EFFECTS OF CONSUMING A FOOD BAR CONTAINING WHEY PROTEIN AND

ISOMALTO-OLIOSACCHARIDES ON GLUCOSE HOMEOSTASIS, EXERCISE

PERFORMANCE AND RECOVERY

A Dissertation

by

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ABSTRACT

The pharmacokinetic study examined the glucose and insulinemic responses of

ingesting a novel protein bar using plant fiber (isomalto-oligosaccharides, IMO). The

purpose of the study was to determine the glycemic index (GI) and glycemic load (GL)

during a 2 hour oral glucose tolerance test (OGTT). The study was performed in two

parts. First, participants ingested a 25 g food bar (FB) or matched 25 g dextrose (PLA).

Later, ten fasted individuals participated the same experiment while ingesting 2 FB's or

50 g of PLA. OGTT results revealed the FB had a GI of 34 [CI 23, 46] and a GL of 8.5

[CI 5.6, 11.6]. Interestingly, the FB elicited a lower glycemic response with a similar

insulin response compared to the PLA. In response, the Exercise Study examined

whether consuming this FB or 25 g PLA prior to, during, and following intense exercise

would affect exercise performance and/or recovery in twelve resistance-trained males.

Participants performed 11 resistance-exercises followed by sprint conditioning drills for

time. Participants donated blood samples, performed isokinetic strength tests, and rated

perceptions of muscle soreness and hypoglycemia prior to, following exercise and after

48 hours of recovery. Data were analyzed by general linear model repeated measures

and are reported as mean change from baseline with 95% confidence intervals. Results

revealed blood glucose was significantly higher 30-min post-ingestion with PLA (PLA

3.1 [2.0, 4.3], FB 0.8 [0.2, 1.5] mmol/L, p=0.001) while post-exercise ratio of insulin to

glucose was greater with FB (PLA 0.04 [0.00, 0.08], FB 0.11 [0.07, 0.15], p=0.013,

η^2=0.25). Total lifting volume was maintained to a greater degree from Set 1 to Set 3

with FB than PLA (PLA -198.26 [-320.1, -76.4], FB -81.7 [-203.6, 40.1] kg, p=0.175, η^2=0.08). Perceived muscle soreness was lower with FB (PLA 1.88 [0.60, 3.17]; FB 0.29 [-0.99, 1.57] cm, p=0.083, η^2=0.13). No significant differences were observed between treatments in sprint performance, isokinetic strength, markers of catabolism, stress and sex hormones, or inflammatory markers. Results indicate that ingestion of this FB can positively affect glucose homeostasis, sustain exercise performance, and lessen perceptions of muscle soreness after intense training.

DEDICATION

       This thesis work is dedicated to my mother Marjorie, father Stephen, and brothers Brandon and Clark Grubic. This work is also dedicated to my fiancé Becca Hall, and the Hall family; Don, Mary, Brandon, and Mary Marvin-Hall. Thank you for your encouragement, patience, love, support and comedic relief throughout this entire process.

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NOMENCLATURE

FB	Food Bar, Fitjoy^{TM}
PLA	Placebo, Dextrose
ESNL	Exercise and Sport Nutrition Laboratory
FAM	Familiarization Session
T1	Testing Session #1 (A or B supplement)
T1rec	48-h Recovery Session following T1
T2	Testing Session #2 (A or B supplement)
T2rec	48-h Recovery Session following T2
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
HR	Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
DXA	Dual X-ray Absorptiometry
BMD	Bone Mineral Density
LM	Lean Mass
FFM	Fat Free Mass
FM	Fat Mass
GPRS	Graphic Pain Rating Scale
VAS	Visual Analog Scale
DOMS	Delayed Onset Muscle Soreness

VM	Distal Vastus Medalis
DVL	Distal Vastus Lateralis
MLVL	Mid-Lateral Vastus Lateralis
NAD	Nebraska Agility Drill
FYD	Forty-Yard Dash
MVC	Maximal Voluntary Contraction
y	Year
m	Meters
cm	Centimeters
g	Gram
bpm	Beats Per Minute
mmHg	Millimeters of Mercury
1RM	1-Repetition Maximum
Reps	Repetitions
Kg	Kilograms
Sec	Second
mmol	Millimoles, Molar Concentration
L	Liter
mL	Milliliter
dL	Deciliter
µIU	Micro Unit of Insulin
ug	Microgram

ng	Nanogram
pg	Picogram
Nm	Newton Meter
N	Newton
W	Watts
J	Joules
BUN	Blood Urea Nitrogen
CRE	Creatinine
BUN:CRE	BUN:Creatinine Ratio
CK	Creatine Kinase
CPK	Creatine Phosphokinase
UA	Uric Acid
LDH	Lactate Dehydrogenase
TEST	Testosterone
CORT	Cortisol
Cort/Test	Cortisol/Testosterone Ratio
IGR	Insulin/Glucose Ratio
kcal	Calories
TNF-α	Tumor Necrosis Factor Alpha
IFN-γ	Interferon Gamma
IL	Interleukin
NO	Nitric Oxide

WBC	White Blood Cell
LYM	Lymphocyte
MID	Mid-Cell Fraction
GRAN	Granulocytes
RBC	Red Blood Cell
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean Corpuscle Volume
MCH	Mean Corpuscle Hemoglobin
MCHC	Mean Corpuscle Hemoglobin Concentration
PLT	Platelet
MVP	Mean Platelet Volume
RDW	Red Cell Distribution Width
P	Pressure
T	Time
SR	Sarcoplasmic Reticulum
SS	Steady State
CRP	C - Reactive Protein
MPS	Muscle Protein Synthesis
GPRS	Graphic Pain Rating Scale
BCKDH	branched-chain alpha-keto acid dehydrogenase

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Nutrient timing in relation to provision carbohydrates and proteins have been reported to play a key role, and be of relative importance in relation to a training session, energy substrate use, and potential recovery [1]. Nutrient timing involves the purposeful ingestion of nutrients prior to, during, and/or following exercise in an effort to favorably impact adaptive responses to acute and chronic exercise such as muscle strength and power, body composition, substrate utilization, and physical performance [2-4]. Studies have shown that endogenous glycogen stores can be maximized following a high carbohydrate diet (8-12g of CHO/kg/day [g/kg/day]), as these stores are typically depleted during strenuous high volume exercise [1]. Carbohydrates are of maximal importance when the training involves one or more glycogen-depleting bouts in a single day, for example a high volume resistance workout followed or preceded by a high intensity cardiovascular training session. The importance of carbohydrates in maintaining blood glucose significantly increases as the exercise time starts to exceed two hours, especially training that approaches three hours [5, 6]. Carbohydrate ingestion throughout resistance exercise, especially sessions targeting major muscle groups (e.g. squats, deadlifts, pullup, bench press) has been shown to promote euglycemia and attenuate the breakdown of glycogen stores. Consumption of carbohydrate by itself, or in combination with protein during resistance exercise increases muscle glucose

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availability, thus contributing to fuel availability necessary for resistance training and/or cardiovascular training.

Protein timing in relation to resistance training in a fasted state seems to provide evidence for maximal importance if consumed more than three hours prior, especially approaching or exceeding four hours prior to exercise. Research has indicated that meeting the total daily intake of protein through strategically spaced feedings (~every 3-h during the day) should be paramount to diets in athletic populations [7]. Moreover, ingestion of essential amino acids (EAA) in the form of a protein bolus of 20-40g has been shown to simulate muscle protein synthesis in skeletal muscle in humans, and has been suggested to attenuate the breakdown of muscle proteins during exercise [8]. Exercise activates the muscle branched-chain α-keto acid dehydrogenase (BCKDH) complex, resulting in BCAA catabolism [9]. Therefore, exercise may require more BCAAs prior to, during, or post exercise to attenuate muscle damage and promote recovery.

Specific timing of carbohydrate plus protein supplements may be used for acute ergogenic effects rather than chronic muscular adaptations. When carbohydrates are ingested, insulin is secreted which normally parallels the elevation in blood glucose. The subsequent insulin secretion is the body's response in attempting to keep blood glucose in the normal range of 70-110 mg/dL [10, 11]. Insulin plays a large role in anabolism inside the muscle cell, but perhaps more importantly, insulin is anti-catabolic thereby inhibiting protein breakdown. If blood glucose levels can be better maintained, one might expect to see a more anabolic and/or a more anti-catabolic environment,

leading to less stress and inflammation, and faster recovery. Ingesting adequate amounts of protein and carbohydrate prior to, during, and/or following intense exercise, could enhance recovery and tissue repair, augment muscle protein synthesis, ameliorate muscle damage, promote euglycemia, facilitate glycogen re-synthesis, improve performance, and improve mood states following high-volume or intense exercise [12, 13]. If rapid restoration of glycogen is required, such as a 48 hour recovery workout, combining carbohydrates with protein could prove highly advantageous [1].

The research surrounding glucose and insulin maintenance prior to and during exercise may have applications to reduce pain and inflammation in diseased and healthy individuals [14-16]. Recently, nutritional research has shifted a focus to maintaining a lower glycemic index (<70 GI) and glycemic load (GL) food strategies, and supplements, as they seem to provide a beneficial effect on blood glucose levels during activity. Food bars offer a quick sufficient way to ingest carbohydrates and/or protein, however most commercially available food bars (FB) are higher glycemic (>70 GI). Carbohydrates and protein may act synergistically with other compounds contained in the supplement, to provide an overall exercise recovery benefit, which might not be just from pure carbohydrates alone. Research has shown that food and supplements containing adequate carbohydrate sources can enhance performance and offer some recovery benefits. However, a paucity of literature exists on nutrient strategies concerning carbohydrate plus protein combinations on resistance-exercise and cardiovascular training.

Food and supplement companies have been working to develop different methods to consume carbohydrate in various forms. Carbohydrate foods differ considerably in the GI, or their effects on glucose and insulinemic responses. This is due in part to different food properties affecting the digestion and absorption of carbohydrate [17, 18]. Moreover, different types of carbohydrate with different GI's have been reported to affect intestinal transport and glucose availability. Towards this end, many supplements and gels combine different forms of carbohydrate (e.g., sucrose, etc.) to strategize nutrient delivery and absorption [19, 20]. Recently, isomalto-oligosaccharides (IMOs) have gained attention in the nutritional supplement industry to due it's low GI & GL, and potential ability to maintain euglycemia, theoretically spurring the potential for exercise-based research on reducing muscle damage and inflammation in combination with protein. The proposed theory behind this study is that administration of a food bar comprised of an adequate amount of carbohydrate (13 g IMO and 4 g sugar) + protein (20 g whey) in combination prior to, during, and post-exercise, may attenuate the muscle damaging and catabolic effects of acute intense exercise on performance and recovery.

Statement of the Problem

Will ingestion of a food bar containing whey protein and isomalto-oligosaccharide as the carbohydrate source more favorably affect glucose homeostasis, exercise performance, or recovery from intense exercise in comparison to a carbohydrate matched placebo?

Purpose of the Research

The purpose of this research was to examine the glycemic insulinemic responses to ingesting this food bar; and to then determine if ingesting this food bar prior to, during, and following intense exercise would affect performance and/or recovery.

General Research Overview

This study was conducted in two phases. First, a Pharmacokinetic Study was performed to assess the effects of ingesting the FB on glucose homeostasis and insulin as well as to determine the glycemic index and glycemic load during a 2 hour oral glucose tolerance test (OGTT). The second study (Exercise Study) involved assessing the effects of ingesting the FB prior to, during, and following intense exercise on performance and recovery.

The Pharmacokinetic Study was conducted in a randomized, counter-balanced, and crossover manner during a 2 hour OGTT. Twenty healthy men and women donated fasting blood samples prior to ingesting a food bar (FB) containing 20 g of a whey protein blend, 25 g of carbohydrate (13 g IMO, 4 g sugar, 8 g fiber), and 7 g of fat (1.5g saturated) or 25 g of dextrose (PLA), and repeated 7 to 10 days later while ingesting the alternative treatment. To accurately obtain a GI and GL, 10 fasted individuals later participated in the same experiment while ingesting 2 FB's or 50 g of dextrose. Blood samples were taken at 10, 20, 30, 60, 90, and 120 min post-ingestion while subjective ratings related to appetite and hypoglycemia were obtained at 0, 60 and 120 min. The independent variable was nutrient intake and dependent variables included blood glucose, insulin, and subjective ratings related to appetite and hypoglycemic side effects.

This Exercise Study examined whether consuming this FB or PLA prior to, during, and following intense exercise (3 treatments) would affect exercise performance and/or recovery. Twelve resistance-trained males participated in an open label, randomized, counterbalanced, cross-over trial, repeated with alternative treatment 7-d later. During testing participants donated venous blood samples, arterialized-venous finger samples, and performed graded pain rating scale (GPRS) measurements, isokinetic leg extension/flexion maximal voluntary contractions (MVCs), Readiness to Perform (RTP), and Eating Satisfaction (ES) surveys were performed with respective treatments. Participants performed 11 resistance-exercises (3 sets x 10 repetitions at 70% of 1RM) followed by agility and sprint conditioning drills for time. After 48 hours of recovery, participants returned to the lab for a fasted venous blood sample and to perform GPRS, MVCs, and RTP assessments. The primary outcome measure was glucose homeostasis. Secondary outcome measures included assessment of performance (i.e., resistance-exercise lifting volume, agility and sprint performance, and isokinetic strength) and recovery as determined by assessing ratings of muscle soreness; markers of catabolism, stress, and inflammation; and, ratings of readiness to perform. Additionally, dietary energy and macronutrient, subjective ratings of appetite, and subjective ratings of hypoglycemia were assessed.

