52(2):158-64.

19. Slivka D, Heesch M, Dumke C, Cuddy J, Hailes W, Ruby B. Effects of post-exercise recovery in a cold environment on muscle glycogen, PGC-1α, and downstream transcription factors. Cryobiology. 2013 Jun;66(3):250-5.

20. Naperalsky M, Ruby B, Slivka D. Environmental temperature and glycogen resynthesis . International Journal of Sports Medicine. 2010;31:561-6.

21. Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, Stager JM. Chocolate milk as a postexercise recovery aid. International journal of sport nutrition and exercise metabolism. 2006 Feb;16(1):78.

22. Lunn WR, Pasiakos SM, Colletto MR, Karfonta KE, Carbone JW, Anderson JM, et al. Chocolate milk and endurance exercise recovery: protein balance, glycogen, and performance. Medicine and science in sports and exercise. 2012 Apr;44(4):682-91.

23. Pritchett K, Bishop P, Pritchett R, Green M, Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Applied Physiology, Nutrition, and Metabolism. 2009 Dec 1,;34(6):1017-22.

24. Thomas K, Morris P, Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Applied Physiology, Nutrition, and Metabolism. 2009 Feb 1,;34(1):78-82.

25. Cramer MJ, Dumke CL, Hailes WS, Cuddy JS, Ruby BC. Postexercise glycogen recovery and exercise performance is not significantly different between fast food and sport supplements. International Journal of Sport Nutrition and Exercise Metabolism. 2015 Oct 1,;25(5):448.

26. Camire ME, Kubow S, Donnelly DJ. Potatoes and Human Health. Critical Reviews in Food Science and Nutrition. 2009 Dec 10,;49(10):823-40.

27. Carlson JJ, Eisenmann JC, Norman GJ, Ortiz KA, Young PC. Dietary Fiber and Nutrient Density Are Inversely Associated with the Metabolic Syndrome in US Adolescents. Journal of the American Dietetic Association. 2011;111(11):1688-95.

28. Kanter M. High-Quality Carbohydrates and Physical Performance: Expert Panel Report. Nutrition Today. 2018 Jan;53(1):35-9.

29. Zaheer K, Akhtar MH. Potato Production, Usage, and Nutrition-A Review. Critical Reviews in Food Science and Nutrition. 2016 Apr 3,;56(5):711-21.

30. Drewnowski A, PhD. New Metrics of Affordable Nutrition: Which Vegetables Provide Most Nutrients for Least Cost? Journal of the Academy of Nutrition and Dietetics. 2013;113(9):1182-7.

31. Ng, Shu Wen, PhD|Slining, Meghan M., PhD|Popkin, Barry M., PhD. Use of Caloric and Noncaloric Sweeteners in US Consumer Packaged Foods, 2005-2009. Journal of the Academy of Nutrition and Dietetics. 2012;112(11):1834.e6.

32. Zytnick D, Park S, Onufrak SJ, Kingsley BS, Sherry B. Knowledge of Sugar Content of Sports Drinks is Not Associated with Sports Drink Consumption. American Journal of Health Promotion. 2015 Nov;30(2):101-8.

33. Devries MC. Sex-based differences in endurance exercise muscle metabolism: impact on exercise and nutritional strategies to optimize health and performance in women. Experimental Physiology. 2016 Feb 1,;101(2):243-9.

34. Tarnopolsky M, Ruby B. Sex differences in carbohydrate metabolism. Current Opinion in Clinical Nutrition and Metabolic Care. 2001 Nov;4(6):521-6.

35. Riddell MC, Partington SL, Stupka N, Armstrong D, Rennie C, Tarnopolsky MA. Substrate utilization during exercise performed with and without glucose ingestion in female and male endurance-trained athletes . International Journal of Sport Nutrition and Exercise Metabolism. 2003;13:407-21.

36. Ruby BC, Coggan AR, Zderic TW. Gender differences in glucose kinetics and substrate oxidation during exercise near the lactate threshold. Journal of Applied Physiology. 2002 Mar 1,;92(3):1125-32.