Hypotheses

The central hypotheses for the Pharmacokinetic Study are:

Ho1:   There will be significant differences in glucose and insulin response during 2
        hour OGTT between the two groups.

Ho2:   There will be significant differences among treatments in reported side

effects.

The central hypotheses for the Exercise Study are:

Ho1:   There will be significant differences in glucose and insulin response during

exercise between the two groups.

Ho2:   There will be significant differences between groups in conditioning and

sprint performance capacity following an intense bout of resistance-exercise

training.

Ho3:   There will be significant differences between groups in recovery from an

intense training bout as determined by assessing markers of inflammation,

muscle damage, muscle soreness, and muscle strength.

Delimitations

Pharmacokinetic Study

1.   Twenty (10 female) recreationally active and healthy males and females ages 18-

35 were recruited for the 2 hour OGTT with 25 g

2.   Ten (4 female) recreationally active and healthy males and females ages 18-35

were recruited for the 2 hour OGTT with 50 g.

3.   Eligible participants took part in a familiarization session during which time they

were informed of the study protocol, filled out necessary paperwork including

informed consent, medical history, exercise performance history forms,

completed a medical screening, and were scheduled for baseline testing.

4. Participants refrained from the consumption of alcohol and any type of physical activity 24-h prior to each testing session.

5. Participants were advised to maintain their normal workout/training regimen over the study duration (on permitted days).

6. Participants fasted for at least 10-h prior to each testing session.

7. Participants completed a 4-d dietary food record prior to baseline testing, and were asked to maintain a consistent diet and turn in a 4-d food records each week they are involved within the study design.

Exercise Study

1. Twelve (n = 12) apparently healthy resistance trained men with current involvement in resistance training consisting of upper and lower body exercises for the past year as well as cardiovascular/sprint conditioning for the past 6 months, ages 18-35, were recruited for the exercise study.

2. Eligible participants took part in a familiarization session during which time they were informed of the study protocol, filled out necessary paperwork including informed consent, medical history, exercise performance history forms, completed a medical screening, and were scheduled for baseline testing.

3. Eligible participants who took part in a familiarization session also performed a DXA assessment, 1RM assessment, and familiarization agility and sprint conditioning.

4.   Participants refrained from the consumption of NSAIDs, analgesics and opioids throughout the duration of the study protocol, and refrained from alcohol and any type of physical activity 24-h prior and 24-h post to each testing session

5.   Participants were advised to maintain their normal workout/training regimen over the study duration (on permitted days).

6.   Participants fasted for at least 10-h prior to each testing session.

7.   Participants completed a 4-d dietary food record prior to baseline testing, and were asked to maintain a consistent diet and turn in a 4-d food records each week they are involved within the study design.

Limitations

1.   Participants were individuals from the Texas A&M University community and surrounding fitness facilities that responded to recruitment fliers and emails; therefore the selection process was not truly random.

2.   Participants were recruited into the study by set minimum study inclusion/exclusion criteria to conduct the crossover study design.

3.   While there may be some variations in testing times and dietary intake, all efforts were made to conduct testing sessions at the same approximate time to account for diurnal variations.

4.   All participants were instructed to maintain their normal training program on permitted days as defined by the study protocol. However, exercise habits during the duration of the study may have changed and therefore changes in

performance measures may have been influenced by individual differences in training rather than the assigned supplement.

5.   There may be some innate limitations of the laboratory equipment that was used for data collection and analysis.

6.   All blood samples were handled and processed uniformly across participants and testing sessions. However, due to multiple laboratory staff working with study subjects there is a possibility that sample handling was not be completely consistent.

Assumptions

Pharmacokinetic Study

1.   Participants answered the entrance questionnaires accurately and honestly prior to being accepted into the study.

2.   The population, which the sample was drawn from, was normally distributed.

3.   The variance among the population sample is approximately equal.

4.   The sample was randomly assigned to the different supplement groups, however, participants and researchers were un-blinded to their supplement during testing sessions in the study.

5.   Participants followed the overall protocol that was explained to them during the familiarization session.

6.   Participants refrained from any alcohol and any type of physical activity 24-h prior to each of the testing sessions.

7. All laboratory equipment was calibrated and functioning properly prior to all testing sessions.

8. Participants fasted for 10-h prior to each testing session that involve a fasting blood draw and maintained a consistent hydration status across all testing sessions within the study protocol.

Exercise Study

1. Participants answered the entrance questionnaires accurately and honestly prior to being accepted into the study.

2. The population, which the sample was drawn from, was normally distributed.

3. The variance among the population sample is approximately equal.

4. The sample was randomly assigned to the different supplement groups, however, participants and researchers were un-blinded to their supplement during testing sessions in the study.

5. Participants followed the overall protocol that was explained to them during the familiarization session.

6. Participants refrained from any alcohol and any type of physical activity 24-h prior to each of the testing sessions in both studies, however, participants also refrained from use of NSAIDs, analgesics, opioids throughout the study design for the Exercise Study.

7. All laboratory equipment was calibrated and functioning properly prior to all testing sessions.

8.   Participants maintained a consistent dietary intake and exercise regimen (when permitted) throughout the duration of their respective studies.

9.   Participants fasted for 10-h prior to each testing session that involved a fasting blood draw and maintained a consistent hydration status across all testing sessions within the study protocol.

10.   Participants honestly answered, to the best of their ability, the graphic pain rating scale (GPRS) in response to algometer quadriceps muscle soreness measurement within each of the testing sessions, and baseline session.

11.   Participants performed at their maximal potential within the primary exercise bout and in subsequent performance testing.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Optimization of training and recovery are paramount for improved performance in the recreationally competitive population, and especially amongst athletes. In order for the human body to chronically adapt to meet the desired demands of sport, resistance training and/or anaerobic sprinting/conditioning training, individuals should participate in specifically designed training regimens which elicit acute physiological responses necessary for adaptation over a prolonged period of time. As a result, the body will progress through a condition of muscular damage, breakdown, and an inflammatory response, referred to as exercise-induced muscle damage. These responses are often the result of highly demanding or unfamiliar exercise selection, and especially present when eccentric exercise is a focal point of the exercise programming. The 24-48 hours following these types of exercises typically are associated with impaired muscle function and symptoms of delayed onset muscle soreness (DOMS). Strength coach professionals attempt to finely tune the art of program designing to implement periodization and proper rest and recovery to account for the physiological demands and stressors of training, and to minimize performance losses. Higher trained individuals expectably have a greater exercise capacity, and a more highly adapted physiological system, to handle the increased training demands of intense resistance training, anaerobic sprint/sport conditioning, and repetitive training loads during a consistent exercise

regimen. Introduction of unfamiliar exercises, changes in exercise tempo, and changes in repetition ranges to training programs typically promote far greater exercise-induced muscle damage as a result of elevated intramuscular damage, stress, and catabolism. It is important for athletes and recreational competitors to accelerate recovery between exercise bouts, especially in terms of muscle function and reductions of pain, soreness, and inflammation. Nutrient timing in healthy, exercising adults and/or elite athletes may play a key role in maintaining blood glucose homeostasis and insulin responsiveness during exercise. Further, the maintenance of fuel availability may assist in augmenting muscle protein synthesis and restoring glycogen. This could lead to a more advantageous exercise-performance, enhanced recovery, and improved mood states and appetite perceptions following high-volume intense exercise or sprint conditioning. The following reviews background information related to delayed onset muscle soreness which is often experienced with resistance-exercise similar to this study protocol.

Delayed Onset Muscle Soreness (DOMS)

Acute bouts of intense exercise produce inflammation, muscle damage, and muscle soreness. Stress responses characterized by mechanical eccentric damage to the muscle and inflammation can almost mimic physiological stress responses associated with adverse cardiovascular episodes and illness [10-12, 21-23]. Intense high volumetric workouts consisting of using large muscle groups, even in one single session, can consequently trigger load-induced responses. These responses can be characterized by structural muscle damage and inflammation which promote the release of intramuscular proteins into the systemic circulation. These markers of muscle damage and breakdown

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are sometimes associated with cardiovascular dysfunction, surgery, and disease [10-12, 21-24].

After prolonged concentric, isometric, and especially eccentric contractions, skeletal muscle becomes fatigued. Although this is relatively short lived, it can decrease athletic performance. Generally, within 1-2 hours most humans have usually recovered full function of the particular muscle group used during the exhaustive exercise [25]. Workouts involving multiple series of repetitive eccentric muscular contractions can lead to the majority of muscular injury which ultimately lead the musculature into the repair sequences of degeneration, inflammation, regeneration, and fibrosis [6, 23-27]. Almost all resistance-training sessions involve series of eccentric contractions as one must lower the bar or load prior to the concentric contraction to complete the lift. The outcome of such eccentric series leads to sarcomeres becoming overstretched, progressively from weakest to strongest. Each time the active muscle is contracted and then relaxed myofilaments are overstretched, leading to more filaments failing to re-interdigitate. This typically results in weaker and more disrupted sarcomeres laying longitudinally the length of the myofibril.

Once one or more sarcomeres have become disrupted, the potential to advance to disrupt adjacent and transverse myofibrils increases. Eventually, a point will be reached where structural malformations can lead to membrane damage, often accompanied by uncontrolled movement of calcium (Ca^{2+}) into the SR. This uncontrolled Ca^{2+} movement facilitates another step in the damage process. These sequential events of eccentric muscle damage begin with disruption of the sarcomeres and lead to membrane damage

and electron coupling interference. The combination of these stressors can trigger

proteolysis proteins, such as calcium activated calpains, which are associated with fiber

breakdown and repair [28].

Muscle soreness following exercise is not a direct result of inflammation and

muscle breakdown, but rather a product of amassed nociceptor and mechanoreceptor

sensitivity to chemicals and by-products released during muscular degeneration. The

inflammation process implicates aggregation of macrophages and monocytes sensitizing

group III and group IV afferent fibers. The onset of swelling and soreness is present at

24 hours after exercise, and depending on the severity, can last up to another 3-4 days. It

is common after bouts of intense eccentric muscle damage for individuals to experience

difficulty in performing finely skilled movements. Consistent experiments have shown

that both the sense of force and position of muscles in the human subject can be

disrupted after eccentric muscle damage [6, 27].

A vast majority of the population, including athletes, choose to treat pain and

inflammation symptoms of exercise with non-steroidal anti-inflammatory (NSAIDs),

such as ibuprofen and naproxen. NSAIDs attenuate the inflammatory response via

inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) that regulate

inflammatory-stimulating prostaglandin production [23, 25]. NSAID use are widely

advertised for acute and chronic painful conditions, even though use as been attributable

to gastrointestinal problems [13]. This concern raises the question if the NSAIDs use is

actually safe and effective in relieving inflammation and perceptions of muscle soreness.

NSAID research has remained controversial due to inconsistent reports following

exercise [23, 29-33], while research has implicated that inhibition of COX enzymes have demonstrated to compromised skeletal muscle protein synthesis and the function of satellite cells, thus presenting a dilemma for hypertrophy, anabolism and recovery. Other studies have found no NSAID effect of anabolic processes post-exercise in the human musculature [34-36]. Due in part to the safety and efficacy controversy in pain relief supplemental research, the topic of nutritional interventions on exercise-recovery has received considerable attention in recent years.

DOMS Timeline

The sensation of pain and stiffness that typically presents itself from 1-5 days following intense bouts of exercise, especially after unaccustomed exercise and eccentric-activity, can adversely affect athletic performance, both from voluntary reduction of effort or inherent loss of ability to create muscular force [37]. DOMS is defined and clinically classified as a type I muscle strain, and can vary from minimal muscle stiffness which disappears quickly during routines of daily living, or and present excruciating debilitating pain, stiffness, and tenderness in the muscle which restricts movement [38, 39]. Generally, the tenderness and sensitivity associated with DOMS is initially concentrated in the distal aspect of the muscle as a result of high concentrations of pain receptors in the myotendinous muscle region, but begins to diffuse progressively by 24-48 hours post exercise [40]. Despite common symptoms of DOMS, the primary mechanism of debilitating muscle-damaging exercise still remains to be elucidated due to the fact that one model cannot explain the complete facets of the DOMS sensation, in response several models have been proposed by researchers [26, 37, 41].

A largely debunked theory in the early development of DOMS research was the lactic acid theory, which was based on the assumption that lactic acid was a toxic metabolic waste product of metabolism and influenced perception of pain [37, 39, 42]. This theory has been largely rejected due to the increased recruitment of type II fibers during concentric contractions failed to result in DOMS-type delayed soreness [43]. Further, blood lactic acid concentrations appear to return to pre-exercising levels within one hour following cessation of exercise. Research has also failed to distinctly identify a correlation between lactic acid concentrations and perceived soreness measurements [44]. Congruently, it is broadly accepted that lactic acid is the end product of glycolysis, and is essential for continuously regenerating NAD+ to support glycolysis, thus providing a drastic change from lactic acid's understanding and role in the human structure as beneficial, rather than detrimental [45]. Lactic acid may however increase acute perceptions to pain as hydrogens are released leading to a more acidic ($pH < 7.4$) environment following intense exercise, however, as mentioned before this cannot be attributed to DOMS pain symptoms experienced 24-48 hours after exercise cessation [46].

Other theories involve the aspects regarding connective tissue and muscle damage, which stem from any type of eccentric muscle action or kinesthetic braking force (downhill running) thus producing higher tensile forces than may be experienced with solely concentric muscle actions [41, 47]. Due to the all-or-nothing firing action of muscle cells, decreased numbers of muscle fibers are recruited during an eccentric contraction compared to the concentric component, of an exercise/lift [48].

Consequently, this results in greater muscular tension produced by the current recruited fibers on the connective tissue located at the myotendinous junction and the immediate associated distal muscle fibers [38, 48]. Connective tissue content and composition differ between type I and type II fibers. Type I (slow-twitch) fibers may demonstrate a lowered susceptibility to stretch-induced injury as compared to type II (fast-twitch) fibers. Excessive stretching or strain on the structure of connective tissue may lead to muscle soreness [49, 50]. Evidence provided by measurements of urine excretion of hydroxyproline and hydroxylysine subsequent to exercise has provided support for this theory as their presence is a result of collagen degradation by overuse, or strain damage [48, 50].

The muscle-damaging enzyme efflux theory centers more the on contractile components of muscle and disruption of Z-line structures [49]. Because of the large amounts of muscular tension produced, the sarcoplasmic reticulum (SR) can be significantly damaged, altering its ability to sequester and maintain calcium levels inside the cell. This can lead to an inhibition of mitochondrial regeneration of ATP production and large calcium accumulations inside the cell activate proteases, such as calpains, which cause degradation of Z-line proteins in the sarcomere and also induce pain sensations from nocireceptor sensory nerve endings [37-39, 41].

The inflammation theory is based on the findings of edema and inflammatory cell presence after repetitive eccentric muscle actions [42, 50, 51]. As a result of tissue damage, muscle fibers release proteolytic enzymes that initiate degradation of protein cell structures, leading to subsequent increases in cell membrane permeability to small

blood vessels and an influx of protein-rich fluid into the muscle [52, 53]. Once the

osmotic pressure is altered in the cell from the influx of exudative fluid, pain is sensed

by activated group IV sensory neurons [54]. Because of the correlation of time course

for peak edema appearing with peak muscle soreness, and the lack of time course for

inflammatory cell aggregation, researchers have elected to also address this method as

the as the "Tissue Fluid Theory" [39]. Whether the development of edema or the

inflammatory enzyme secretions are the main causes for the response in DOMS

symptoms, both provide evidence to induce monocyte and macrophage aggregation at

the site of muscle damage and produce substances that are sensed by the group III and

group IV sensory nerve endings within a 24-48 hour time frame [37, 39].

As a result of the exact mechanisms not being fully elucidated yet by science,

researchers have gathered a general consensus that a single theory alone cannot account

the onset of DOMS. Instead, researchers have proposed that the theories described

above may present sequence order to account for the DOMS phenomenon [26, 37, 41,

53]. The proposed sequence of DOMS begins with the assumption that high tensile

forces, such as seen with eccentric contractions, cause damage to muscle via disruption

of structural proteins, specifically at the Z-line. This is followed by strain on the

connective tissue near the myotendinous junction and accompanying muscle fibers.

Subsequent damage to the SR leads to an accumulation of calcium that hinders ATP

resynthesis as mitochondrial calcium homeostasis is disrupted. Elevated calcium

concentrations activate proteolytic calcium-dependent enzymes that degrade desmin and

perhaps titin proteins at the Z-line [38, 39, 41]. In a period of a few hours, inflammation

has begun as there is an increase in circulating neutrophils. Shortly thereafter, intracellular markers of connective tissue damage (e.g CK) diffuse into the cell plasma and interstitial space, attracting monocytes that transition into macrophages around 6-12 hours. Monocytes and macrophages appear to reach peak concentrations near 48-h. Macrophages seem to be responsible for the stimulation of pain sensations in the group III and group IV nerve endings, as macrophages yield prostaglandin (PGE_2) [37, 39]. At present, it appears these sequential events lead to the sensation of the DOMS phenomenon, and supplementary pain may be increased with mechanical movement of muscles as increased muscle pressure augments a stimulus for nocireceptors already excited by PGE_2 [42, 50, 51].

Hormonal Adaptations

Testosterone & Cortisol

Strength, power, fatigue, and endurance in athletes can be directly affected by the recovering muscle status. Insufficient recovery is likely to result in decreased performance, which can be indicated in blood biomarker muscle assessments. The research suggests that endocrine regulation of muscle repair/adaptions function should focus on anabolic/catabolic balance, specifically testosterone and cortisol [55]. Testosterone and cortisol are well documented, and validated, markers relating to fatigue, recovery, protein synthesis, and homeostasis. Due to the natural variations in individual blood concentrations of testosterone and cortisol, it is prudent to monitor progressive changes from baseline at different timelines throughout training/testing (i.e., fasted, before training, post training, the day after a rest day). A decrease in testosterone

may indicate that training has exceeded an individual's tolerance and has reduced the anabolic potential [56]. Likewise, in opposition, an increase in cortisol my suggest an impaired capacity for protein synthesis leading to a reduction in glycogen replenishment, and an increase in protein breakdown and red blood cell production [57]. Seeing how testosterone and cortisol work antagonistically, we can get a fairly good idea of anabolic status for protein synthesis, red blood cell production, glycogen replenishment. In addition, monitoring a testosterone and cortisol ratio (T:C ratio) during a training session may provide further evidence to a relative anabolic-catabolic atmosphere in the body, especially in male athletes [55, 58]. The T:C ratio is considered more sensitive to training stressors, than either measurement alone. A pronounced reduction in the T:C ratio (30%) has been suggested to be an good indicator of inadequate recovery [59, 60].

Insulin

Insulin has been shown to be extremely anabolic, as well as anti-catabolic, and can significantly affect the overall anabolism/catabolism in the muscle [61, 62]. Serum insulin concentrations parallel response to changes in blood glucose, however the response is augmented when protein and/or carbohydrates are ingested prior to, during, or post workout [61-65]. Without protein/carbohydrate supplementation, insulin concentrations have been shown to reduce levels during an acute bout of resistance-exercise [66]. Insulin appears to only be anabolic if it is in, or above, the normal range as a dose response does not appear to exist. It also appears to be mostly sensitive to blood glucose concentrations and dietary intake. It is therefore suggested that supplementation of carbohydrates, amino acids, or combinations of both, to be ingested

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prior to, during, and/or immediately following a resistance-exercise bout to maximize

muscle tissue anabolism and/or prevent muscle catabolism [63]. Moreover,

supplementation prior to, or during, resistance-exercise may be especially promising due

to taking advantage of the exercise-induced increase in blood flow (hyperemia) and

amino acid delivery.

Muscle Enzymes

Intense exercise bouts may damage muscle tissue resulting in metabolic markers

to increase concentration in the human blood serum. Serum levels of skeletal muscle

enzymes or proteins can be useful markers to identify the functional status of the muscle

tissue and physiological conditions. Creatine kinase, blood urea nitrogen, creatinine,

lactate dehydrogenase, are among the most useful serum markers of stress, muscle injury

or catabolism. These various markers can provide researchers with a composite picture

of the muscle status. It is broadly recommended to use multiple markers to better

estimate human physiological conditions.

Creatine Kinase

After an intense exercise bout, especially following muscle damaging resistance-

exercise, the enzyme creatine kinase (CK) is released from the muscle tissue into the

blood. It is very typical for athletes to have an elevated level of CK after training, as

compared to their baseline serum values. Serum CK has been proposed to be one of the

best indirect indicators of the training intensity and overtraining [67, 68]. CK levels peak

approximately 24 hours post-exercise, but may remain in high circulation for up to a

week [55].

Ironically, there are still complications regarding the use of CK in this manner, as there is great interindividual variability in serum CK. This provides a problem when trying to figure out reliable reference values for athletes. Moreover, certain factors such as gender, training level, muscle mass involved can all influence CK levels to a greater extent. Elevated CK levels for multiple days after a workout may suggest incomplete or insufficient recovery.

Blood Urea Nitrogen & Creatinine

Markers such as blood urea nitrogen (BUN) can demonstrate comprehensive protein synthesis/breakdown [69]. Blood urea nitrogen increases with greater protein degradation. Creatinine is a compound which is synthesized through the metabolism of creatine and excreted in the urine. A high creatinine measurement in healthy adults is usually indicative of a very strenuous workout as high rates of ATP and creatine are broken down to sustain work. Increases in creatinine reflect greater nucleotide degradation and is often used to assess hydration. Creatinine to BUN ratio (BUN:creatinine) is a general marker of whole body catabolism. Increases in the BUN:creatinine suggest greater whole body protein degradation. These markers in combination are useful to determine an athlete's overall recovery following a muscle damaging bout, especially intense resistance-training.

Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the reaction of lactate to pyruvate. This is a critically important reaction in the cell for ATP synthesis and anaerobiosis. Many different cells in the body contain this enzyme which can reflect

intensity or long duration exercise. Some key organs highly rich in LDH are the heart, kidney, liver, and skeletal muscle. This enzyme is important for glycolysis both in anaerobic respiration and aerobic respiration. Short-term intense effort exercise usually causes increases in LDH in the human. Research demonstrates that LDH activity can increase up to 3-5 hours during heavy muscular activity [70]. The half-life of LDH released from skeletal muscle into the blood is usually around 10 hours.

Markers of Inflammation

Regular physical activity is associated with mild trauma followed by recovery [71]. When sufficient recovery is allowed to occur, usually an adaptation from training occurs and performance will increase over time. However, when exercise intensity or volume is increased without proper recovery, mild trauma can become more severe which is an often time called "overtraining". Markers of oxidative stress and inflammation have been associated with overtraining and lack of proper recovery [72]. The literature has demonstrated that overtraining, and lack of recovery, can induce significant rises in inflammatory markers.

The blood provides biomarkers which express pro-inflammatory macrophage activity released by macrophages. These include growth factors and cytokines, which are signaling molecules that are very diverse and numerous, and have wide ranges in resting levels. This creates a struggle to properly measure the inflammation in an athlete as a direct measure of blood cytokine presence [55]. However, a good measurement used to assess inflammation in an individual is to monitor the increases in cytokines from baseline.

Interleukins (IL) are a group of naturally occurring proteins which assist in signaling between cells. Interleukins are especially important in stimulating growth, differentiation, and immune responses such as inflammation. Notable classic pro-inflammatory cytokines include IL-1ß, TNF-α, IL-6, and IL-8 [55]. TNF-α (tumor necrosis factor alpha) is involved in systemic inflammation and is responsible for a wide array of signaling events within the cell leading to necrosis or apoptosis. Other common cytokines include IL-4, IL-13 and IFN-γ to be measured in this study. IFN-γ is a is a major pro-inflammatory cytokine and a member of the interferons.

It has been well documented that intense and strenuous exercise produces an inflammatory response of cytokines. Some researchers suggest that this response is induced from the mobilization and augmentation of neutrophils and monocytes. In response, cytokines are released into the circulation to mediate this phenomenon. Among these cytokines, TNF-α and IL-1 have traditionally been thought of to be the main inducer of the acute phase of inflammation. [73]. The majority of research on these two cytokines have revealed that a circulating concentration post exercise is either unchanged, or exhibits small delayed increments. Plasma IFN-γ do not appear to alter as significantly as IL-6 with exercise. IL-6 appears to greatly increase following exercise, and may be influenced by cytokine inhibitors such as cortisol. Exercise studies have commonly observed significant increases in TNF-α, IL-1, and IL-6 [74].

It has recently been demonstrated that endurance exercise augments the release of IL-8. Moreover, it has recently been shown that IL-4 concentrations were significantly heightened 2 hours after exercise. Although the exact time course is not

well understood concerning the appearance of cytokines in the blood, it is conceivable that limiting certain aspects of inflammation through nutrition or supplementation might present new treatment strategies related to recovery [75].

As stated, there is no universally agreed upon threshold for which cytokines above is considered elevated. Therefore, it is recommended to use repeated testing at multiple time points on healthy athletes/individuals; with baseline measurements, and at precise points during or after training or recovery periods [63, 76]. The following reviews background information related to nutrient timing strategies which might enhance performance and/or recovery.

Nutrient Timing

Consumers often ingest carbohydrate and protein energy bars in between meals as snacks or prior to exercise in order to increase amino acid availability and/or maintain blood glucose during exercise [77-80]. However, many energy bars or drinks have a relatively high glycemic index (GI) and therefore may not be not suitable for individuals who are glucose intolerant and/or diabetic [79, 81]. Additionally, while it is recommended that athletes ingest carbohydrate and protein prior to exercise [77, 80], ingesting foods, gels, and/or beverages that have high GI's may promote hypoglycemia during exercise and thereby hasten fatigue [77, 79, 80, 82, 83]. It appears that adding different types of carbohydrate with low to high GI's to whey protein has differential effects on glucose and insulin responses following intense resistance-exercise [82].