37. Roepstorff C, Steffensen CH, Stallknecht B, Kanstrup I, Richter EA, Kiens B. Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. The American Journal of Physiology. 2002 Feb 1,;282(2):E435.

 James AP, Lorraine M, Cullen D, Goodman C, Dawson B, Palmer TN, et al. Muscle glycogen supercompensation: absence of a gender-related difference
European Journal of Applied Physiology. 2001;86:533-8.

39. Paul DR, Mulroy SM, Horner JA, Jacobs KA, Lamb DR. Carbohydrate-loading during the follicular phase of the menstrual cycle: effects on muscle glycogen and exercise performance . International Journal of Sports Nutrition and Exercise Metabolism. 2001;22:430-41.

40. Walker JL, Heigenhauser JF, Hultman E, Spriet LL. Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. Journal of Applied Physiology. 2000 Jun 1,;88(6):2151-8.

41. Tarnopolsky MA, Zawada C, Richmond LB, Carter S, Shearer J, Graham T, et al. Gender differences in carbohydrate loading are related to energy intake. Journal of Applied Physiology. 2001 Jul 1,;91(1):225-30.

42. Goldman HI, Becklake MR. Respiratory function tests; normal values at median altitudes and the prediction of normal results

. American Journal of Tuberculosis. 1959;79(4):457-67.

43. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription . Ninth ed. Lippincott Williams & Wilkins; 2013.

44. Kissileff HR, Carretta JC, Geliebter A, Pi-Sunyer FX. Cholecystokinin and stomach distension combine to reduce food intake in humans

. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2003;285(5):R998.

45. Bergstrom J. Percutaneous needle biopsy in physiological and clinical research . Scandinavian Journal of Clinical Lab Investigations. 1975;35(7):609-16.

46. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size . Medicine and Science in Sports and Exercise. 1982;14(1):101-2.

47. Ruby BC, Gaskill SE, Slivka D, Harger SG. Addition of a fenugreek extract to glucose feedings increases muscle glycogen resynthesis after exercise . Amino Acids. 2005;28(Supplement):71-6.

48. Burke LM, van Loon L, Hawley JA. Post-exercise muscle glycogen resynthesis in humans. Journal of Applied Physiology. 2017;122:1055-67.

49. Jentjens R, Jeukendrup AE. Determinants of Post-Exercise Glycogen Synthesis During Short-Term Recovery. Sports Med. 2003;33(2):117-44.

50. Keck N, Cuddy J, Hailes W, Dumke C, Ruby B. Effects of Commercially Available Pneumatic Compression on Muscle Glycogen Recovery After Exercise. Journal of Strength and Conditioning Research. 2015 Feb;29(2):379-85.

51. Rietschier H, Henagan T, Earnest C, Baker B, Cortez C, Stewart L. Sun-Dried Raisins are a Cost-Effective Alternative to Sports Jelly Beans in Prolonged Cycling. Journal of Strength and Conditioning Research. 2011 Nov;25(11):3150-6.

52. Macdonald IA. Physiological regulation of gastric emptying and glucose absorption . Diabetic Medicine. 1996;13(9).

53. Frost GS, Brynes AE, Dhillo WS, Bloom SR, McBurney MI. The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. European Journal of Clinical Nutrition. 2003 Feb;57(2):293-8.

54. Vincent R, Roberts A, Frier M, Perkins AC, MacDonald IA, Spiller RC. Effect of bran particle size on gastric emptying and small bowel transit in humans: a scintigraphic study. Gut. 1995;37:216-9.

55. Hellström PM, Grybäck P, Jacobsson H. The physiology of gastric emptying. Best Practice & Research Clinical Anaesthesiology. 2006;20(3):397-407.

56. Lundsgaard A, Kiens B. Gender differences in skeletal muscle substrate metabolism: molecular mechanisms and insulin sensitivity. Frontiers in endocrinology. 2014;5:195.

57. De Bock K. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. The Journal of Physiology. 2005 Apr 15,;564(2):649-60.