Carbohydrate

The literature demonstrating the effects of carbohydrate timing on resistance training are limited, however there have been multiple studies revealing that resistance training significantly decreases muscle glycogen stores [84-87]. These decreases are moderate in comparison to intensive endurance exercise, and benefits are not seen with consumption of pre-exercise carbohydrates when performing resistance training in moderately glycogen depleted circumstances. Currently, only one study thus far has been able to document that carbohydrate ingestion prior to and during resistance exercise has led to improved performance outcomes, but these benefits were only noted in the second training session performed later in the same day [88]. In opposition, multiple studies have been unable to support any improvements in resistance exercise performance [2, 89, 90]. The referenced study by Haff and colleagues [2] did however seem to see 49% reduction in loss of muscle glycogen following a 40 min resistance training workout where a carbohydrate dose (1.0 g/kg) was given pre-workout and every 10 min (0.5 g/kg) through the duration of training when compared to a placebo beverage. Participant's isokinetic muscle performance did not show any significant change.

Glycemic Index

The Glycemic Index (GI) concept first began in the 1980's as a way to rank carbohydrates based on the measured glucose responses to the ingested food compared with a reference carbohydrate, like glucose or white bread [91]. The GI of a carbohydrate is a measure of the integrated area under the curve (AUC) resulting from an ingested food by that of a standard food. The GL is calculated as the product of the

amount of available carbohydrate in a food and the GI value (using glucose as the reference food), divided by 100. Foods are often ranked by their GI or ability to alter glucose homeostasis. Carbohydrate foods with a higher GI will raise blood glucose more so than a moderate GI and a low GI food. The international tables rank foods with a low GI as less than 55, moderate GI between 55-70, and a high GI when greater than 70. The reference GI value for glucose is 100. The reported GI values for common sugars are maltose (105), dextrose (96), xylose (75), trehalose (70), sucrose (58), honey (55), lactose (43), IMO (35), fructose (20), and stevia (0). Other common carbohydrate GI values commonly ingested around exercise are Pop Tarts (70), Skittles (70), white rice (69), Power Bar (58), potato (54), banana (51), brown rice (50), apple (40), milk (40), yogurt (36), spaghetti (32), and peanuts (23) [92].

Nutritional recommendations and guidelines for general health and optimal performance are often based on the information relating to the GI of a carbohydrate food source. Investigations into pre-exercise meal strategies have provided evidence that metabolism and substrate utilization can be effected by low and high GI meals [91]. It appears the primary benefit of the low GI carbohydrate is the lowered resulting post-ingestion hyperglycemia and hyperinsulinemia. Moreover, the combination with the reduction in free fatty acid oxidation, which assists in maintaining euglycemia and may result in a more sustained carbohydrate availability during the exercise bout. Although a few studies have shown augmented exercise performance, the majority of studies have failed to report a metabolic difference between low and high GI's translating to enhanced exercise capacity or performance [91, 93-95].

It is recommended that athletes do not ingest high GI carbohydrates prior or during exercise. This recommendation is a strategy to avoid the spike in insulin leading to hypoglycemia. Exercise can further induce hypoglycemia following a high GI meal with exercise-stimulated glucose uptake, resulting in fatigue, discomfort, and nausea.

A study by Wee and colleagues [96] examined the effects of ingesting high and low GI carbohydrates on pre-exercise meals on running capacity. Eight subjects participated in a $70\% VO_{2max}$ treadmill run to exhaustion after an overnight fast. Each subject received a 67% carbohydrate, 30% protein, and 3% fat meal containing either a high or low GI carbohydrate, and repeated the experiment 7 days later with the alternate treatment. There was no difference in endurance capacity between the two treatments, however the authors reported a 12% lower carbohydrate, and a 118% increase in fat, oxidation when the low GI was ingested during the first 80 min of exercise.

Wu et al [95] performed a randomized crossover study design with 8 male recreational runners who ingested a high (77) and low (37) GI isocaloric meal 3 hours before running at $70\% VO_{2max}$ until exhaustion following an overnight fast. The authors reported a significantly improved running time to exhaustion for the low GI compared to the high GI (Low GI 108.8 ± 4 min; High GI 101.4 ± 4 min, p=0.038). The authors also observed a higher fat oxidation rate with the low GI group compared to the high GI group (p<0.05).

DeMarco and colleagues performed a study after an overnight fast using 10 trained cyclists who consumed a low or high GI meal 30 min prior to performing a 2 hour $70\% VO_{2max}$ until exhaustion. The authors reported significantly lower insulin levels

in the low GI group and significantly higher respiratory exchange ratios in the high GI group (p<0.05). The low GI group also reported lower subjective ratings of perceived exertion and observed a 59% longer time to exhaustion compared to the high GI treatment group. The results from this study suggest an enhanced maximal performance following a low GI meal 30 min prior to exercise.

Further research is needed before recommendations or manipulation to carbohydrate GI sources in an athlete's diet can be set. It appears that the timing, the type, and the amount of carbohydrate may need to be individualized to suit the athlete's sport specific needs, gut distress, and preferences. The current literature seems to suggest a benefit for low GI foods on resistance-exercise adaptations in diabetic and pre-diabetic individuals [97-99], however more research is needed pertaining to the effects of low to high GI nutrient strategies on resistance training in healthy adults. If insulin and blood glucose can be better manipulated with low GI foods prior to, and during exercise, one might have better success offsetting exercise induced catabolism often observed with hypoglycemia and lowered insulinemic responses.

Isomalto-oligosaccharides

Isomalto-oligosaccharides (IMO) are a prebiotic high fiber, low calorie source of carbohydrate that has been used as a functional food and prebiotic sweetener in Asia for over 3 decades [100-104]. The glycemic index for IMOs is 34.66 ± 7.65, which represents a low GI [100]. IMOs can be found naturally in assorted fermented foods including miso, sake, soy sauce, as well as honey [105]. Commercially available IMOs are the market leader in the dietary carbohydrate sector of functional foods in Japan.

Isomalto-Oligosaccharides are enzymatically produced and typically are obtained from starch hydrolysates such as maltose and maltodextrins, from the action of the α-transglucosidase, or other alternative methods implying the use of α-amylase and an α-glucosidase combined with a pullulanase [100, 106, 107] and is typically in an α-(1→6) linkages. Classification of IMO structure can be indicated by linkage types (α-1→2, 3, 4, or 6) and the proportion and position of each type of linkage (only α-(1→6) or combined) [108-110].

Basic animal studies indicate that IMO's serve as a soluble dietary fiber and can stimulate activity of probiotic gut flora, improve gut function, and help manage cholesterol in animals fed on a high fat diet [100, 102, 111-113]. Regulation of the gut microbial ecology has gained extensive interest in the scientific community, as well as among consumers, in the use of probiotics and prebiotics [114-116]. The use of prebiotics seem to overcast an advantage to probiotics due to the various nature of being cheaper, carrying less risks, resistance to digestive barrier, and is much easier to incorporate into the diet [117-119].

Prebiotics are nondigestible dietary components that effectively pass through the digestive tract to the colon, while en route can successfully stimulate proliferation, or increase the activity, of select populations of bacteria in the human or animal [120, 121]. IMOs are among the class of nondigestible oligosaccharides that have gained the most attention, and have mostly been developed in Asia countries due to its valuable properties and favorable applications to the food industry. IMOs are low-digestibility

glucosyl anomalously linked oligosaccharides (ALOs) [122] which are considered as

prebiotics and anticariogenic (tending to prevent tooth decay) agents [123].

Recent data published by Delzenne and Williams [124] support the favorable

evidence in humans that dietary oligosaccharides can influence hormone production,

lipid and carbohydrate metabolism, and immune responses in situ, especially in the

intestinal tract as well as outside the gastrointestinal tract. IMOs have also revealed a

positive effect on non-competitive inhibitors of α-glucosidase, which aids in delaying the

digestion of starch and saccharose, thus promoting an application to several diseases

including obesity, diabetes, gastritis, gastric ulcers, duodenal ulcers, cancer, and viral

diseases including hepatitis B and C, HIV, and AIDS.

Diets including 5-20% IMO have demonstrated an ability to lower abdominal fat

in mammals [125]. These findings suggest a potential application for individuals

involved in exercise training to maintain blood glucose availability, prevent

hypoglycemia, minimize exercise induced protein degradation during exercise, and

stimulate protein synthesis. Additional research is needed to further evaluate IMOs and

their role in exercise, as well as the timing of ingestion pertaining to performance.

Protein and Amino Acids

It appears leucine, isoleucine, and valine (BCAAs) catabolism in skeletal muscle

is regulated by the branched-chain alpha-keto acid dehydrogenase (BCKDH) complex,

which may have a contributing effect to muscle protein synthesis and muscle growth,

thus aiding in recovery and preventing the DOMS effect. It has been proposed that

exercise training activates the muscle BCKDH complex, which may result in an elevated

BCAA catabolism. As a result, exercise may require an elevated supply of BCAAs to meet the requirements of the heightened demands. A study by Shimomura et al. [8] reported that oral BCAA supplementation of ~5 g (males 77 ± 3 mg/kg, females 92 ± 2 mg/kg body weight) before and after squat exercise (seven sets of 20 repetition squats) in 30 male and female participants, can effectively reduce DOMS and muscle fatigue for several days after exercise. Cockburn and colleagues [126] reported that consuming milk, or milk-based carbohydrate + protein supplements immediately after unilateral eccentric-concentric knee flexions were able to attenuate symptoms of DOMS for 24 and 48 hours post-exercise. The reductions in muscle soreness are likely due to the ingestion of high-quality protein from the milk, such as whey. Gilson et al. [127] found that the 5 g of essential amino acids (EAA) contained in chocolate milk was able to successfully reduce DOMS symptoms more so than an isocaloric carbohydrate drink in 13 intercollegiate soccer players following a period of increased training duration involving resistance-training and aerobic/conditioning drills.

Skeletal muscle glycogen stores are utilized and can be relatively depleted during resistance training and/or cardiovascular training. A single resistance training session has the capacity to reduce endogenous muscle glycogen stores from 24-40%, depending on the duration and intensity of the exercise bout [128]. If glycogen is depleted and not adequately restored, subsequent exercise training may be compromised and delayed recovery symptoms may flourish [129]. Currently, there is not a general agreement in relative, or total, amounts of protein or carbohydrate, or carbohydrate + protein combinations necessary due to the lack in available literature. Determination in efficacy

of nutrient timing methods involving carbohydrate + protein to enhance recovery and performance show promise but need to be further elucidated with research. More research is also needed to investigate the effects of nutrient timing using resistance-trained individuals as these are the athletes who might require additional nutrient delivery surrounding exercise.

The literature suggests that timing in relation to carbohydrates and proteins may play a key role, and be of relative importance in relation to a training session, energy substrate use, potential recovery and symptoms of DOMS. Nutrient timing involves the purposeful ingestion of nutrients to favorably impact adaptive responses to acute and chronic exercise such as muscle strength and power, body composition, substrate utilization, and physical performance. The initial work examining the strategies behind nutrient timing began in the 1970-80's, which examined the effects of carbohydrate feedings on glycogen bioavailability, exercise performance, and rates of glycogen resynthesis [130-132].

It is well documented that glycogen stores are limited in the human body with about ~80-100 g being stored in the liver and 300-400 g stored in the skeletal muscle [133, 134]. It is critically important to build, and resynthesize glycogen stores prior to, and after, exercise as glycogen is the preferred fuel source during moderate to high intensity endurance (e.g., 65-80% VO_2max) or resistance-training (e.g., 3-4 sets of ~6-20 repetitions maximum [RM]) type workouts [135]. Research has indicated that carbohydrate operates as the primary source of fuel for up to a few hours during moderate to intense exercise. During resistance training, one study demonstrated a 39%

reduction in vastus lateralis muscle glycogen by performing six sets of 12-RM leg extension [86]. The importance behind this immense glycogen reduction is that exercise performance intensity and output largely decreases as muscle glycogen levels decline [134]. Rates of tissue breakdown also increase as glycogen levels decrease, leading to the creation of guidelines aimed at maximizing maintenance of stored carbohydrates relative to the amount of work necessary for performance [136, 137].

The literature suggests that the most straightforward guideline to maximizing endogenous glycogen stores is to ingest an adequate amount of carbohydrates necessary for the intensity and volume of the upcoming training. Current guidelines suggested by the ISSN position stand on nutrient timing, recommend daily intakes of 5-12 g/kg/day of carbohydrates, with endurance athletes training at moderate to high intensities ($\geq 70\%$ VO_2max) for more than 12 hours per week, on the upper end of the range (8-12 g/kg/day) [138-140]. The percentage-based recommendations of 60-70% carbohydrates of daily intake have recently decayed because of a lack of ability to meet the required demands of carbohydrates needed to be prescribed for athletes whose diets consist of high amounts of total food intake or diets restricting caloric intake [135].

It is notable to point out that the majority of recommendations for carbohydrate intake are based off endurance athletes, and specifically in male endurance athletes. A paucity of research surrounds the carbohydrate intake with female athletes, furthermore studies have shown that trained female athletes do not oxidize fat and carbohydrate at the same rates as their male counterparts, and may even deplete glycogen stores to different degrees [141-144].

There is novel evidence to suggest that higher protein intakes (> 3 g/kg/day) may be necessary for resistance-trained individuals, and optimal protein ingestion might not be practical by diet alone. While it is possible to achieve adequate protein needs through diet for trained-individuals, supplementation might be prudent as a practical application to employ carbohydrate and protein in reference to food tolerance, digestion, and promoting optimal performance and recovery.

Role of Protein in Recovery

Since the early 1990's, research has cemented the idea that exercise and macronutrient ingestion interact synergistically to provide a far greater net anabolic effect than what exercise or food could deliver alone [61, 145, 146]. Without adequate protein feedings in close proximity to an exercise bout, the overall muscle protein balance will remain negative following an acute bout of resistance-training [147].

Protein feedings following an acute resistance bout significantly increases amino acid availability, thus significantly increases the rates of muscle protein synthesis (MPS) [148]. Further, the muscle's anabolic responsiveness and sensitivity to whey protein is heightened following an acute resistance-exercise bout [149]. A study by Borsheim et al. reported a dose-response outcome of net protein balance in response to 3-6 g dose of EAA [150]. Moreover, Tipton and colleagues [151] documented that a 9-15 g EAA dose taken pre and post-resistance training augmented a higher net protein increase at 3, 4, and 24 h post exercise.

The strategic feedings of protein, in various forms, taken pre, during, and post-workouts have been shown to maximize skeletal muscle recovery and repair and

optimize strength and cross-sectional area adaptations [77, 152]. Recent investigations have shown that protein supplements such as eggs, whey, casein, beef, and whole milk can express an identical, or greater, anabolic response to ingestion of free amino acids when taking in equal amounts of EAA's [153-155].

Whey protein ingestion in close proximity to resistance-training has been shown to augment the phosphorylation of mTOR and its downstream mRNA translational proteins (p70s6K and eIF4BP). These higher activated proteins and signaling cascades further suggest that protein feeding in relation to timing may significantly promote muscle hypertrophy [153, 156]. Research from Coffey et al. [157] and others [158-160] revealed that the timing of protein feedings near ± 2 h anaerobic exercise appears to promote enhanced activation of molecular signaling pathways regulating myofibrillar and mitochondrial protein synthesis, while also augmenting glycogen synthesis.

Muscle glycogen stores are critically important for exercise-performance and anaerobic conditioning fuel availability. Research has illustrated that adding whey protein (0.4 g/kg) to a moderate carbohydrate-containing supplement augments the rate of glycogen synthesis [146]. As a further matter, the incorporation of protein facilitates the repair and recovery of the muscle post-exercise [161]. These findings are thought to be related to the insulin signaling pathway, and are further suspected to be related to a greater insulin response post-exercise. Interestingly, whey protein has also demonstrated an ability to promote glycogen synthesis in the liver and muscle to a greater insulin-dependent fashion than casein [162]. These findings may be due to whey's capacity to upregulate glycogen synthase activity. In conclusion, the addition of whey protein may

enhance recovery, augment protein balance, and improve glycogen replenishment. To date, even amongst a substantial amount of literature discussing the concept of protein timing, only a very limited number of training studies have been able to assess whether strategies implementing pre, during, or post-exercise protein feedings provide advantages compared to other time points [163].

Pre Exercise - Protein

The majority of literature on protein feeding on resistance training have employed some fashion of protein or EAA prior to the start of exercise [135]. Typically these studies also administer some form of protein or EAA of an identical dose during the exercise period as well. Towards this end, one study by Tipton et al. [164] examined resistance exercise and responses of muscle protein balance with a 20 g dose of whey protein taken prior to and immediately post a bout of lower body resistance training. The authors reported that MPS rates were similar and that both groups increased between pre-exercise, and post-exercise, but was not significantly different from one another, suggesting that the response of net protein balance due to timing of intact protein administration alone does not respond to the degree of a combination of free amino acids and carbohydrates.

Anderson and colleagues [165] were among the first to conduct a study looking at the effects of protein consumption immediately before and after resistance training over multiple weeks. This study compared the effects of 14 weeks of resistance training combined with a timed consumption of a 25 g protein blend (16.6 g whey, 2.8 g casein, 2.8 g egg white, 2.8 g glutamine) vs carbohydrate (maltodextrin) immediately before and

after each workout. Participants experienced the greatest significant increases in Type I

(18% ± 5%) and type II (26% ± 5%) muscle fibers size, and squat jump height only

significantly increased, in the treatment group that ingested the protein blend. Utilizing

a similar design approach, Hoffman et al. [166] examined the effect of ten weeks of 42 g

of hydrolyzed collagen protein supplement timing on the strength, power, and body

composition in resistance-trained collegiate football athletes. The results indicate that

the timing of protein intake immediately before or immediately after exercise, or in the

morning and evening over the course of ten weeks of resistance-training did not provide

any added benefit between groups. It should be noted that collagen hydrolysate is not a

high quality protein source, further, the DEXA may not have the sensitivity needed to

detect minute hypertrophic adaptations to skeletal muscle [167]. The study by Anderson

and colleagues [165] used histochemical approaches to measure muscle hypertrophy,

and also fed their participants 20% more calories per day (~36.6 kcal/kg/day), which

may reveal some of the difference in outcomes between the Hoffman study (~30.4

kcal/kg/day).

Recently, Schoenfeld and colleagues [168] compared the effects of consuming 25

g of whey protein isolate immediately pre- versus post-resistance training (3 sets of 8-12

RM) in 21 resistance-trained men (> 1 year RT experience). This study was the first to

directly compare the long-term effects of protein timing administration before and after

each resistance-training workout. Schoenfeld and colleagues helped to further evoke

questions regarding the quantity, composition, and timing of the meal prior to exercise

which may play a larger role than previously thought, and may influence the extent of

training adaptations seen in studies like this. Another question raised by the authors was the amount of training actually involved in these studies might be inadequate compared to individuals who would most likely benefit from protein timing in reference to their workouts. Collegiate athletes train on average four hours per day to reach a total allowance of 20 hours per week as deemed by NCAA Bylaw 2.14 (20 hour Rule) [169] whereas participants in this type of study category may only train 30 hours over the entire exercise-training protocol period.

During Exercise - Protein

A limited amount of research exists with the ability to examine the effects of protein delivery throughout an acute bout of resistance training. A greater limitation in the literature comes from studies designed to specifically compare the impact of protein ingestion during exercise being more beneficial than other times of ingestion. The study by Bird et al. [170, 171] (also mentioned later in the carbohydrate + protein section on resistance training) saw an increase in post-exercise insulin levels and reductions of cortisol and reductions of markers of muscle breakdown (3-methyl-histidine), alluding to a potential benefit in recovery. However, it should be noted that when investigated over a 12-week study, the 6 g of EAA supplement resulted in fewer increases to skeletal muscle fiber size, than when combined in a carbohydrate + protein supplement [172].

Post Exercise-Protein

Post-exercise protein feeding has been deeply examined in attempt to uncover any potential advantages to enhance training adaptations or outcomes dealing with resistance training. A large array of acute studies on exercise and protein timing

administration has contributed to many theories and mechanistic explanations for why post-resistance training administration might be advantageous to athletes [173-177]. Other studies reveal that adaptations or enhancements might been seen over much longer durations, such as weeks to months, especially following initial exposure to resistance training in novice populations [178].

As mentioned in the "Protein Feeding Pre Resistance Training" section, the vast majority of literature examining some feature of protein timing in reference to post exercise has also offered an identical bolus of protein immediately prior to that resistance training bout [165, 166, 179, 180]. Of these mentioned studies, the ingestion of protein [165] or carbohydrate + protein [179] immediately prior to or following resistance exercise has resulted in beneficial training outcomes and adaptations. The only study to reveal results in opposition was the study performed by Hoffman and colleagues [166] where the participants consumed 42 g of hydrolyzed collagen protein (not a high quality protein source) and were in a caloric deficit throughout several weeks of resistance training in highly-trained collegiate football players.

A small number of studies have solely inspected the impact of protein administration post exercise. Tipton et al. [164] examined if ingestion of whole proteins before exercise would have the same impact as previously demonstrated by their work with EAA + carbohydrates [181]. This study used an acute model to measure the response of MPS when a 20 g bolus of whey protein was ingested both before and following lower-body resistance training. The results indicated that MPS rates were increased significantly in both groups (Pre or Post), with no difference between groups.

A study performed by Esmarck and colleagues [182] looked at the effects of post-exercise protein ingestion in a longitudinal fashion on 13 elderly men (age, 74 ± 1 year). The participants consumed an oral liquid dose of carbohydrate (7 g), protein (10 g), and fat (3 g) either immediately post-exercise (within 30 min) or delayed after each exercise bout (within two hour) performed three times per week for 12 weeks. The authors reported that protein ingestion immediately post resistance training led to far greater enhancements in muscle strength and muscle cross-sectional area (CSA) than when the same dose of nutrients was ingested 2 hours delayed. The authors also reported that no measureable increase in muscle CSA followed completion of the study in the delayed two hour group, which led reviewers to somewhat question the outcomes from this particular study [183, 184].

Candow and colleagues [185] produced a study which demonstrates that 10 g of protein was most likely an inadequate dose of protein for this age group performed in the aforementioned study. Schoenfeld and colleagues [168] performed a study investigating muscle strength, hypertrophy, and body composition changes in response to an equal dose of protein (25 g of whey), which offers support that whey protein consumed before or after resistance workouts can promote beneficial strength and hypertrophy improvements, however the timing of such protein ingestion strategies do not necessarily take precedence over other feeding strategies.

Other reviews by Aragon and Schoenfeld [186] and Schoenfeld and colleagues [187] looked to extensively examine the efficacy of protein timing following post resistance training ingestion. The aforementioned authors suggest that protein intake

timing may have little effect if adequate or recommended protein levels have already been consumed. The literature does however demonstrate that skeletal muscle remains sensitive to protein nutrient delivery for at least 24 hours post resistance training, alluding to the possibility that protein timing, quantity, or bolus may have an impact to some level in training adaptations. Further, MacNaughton et al. [188] investigated the influence of 20 or 40 g of whey protein following a bout of whole-body resistance exercise in resistance-trained males. The authors reported that the acute bolus of 40 g of whey protein resulted in greater increases in MPS when targeting full body major muscle groups. This data suggests more of a protein dose effect; however these findings do suggest a significance in a timing interaction despite the ability of the higher dose to seemingly elicit more of a beneficial response. In conclusion, the amount of studies that have truly investigated the timing response is still rather small. In addition, a very small number of studies have examined the nutrient timing used by highly-trained athletes in reference to benefits including skeletal muscle outcomes, performance, or recovery [135].

Carbohydrate and Protein

If rapid restoration of glycogen is necessary for a subsequent exercise bout, aggressive carbohydrate refeeding (1.2 g/kg/h) or combining carbohydrates (0.8 g/kg/h) with protein (0.2-0.4 g/kg/h) might be considered. Notably, extended (> 60 min) bouts of high intensity (> 70% VO_2max) challenges bioavailability of fuel supply, hence a carbohydrate should be ingested at a rate of ~30-60 g of carbohydrate/hour. If carbohydrates are not available, or delivery of such is inadequate, the ingestion of

protein may aid to attenuate muscle damage, maintain euglycemia, and enhance glycogen restoration [135]. Further, pre-, during, and post exercise strategies involving carbohydrate in combination with protein may work as an effective strategy to increase strength and promote recovery, however, the timing and quantity of the meal may impact the subsequent exercise. More research is needed to fully elucidate the strategies.

Pre Exercise - Carbohydrate and Protein

A small group of studies are available examining carbohydrate plus protein administration prior to resistance training. Kraemer et al. [189] investigated a combination of carbohydrate, protein, and fat or a matched placebo for seven days prior to two consecutive days of heavy resistance training. The supplement was taken 30 min prior to the exercise bout on both training days, and led to improved vertical jump power, repetitions at 80% RM, and potentiated endocrine signaling, such as testosterone, following heavy resistance exercise.

Comparable outcomes were reported by Baty et al. [190] where they recruited 34 males to perform an acute bout of resistance training (3 set of 8 repetitions at 90% RM) while consuming a carbohydrate (6.2 % CHO) or a carbohydrate with protein (6.2% CHO + 1.5% PRO) 30 min prior to exercise, as well as during, and post-exercise. There were no relative differences between groups on exercise performance, however the carbohydrate with protein treatment led to significantly greater insulin and lower cortisol levels, and reduced the markers of muscle damage (e.g. CK) during the initial 24 hours of recovery.

In contrast, White and colleagues [191] completed a study examining the specific timing of carbohydrate plus protein consumption had any impact on markers of muscle damage and isokinetic maximal voluntary force production. Twenty-seven untrained men were given a non-caloric sweetener or a carbohydrate (75 g) + protein (23 g) combination 15 min prior and 15 post-exercise during a bout of damaging resistance training. The results indicated that the nutritional strategies and the timing had no difference in effect on force production or markers of muscle damage.

During Exercise - Carbohydrate and Protein

As of current literature, the examination of carbohydrate and protein or amino acid combination on acute effects during resistance training has been studied, although no studies have truly focused on the question of carbohydrate plus protein nutrient timing on such [170-172, 192, 193]. With that being said, carbohydrate with protein combinations during resistance training have been proposed to improve skeletal muscle development due to an augmented insulin response, as insulin has anti-catabolic effects on skeletal muscle growth [194, 195].

Bird and colleagues [170-172, 193] completed a series of studies examining the effects of ingesting a carbohydrate or carbohydrate with EAA on measures of acute performance, as well as markers of muscle damage. One of the series of studies by Bird et al. [170] recruited 32 participants to consume a 6% carbohydrate solution, a 6% carbohydrate plus 6 g EAA solution or a placebo beverage routinely during a 60 min resistance training bout. After examination, the carbohydrate + EAA combination solution led towards a more favorable anabolic environment by stimulating insulin

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release and lowered muscle protein breakdown markers by 27%, including a suppression of exercise-induced cortisol response.

Another study by Bird et al. [193] utilized a triphasic protocol inside a crossover study design where a multinutrient carbohydrate with an amino acid combination or similar placebo was given prior to, during and after a bout of resistance exercise to 15 male strength-trained athletes. Results indicated that resistance exercise performance was significantly improved with the nutrient delivery (as compared to none at all) and markers of muscle damage were attenuated (creatine kinase and C-reactive protein).

A study by Beelen et al. [192] examined the potential to modulate protein synthesis during exercise. The study investigated participants in a fed state and administered a carbohydrate combination with hydrolyzed casein protein combination bolus (0.15 g/kg body mass) prior to the start of a two hour resistance-training session and at 15 min increments throughout the exercise. As a consequence, the carbohydrate and protein combination led to significantly lower protein breakdown rates, stimulated an increase in whole body and muscle protein synthesis rates by $49 \pm 22\%$ during resistance exercise, and achieved a positive net protein balance ($16.3 \pm 0.4 \, \mu m$) whereas whole body net protein balance was negative in the carbohydrate only group ($-4.4 \pm 0.3 \, \mu m$).

Bird and colleagues [172] conducted a study pertaining to the chronic effects of examining 6% carbohydrate with 6 g EAA solution administered throughout resistance exercise (two bouts/week) over twelve weeks. Their results indicated urinary concentrations of histidine were 26% lower with the carbohydrate plus EAA

combination compared to a 52% increase seen in the placebo group. However, these findings may be limited to the amount of EAA consumed as other research has indicated that MPS may be maximally stimulated with 12 g of EAA. Future research in nutrient delivery should look to analyze different doses of EAA and/or combining with different doses of carbohydrate to determine if performance benefits are further enhanced from the protein that is supplied or the amount of carbohydrate combined. In reference to this, Hulmi et al. [196] found no benefit in resistance training adaptations by adding a combination of carbohydrate (34.5 g) + whey protein concentrate (37.5 g) compared to protein supplement alone in a 12 week resistance-exercise protocol.

Post Exercise - Carbohydrate and Protein

A post workout supplement would ideally contain at least 45 g of whey protein to increase the insulin concentration in the optimal range of ~15-30 μIU/mL to reduce proteolysis [197]. This gives rise to the question of efficacy to added carbohydrate supplementation to influence muscle development when sufficient protein is already supplied. In this respect, Staples et al. [198] recruited nine men to perform a single bout of four sets of 8-12 repetitions to failure on knee extension followed by consumption of protein (25 g whey), carbohydrate (25 g maltodextrin), or a combination of said protein with carbohydrate supplements on rates of MPS. The study reported that the carbohydrate plus protein combination failed to produce an added increase in MPS when compared to only protein administration. Further, Rasmussen et al. [199] also reported no difference in amino acid net balance after a bolus ingestion of a drink containing sucrose (35 g) with EAA (6 g) at one or three hours post resistance training. It would

appear that much more literature is needed under this topic to further provide scientific knowledge and practical applications to use these feeding strategies following resistance-exercise.

Recovery Markers

Small molecules, such as proteins, metabolites, and electrolytes, can serve as key biomarkers for athletes and recreationally active individuals in assessing recovery for health and/or performance [55]. Current advances in scientific technology advocate that using intrinsic data such as biochemical and hematological markers can employ a powerful role in identifying stress and recovery in each unique individual. Many commercially available kits are used to assess biomarkers of health, performance, and recovery, in hopes of reducing overtraining or risk of injury, while attempting to better understand recovery and aspects of enhancing it. With this being said, there are still many challenges of biomarker testing; including single biomarkers are not definitive for diagnosing broad physiological "recovery", reference ranges for athletes and recreational individuals are not yet specifically defined, as well as interindividual variance in absolute values and relative changes in biomarkers.

Most researchers agree that multiple cytokines should be measured together to assess physiological function of inflammation [200]. Data utilizing multiple inflammatory cytokines, endocrine markers of dysregulation like testosterone and cortisol, and muscle damage markers such as creatine kinase (CK), can assist in identifying precise and accurate measurements of an athlete's health. Moreover, simply relying on a single marker for accurate information is not well received, given the

simplistic nature of most human biomarkers [71]. Since athletes may display a greater

range or variability in their values compared to non-exercising or average individuals,

absolute or one-time measurements in values of cytokines and biomarkers may not give

meaningful data and responsiveness. A more representative value of biomarkers might

be taken before and after an acute challenging exercise bout. The absolute resting levels

of the biomarkers may not change from baseline, although the stress could be atypical.

In response, the timing of measurement of markers should be assessed over multiple

time points to really understand the fluctuation of biomarkers of an individual in

response to an acute bout of exercise and recovery over the course of hours, days, and

weeks [55]. Testing prior to, and post-exercise will assist in elucidating biomarker

responses to acute exercise. This could be very valuable when resting values fail to

pinpoint any changes.

Rationale of Study

Ingestion of nutrients prior to exercise contributes to fuel availability which may

reduce catabolism during exercise and promote recovery. It appears the implementation

of carbohydrate with protein (or EAA) surrounding, or during, both endurance and

resistance training may be an effective nutrient strategy to positively enhance exercise

performance, adaptations to training, or favorably enhance recovery. Furthermore,

favorable performance benefits to training have been reported with carbohydrate and

protein supplementation in close proximity to exercise bouts, in particular if less than

adequate amounts of carbohydrate are consumed beforehand.

When adequate carbohydrates are consumed, the influence of additional protein appears to have little to no enhancing effect on endurance or resistance exercise performance and/or recovery of muscle glycogen. It seems that if total protein priorities are met throughout the day and therefor daily protein levels are met, the importance of added carbohydrates may be limited. One area of future studies might be in highly trained athletes, where total energy needs might also need to be met because the athletes experience large volumes of training and/or have large amounts of lean body mass. These athletes may require the addition of a carbohydrate + protein to allow the athlete to meet an appropriate energy obligation to impact recovery or muscular adaptations before, during, or after exercise. Towards this end, athletes who must combine resistance training and endurance type training (or sport-specific training) might warrant additions of carbohydrate with protein supplementations in close timing to their sessions to optimize recovery periods and decrease muscle breakdown. Other athletes who might consider the supplementation of carbohydrate with protein supplements to their diets are athletes who must train in the early mornings, and may not have time to consume an adequate meal prior to vigorous exercise expenditure.

It is recommended that athletes consume low to moderate sources of carbohydrate and 10-20 g of high quality protein prior to intense and prolonged training in order to maintain blood glucose availability, prevent hypoglycemia, minimize exercise induced protein degradation during exercise, and stimulate protein synthesis following exercise [1, 201]. However most commercially available energy/food bars contain large amounts of high glycemic carbohydrate and/or low amounts of quality

protein which may not be optimal for athletes to ingest prior to exercise. Additionally, they are typically marketed as in-between meal snacks [202].

We have been working with Nutrabolt to determine the glycemic index (GI) and glycemic load (GL) of an innovative new energy/food bar that contains low-glycemic sources of carbohydrate (IMO and plant fiber) and 20 g of high quality whey protein that would provide more than 6 g of EAA. We have found that his energy/food bar has a low GI of 34 and GL of 8.5 while promoting a similar insulin response to a high GI carbohydrate (dextrose) [203]. Theoretically, this may serve as an optimal pre-exercise source of carbohydrate while stimulating an insulin response thereby reducing catabolism during or post-exercise. Current literature suggests there may be a dose and/or significance in timing effect, however the amount of literature available is rather small, even more so when examining nutrient timing in highly-trained individuals. Additionally, there is a paucity of research surrounding the role nutrient timing, specifically carbohydrate in combination with protein on both endurance and resistance-training.

There is also a need to investigate the effects of ingestion of IMO and exercise performance. IMOs could present an advantage on blood glucose and insulin in healthy adults. Isomalto-oligosaccharides are produced from highly available and relatively low-cost plant materials through simple enzymatic processes. They have also proven their enhancing effects on metabolism, bifidogenic flora, bowel functions, and the immune system. In this context, IMOs could theoretically serve as a low glycemic food option for individuals on a low glycemic diet and/or athletes.

This study will determine whether ingesting this innovative energy/food bar that displays a IMO carbohydrate and protein combination strategy prior to, during, and post exercise will affect performance and/or recovery. In this study, we are using an exercise and recovery protocol that has previously studied the effects of ingesting protein with various forms of carbohydrate on resistance-exercise and recovery [204] to examine the acute effects of ingesting a commercially available low glycemic carbohydrate / whey protein food bar on exercise capacity and recovery from an intense resistance-exercise and sprint conditioning training bout. Successful completion of the study aims could influence the scientific knowledge and practical applications of use of these types of energy/food bars around exercise. Further, this study will add to the understanding of nutrient timing and whether or not it may influence acute intense resistance/conditioning training and recovery.

CHAPTER III

PHARMACOKINETIC STUDY

GLYCEMIC AND INSULINEMIC RESPONSE TO INGESTION OF A NOVEL

FOOD BAR CONTAINING WHEY PROTEIN AND ISOMALTO-

OLIGOSACCHARIDES*

Introduction

Consumers often ingest carbohydrate and protein energy bars in between meals as snacks or prior to exercise in order to increase amino acid availability and/or maintain blood glucose during exercise [77-80]. However, many energy bars or drinks have a relatively high glycemic index (GI) and therefore may not be not suitable for individuals who are glucose intolerant and/or diabetic [79, 81]. Additionally, while it is recommended that athletes ingest carbohydrate and protein prior to exercise [77, 80], ingesting foods, gels, and/or beverages that have high GI's may promote hypoglycemia during exercise and thereby hasten fatigue [77, 79, 80, 82, 83]. For example, we previously reported that ingestion of moderate to low GI carbohydrate gel during prolonged cycling maintained blood glucose and insulin levels to a greater degree than a higher GI gel [83]. Additionally, that adding different types of carbohydrate with low to high GI's to whey protein had differential effects on glucose and insulin responses following intense resistance-exercise [82].

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*Reprinted with permission from "Glycemic and insulinemic response to ingestion of a novel food bar containing when protein and isomalto-oligosaccharides" by Tyler Grubic et al. 2018. *Austin Journal of Nutrition and Food Sciences*, Vol. 6, pp 1-10, Copyright 2018 by Tyler Grubic.

Isomalto-oligosaccharides (IMO) is a prebiotic high fiber, low calorie source of carbohydrate that has been used as a functional food and prebiotic fiber sweetener in Asia for over 3 decades [205-209]. Basic animal studies indicate that IMO's serve as a soluble dietary fiber and can stimulate activity of the probiotic gut flora, improve gut function, and help manage cholesterol in animals fed on a high fat diet [205, 208, 210-212].

Given the interest in developing food and energy bars that provide quality protein with a low to moderate glycemic profile, we sought to determine the glycemic and insulinemic responses of ingesting a whey protein food bar with IMO as the source of carbohydrate. Our primary outcome was assessment of the glycemic insulinemic responses to ingesting this food bar (FB). The secondary outcome was assessment of how ingestion of this FB affected appetite related variables and subjective ratings of hypoglycemic symptoms. We hypothesized that ingestion of a mixed ingredient food bar containing IMO would promote a low to moderate glycemic response and positively affect perceptions about appetite with no evidence of hypoglycemia.

Methods

Experimental Design

This study was conducted with approval by an Institutional Review Board (IRB2016-0830D) and was registered with clinicatrials.gov (#NCT03166514). This study was conducted in two parts at a university-based research setting in randomized, counter-balanced, and crossover manner.
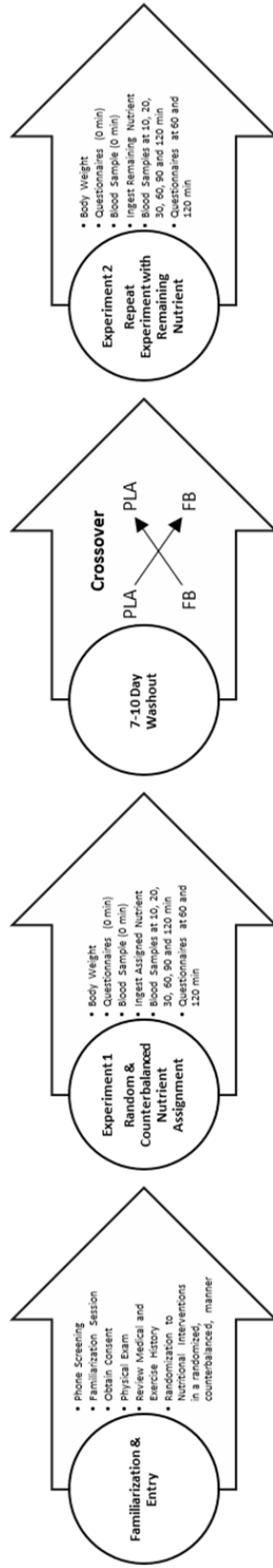
Figure 1: Overview of pharmacokinetic study design. Reprinted with permission from (Grubic, 2018)
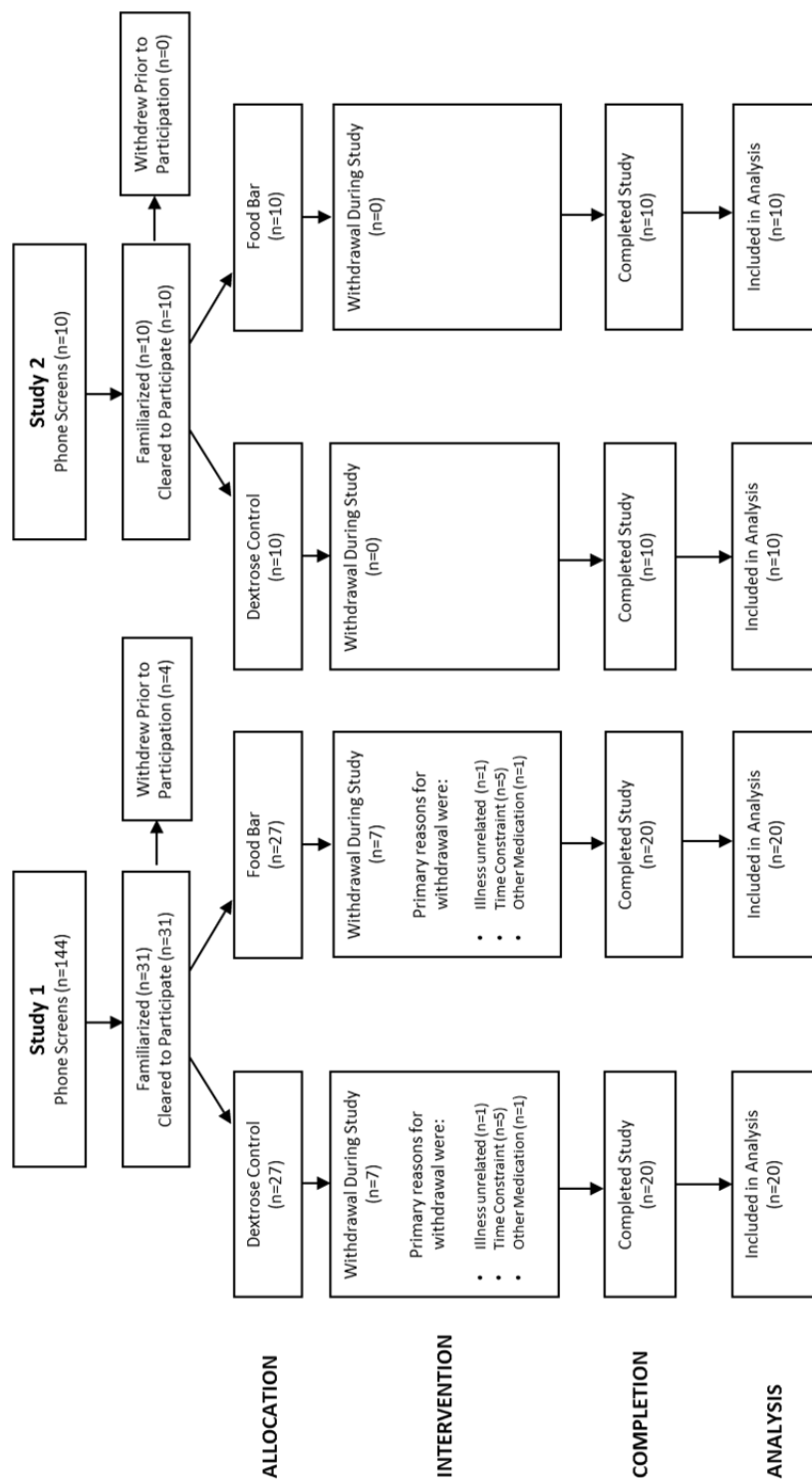
Figure 2. Consort diagram for pharmacokinetic study. Reprinted with permission from (Grubic, 2018)

In both studies, the independent variable was nutrient intake and dependent variables included blood glucose, insulin, and subjective ratings related to appetite and hypoglycemic side effects.

Participants

Apparently healthy men and women between the ages 18–35 years with a body mass index (BMI) less than 25 kg/m2 were recruited to participate in this study. Individuals who expressed interest in participating were screened by phone to determine if they met initial eligibility to participate in this study. Qualified individuals were invited to attend a familiarization session in which participants received a written and verbal explanation of the study design, testing procedures, and read and signed informed consent statements. Those giving consent completed personal and medical histories and had height, weight, blood pressure, and heart rate determined. The research coordinator reviewed medical history forms, physical examination measurements, and determined eligibility to participate. Participants were excluded from the study if they reported: 1.) any uncontrolled metabolic disorders or cardiovascular disorder, including heart disease, a history of hypertension, diabetes, thyroid disease, hypogonadism; 2.) hepatorenal, musculoskeletal, autoimmune, or neurological disease; 3.) they were currently taking prescribed medication or dietary supplements for thyroid, hyperlipidemia, hypoglycemia, anti-hypertensive, anti-inflammatory, or weight loss (e.g. thermogenic compounds) within three months before the start of this study; or, 4.) had any known allergies to some of the nutrients contained in the food bar (i.e., almonds, milk, soy, peanuts, tree nuts, egg, and wheat).

Nutritional Intervention

In a placebo controlled, counterbalanced, and crossover manner, participants ingested a carbohydrate and protein food bar (FB, FitJoy™, Nutrabolt, Bryan TX) containing 20 g of a whey protein blend, 25 g of carbohydrate (13 g fiber and 4 g of sugar) as IMO plant fiber (VitaFiber™, BioNutra North America, Inc. Edmonton, Alberta, Canada), and 7 g of fat (1.5 g saturated) or 25 g of dextrose (PLA, Valeant Pharmaceuticals North America LLC, Bridgewater, NJ, USA). After a 7 to 10-day washout period, participants repeated the experiment while ingesting the remaining nutrient. In Study 1, participants ingested one food bar (FB) containing 220 calories and one 25 g serving of the PLA providing 100 calories (i.e., typical serving size) while in Study 2 participants ingested two FB's and two 25 g servings of the dextrose PLA in order to assess the glycemic responses to ingesting a standard oral glucose tolerance test dose (i.e., 50 g). Participants were given as much time as need to ingest the nutrients but this typically was less than 3-5 minutes.

Testing Sequence

Figure 1 presents the general experimental design employed in both studies. For each experiment, participants were instructed to refrain from exercise for 24 h and fast for 10 h prior to reporting to the lab for testing. Once arriving at the lab, body weight was determined, participants completed appetite and hypoglycemia symptom related questionnaires, and they donated a fasting blood sample. Participants then ingested their assigned nutrient and a timer was started. Blood samples were obtained at 10, 20, 30, 60, 90 and 120 min post-ingestion while responses to questionnaires were obtained 60 and

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120 minutes after ingestion of the assigned nutrient. Participants observed a 7 to 10-day washout period and then repeated the experiment in a crossover manner while ingesting the remaining nutrient.

<div align="center">*Procedures*</div>

Anthropometrics

Body weight and height was determined on a Healthometer Professional Scale model 500KL (Pelstar LLC, Alsip, IL, USA). Heart rate was taken at the radial artery and systolic and diastolic blood pressure was measured using standard procedures [213].

Blood Collection Procedures

Venous catheters were placed in the participant's arm using a BD Insyte Autoguard 20 gauge intravenous (IV) catheter (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) using standard procedures [214, 215]. Blood samples were collected in 8.5 mL BD Vacutainer® serum separation tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were left at room temperature for 15 min prior to being centrifuged at 3,500 rpm for 10 min using a refrigerated (4°C) Thermo Scientific Heraeus MegaFuge 40R Centrifuge (Thermo Electron North America LLC, West Palm Beach, FL, USA) [216]. Serum was then aliquoted into serum storage containers (Eppendorf North America, Inc., Hauppauge, NY, USA) and frozen at -80°C for subsequent analysis.

Blood Chemistry Analysis

Blood glucose was analyzed using a Cobas c111 (Roche Diagnostics, Basel, Switzerland) automated clinical chemistry analyzer. Quality control was performed daily

to determine whether the system calibrated to acceptable standards using two levels of controls. Serum samples were re-run if values were outside the control values or clinical normality. The test-to-test reliability of performing glucose analysis was 2.3±0.03% with a coefficient of variation (CV) of 1.1%. Insulin was assayed in duplicate by using an enzyme-linked immunosorbent assay (ELISA) kit (ALPCO, Salem, NH) and assaying samples with a BioTek ELX-808 Ultramicroplate reader set at an optical density of 450 nm with BioTek Gen5 Analysis software (BioTek Instruments Inc., Winooski, VT). The intra-assay CV for insulin ranged from 2.9% to 6.2%. Glycemic Index (GI) was calculated using the integrated area under the curve (iAUC) change from baseline after FB ingestion divided by the iAUC of the dextrose PLA control normalized to 100 [217, 218]. Glycemic load (GL) values were calculated as the product of the amount of available carbohydrate in the FB times the GI value divided by 100 [217, 218].

Side Effects and Eating Satisfaction Questionnaires

Participants were asked to subjectively rate appetite, hunger, satisfaction from food, feelings of fullness, and amount of energy using a 0 to 10 Likert scale where 0 was none, 2.5 was low, 5 was moderate, and 7.5 was high, and 10 was severe. Participants were also asked to rank the frequency and severity of their symptoms (i.e., hypoglycemia, dizziness, headache, fatigue, stomach upset) using the following scale: 0 (none), 1-4 (light), 5-6 (mild), 7-9 (severe), or 10 (very severe).

Statistical Analysis

Data were analyzed using IBM® SPSS® Version 24 software (IBM Corp., Armonk, NY, USA). The sample size was based on prior research we conducted that

indicated an n-size of 10 – 20 would yield a power of 0.80 on changes in glucose and insulin in response to an oral glucose challenge [82, 83]. Baseline demographic data were analyzed using one-way ANOVA. Data were analyzed using univariate, multivariate and repeated measures general linear models (GLM) with and without gender as a covariate. Since no gender interactions were observed, we report GLM data without the covariate. Wilks' Lambda multivariate tests are reported to describe overall effects of related variables analyzed. Greenhouse-Geisser univariate tests with least significant difference post-hoc comparisons are presented for individual variables analyzed. Delta changes (post-pre) were calculated and analyzed by one-way ANOVA post-hoc analyses. Data are reported as mean (SD) and mean change from baseline with 95% confidence intervals (CI). Changes from baseline were calculated by integrated area under the curve (iAUC) using procedures previously described [219, 220]. Data were considered statistically significant when the probability of type I error was 0.05 or less. Mean changes with 95% CI's completely above or below baseline were considered significantly different [221].

Results

Participant Characteristics

Figure 2 presents a CONSORT diagram for both studies. In study 1, a total of 31 individuals met initial screening criteria and consented to participate in this study. A total of 20 completed the study. In Study 2, a total of 10 individuals met initial screening criteria and consented to participate in this study. A total of 10 completed the study. Table 1 presents participant demographics for the studies. In study 1, participants were

24.3±4.2 yr, 73.1±11.4 kg, and had a body mass index (BMI) of 22.6±3.2 kg/m2. Men were significantly taller, heavier, and had a higher BMI. In study 2, participants were 26.3±3.2 yr, 73.1±11.4 kg, and had a BMI of 21.8±2.0 kg/m2 with men weighing more and having a higher BMI.

Glucose and Insulin

Pharmacokinetic Participant Demographics

	Study 1				Study 2			
	Male	Female	Mean	p-Level	Male	Female	Mean	p-Level
N	10	10			6	4		
Age (y)	25.1±3.1	23.5±5.0	24.3±4.2	0.230	26.2±4.2	26.4±3.2	26.3±3.2	0.894
Height (m)	1.63±0.04	1.52±0.05	1.57±0.04	0.001	1.73±0.07	1.70±0.08	1.72±0.08	0.417
Weight (kg)	70.9±4.7	60.6±7.8	73.1±11.4	0.001	76.6±9.0	66.9±12.6†	73.1±11.4	0.001
BMI (kg/m^2)	23.6±1.3	21.7±1.7	22.6±3.2	0.001	20.8±1.5	22.8±2.2	21.8±2.0	0.023

Data are mean ± SD.

Table 1. Pharmacokinetic participant demographics. Reprinted with permission from (Grubic, 2018)

Table 2 presents glucose and insulin data observed by treatment and gender in Study 1 and 2 while Figure 3 shows mean responses to the treatments over time. Multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.003) effects in study 1 with no gender effects. Univariate analysis revealed significant time and treatment x time interactions in glucose responses. Post-hoc analysis revealed that while blood glucose levels increased in both groups, values in the FB treatment were significantly lower than PLA responses during the first 60 minutes after ingestion. Insulin levels increased over time with no significant differences observed between treatments. In study 2, multivariate analysis revealed overall Wilks' Lambda

time (p=0.001) and treatment x time (p<0.001) effects. In both experiments, glucose and insulin levels peaked 30 minutes after ingestion. Figure 4 presents mean changes with 95% CI's for both studies. Glucose generally increased to a greater degree and for a longer period of time after ingesting the PLA. Interestingly, FB ingestion was only marginally increased from baseline for the first 30 minutes in Study 1 and 10 minutes in Study 2.

The overall AUC for glucose was significantly lower in FB treatment in Study 1 (FB 599±50, PLA 688±78 mmol-h/L, p<0.001) and Study 2 (530±48, PLA 697±67 mmol-h/L, p<0.001). Using the Study 2 values, the FB GI was 76.7±10 with a GL of 19.2±2.5. No significant differences were observed between treatments in the overall insulin AUC (Study 1: FB 2,136±1,073, PLA 1,848±971 µIU/mL-h/L, p=0.38; Study 2: FB 4,185±1,934, PLA 3,888±707 µIU/mL-h/L, p=0.65).

Figure 5 presents iAUC changes from baseline for glucose and insulin. In both studies, the iAUC change from baseline was significantly greater after PLA ingestion (Study 1 FB 60 [CI 48, 71], 160 [134, 186], p<0.001; Study 2 FB 65 [49, 82], 209 [170, 244] mmol-h/L, p<0.001). No significant differences were observed between treatments in insulin iAUC responses (Study 1: FB 1,436 [1,061, 1,811], PLA 1,302 [1,019, 1,585] µIU/mL-h/L, p=0.55; Study 2: FB 1,434 [917, 1,950], PLA 1,236 [842, 1,630] µIU/mL-h/L, p=0.50). In comparison to consuming 50 g of dextrose normalized to 100, the FB had an iAUC derived GI of 34 [CI 23, 46] and a GL of 8.5 [CI 5.6, 11.6].
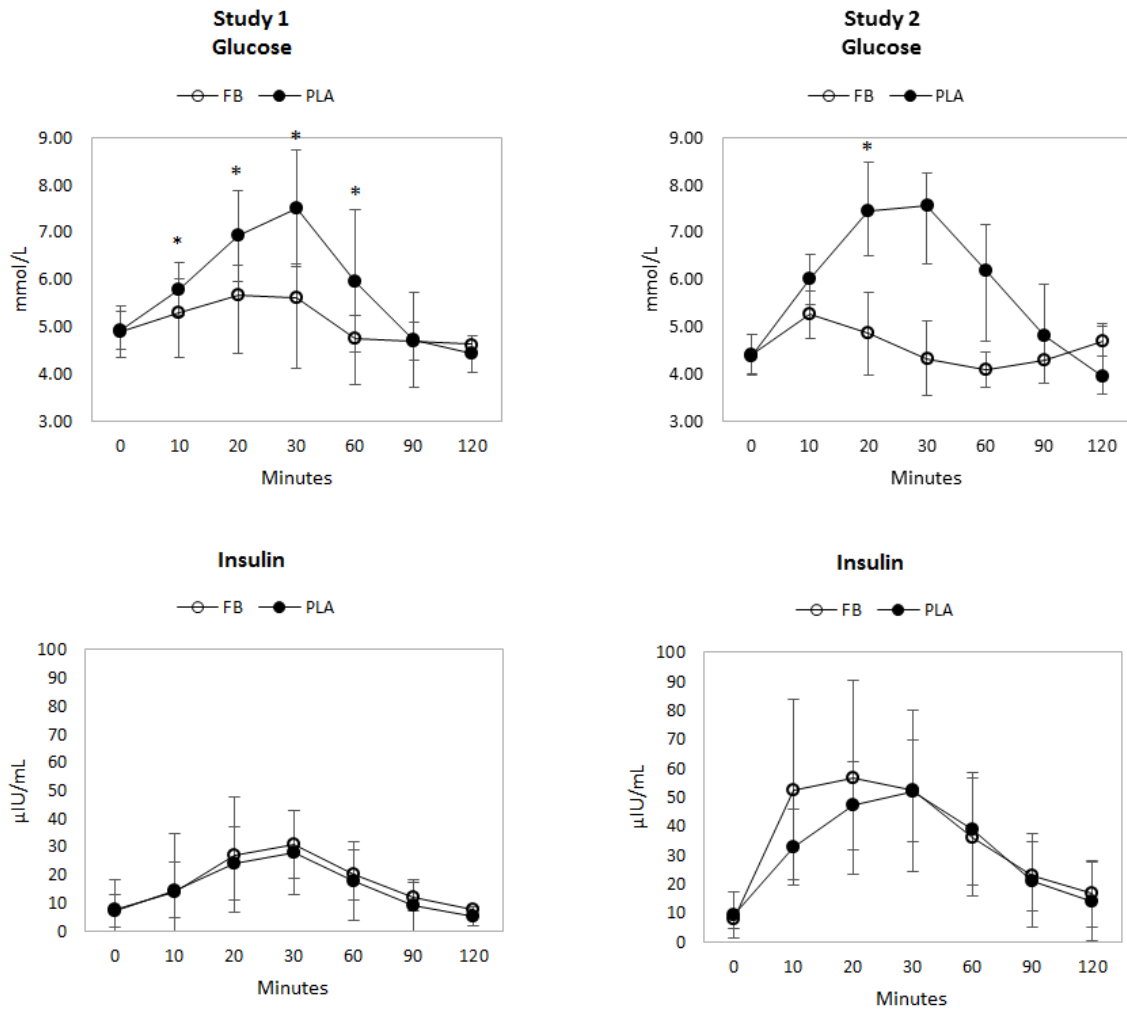
Figure 3. Glucose and insulin values observed in study 1 and study 2 for the placebo (PLA) and Food Bar (FB) treatments. *represents p<0.05 difference between PLA and FB. Reprinted with permission from (Grubic, 2018)

Pharmacokinetic Glucose and Insulin response to an oral glucose challenge.

|	Variable	Treatment	0	10	20	30	60	90	120	Effect	p-Level
Study 1	Glucose (mmol/L)	Time	4.91 ± 0.38	5.63 ± 0.63 †	6.29 ± 1.05 †	6.56 ± 1.36 †	5.36 ± 1.31 †	4.70 ± 0.77 †	4.52 ± 0.40 †	Time	0.001
		FB	4.90 ± 0.36	5.30 ± 0.54 †*	5.67 ± 0.71	5.61 ± 0.62 †*	4.76 ± 0.71 *	4.68 ± 0.49	4.61 ± 0.40 †	Treatment	0.001
		PLA	4.92 ± 0.40	5.79 ± 0.57 †	6.92 ± 0.96 †	7.51 ± 1.24 †	5.96 ± 1.50 †	4.71 ± 1.00 †	4.42 ± 0.39 †	Treatment x Time	0.001
		Male	5.01 ± 0.43	5.76 ± 0.56	6.60 ± 0.92	6.94 ± 1.37	5.49 ± 1.38	4.63 ± 0.59	4.61 ± 0.35	Gender	0.021
		Female	4.81 ± 0.30	5.32 ± 0.57	5.99 ± 1.10	6.18 ± 1.28	5.23 ± 1.26	4.76 ± 0.94	4.42 ± 0.44	Time x Gender	0.129
		FB M	4.97 ± 0.43	5.49 ± 0.47	5.90 ± 0.45	5.82 ± 0.52	4.97 ± 0.80	4.83 ± 0.46	4.80 ± 0.23	Treatment x Gender	0.855
		FB F	4.82 ± 0.29	5.11 ± 0.57	5.43 ± 0.85	5.41 ± 0.67	4.54 ± 0.56	4.54 ± 0.49	4.43 ± 0.46	Treatment x Time x Gender	0.247
		PLA M	5.05 ± 0.45	6.03 ± 0.53	7.29 ± 0.71	8.06 ± 0.95	6.00 ± 1.67	4.44 ± 0.66	4.42 ± 0.35		
		PLA F	4.80 ± 0.33	5.54 ± 0.51	6.54 ± 1.06	6.96 ± 1.29	5.92 ± 1.40	4.98 ± 1.22	4.42 ± 0.44		
	Insulin (µIU/mL)	Time	7.38 ± 5.18	14.23 ± 9.94 †	25.47 ± 16.96 †	29.35 ± 17.96 †	18.82 ± 12.94 †	10.43 ± 9.11 †	6.24 ± 4.42 †	Time	0.001
		FB	7.71 ± 4.66	14.03 ± 10.25	27.05 ± 20.32	30.87 ± 20.68	19.92 ± 12.02	12.03 ± 9.00	7.38 ± 4.95	Treatment	0.453
		PLA	7.04 ± 5.76	14.44 ± 9.89	23.89 ± 13.13	27.83 ± 15.17	17.73 ± 14.03	8.83 ± 9.16	5.09 ± 3.59	Treatment x Time	0.833
		Male	7.87 ± 4.16	15.70 ± 7.95	28.11 ± 13.60	34.24 ± 15.23	19.75 ± 11.91	9.15 ± 8.39	6.13 ± 2.93	Gender	0.001
		Female	6.88 ± 6.11	12.77 ± 11.62	22.83 ± 19.77	24.46 ± 19.49	17.90 ± 14.14	11.71 ± 9.83	6.35 ± 5.62	Time x Gender	0.163
		FB M	7.38 ± 2.93	15.95 ± 9.94	28.95 ± 16.41	34.23 ± 16.28	22.80 ± 9.46	11.98 ± 10.54	7.60 ± 3.21	Treatment x Gender	0.928
		FB F	8.05 ± 6.09	12.10 ± 10.71	25.15 ± 24.38	27.51 ± 24.75	17.05 ± 14.04	12.09 ± 7.75	7.17 ± 6.43	Treatment x Time x Gender	0.527
		PLA M	8.37 ± 5.23	15.44 ± 5.89	27.28 ± 10.93	34.25 ± 15.00	16.71 ± 13.77	6.33 ± 4.44	4.66 ± 1.75		
		PLA F	5.72 ± 6.22	13.43 ± 13.02	20.51 ± 14.79	21.41 ± 13.00	18.74 ± 14.95	11.32 ± 11.99	5.53 ± 4.87		
Study 2	Glucose (mmol/L)	Time	4.39 ± 0.42	5.63 ± 0.63 †	6.15 ± 1.63 †	5.94 ± 1.81 †	5.14 ± 1.30 †	4.54 ± 0.86	4.31 ± 0.89 †	Time	0.001
		FB	4.40 ± 0.42	5.25 ± 0.51 †*	4.85 ± 0.87	4.32 ± 0.79 *	4.08 ± 0.38 *	4.28 ± 0.49	4.69 ± 0.32	Treatment	0.001
		PLA	4.38 ± 0.46	6.02 ± 0.50 †	7.44 ± 1.04 †	7.57 ± 0.67 †	6.19 ± 0.98 †	4.80 ± 1.08	3.94 ± 1.12	Treatment x Time	0.001
		Male	4.56 ± 0.38	5.77 ± 0.70	6.39 ± 1.77	6.08 ± 1.84	5.01 ± 1.25	4.59 ± 1.01	4.27 ± 0.76	Gender	0.334
		Female	4.14 ± 0.37	5.43 ± 0.48	5.78 ± 1.40	5.74 ± 1.88	5.32 ± 1.44	4.47 ± 0.60	4.38 ± 1.10	Time x Gender	0.337
		FB M	4.57 ± 0.36	5.28 ± 0.57	5.06 ± 1.03	4.49 ± 0.94	4.10 ± 0.37	4.41 ± 0.53	4.75 ± 0.28	Treatment x Gender	0.675
		FB F	4.14 ± 0.40	5.20 ± 0.50	4.53 ± 0.52	4.07 ± 0.49	4.06 ± 0.46	4.08 ± 0.39	4.59 ± 0.40	Treatment x Time x Gender	0.697
		PLA M	4.54 ± 0.44	6.26 ± 0.42	7.72 ± 1.27	7.66 ± 0.69	5.93 ± 1.13	4.76 ± 1.38	3.79 ± 0.80		
		PLA F	4.14 ± 0.41	5.65 ± 0.39	7.03 ± 0.42	7.42 ± 0.71	6.58 ± 0.61	4.87 ± 0.52	4.17 ± 1.60		
	Insulin (µIU/mL)	Time	8.44 ± 5.96	42.50 ± 25.47 †	51.84 ± 25.89 †	52.10 ± 22.72 †	37.51 ± 19.47 †	21.87 ± 13.88 †	15.18 ± 12.31 †	Time	0.001
		FB	7.68 ± 3.01	52.54 ± 31.21	56.69 ± 33.64	52.18 ± 27.96	36.07 ± 20.49	22.67 ± 12.06	16.59 ± 11.47	Treatment	0.509
		PLA	9.20 ± 8.05	32.47 ± 13.09	46.99 ± 15.18	52.02 ± 17.55	38.96 ± 19.38	21.07 ± 16.13	13.77 ± 13.56	Treatment x Time	0.410
		Male	6.49 ± 4.20	43.15 ± 31.86	52.04 ± 30.89	55.14 ± 22.63	31.98 ± 18.20	20.00 ± 14.47	11.75 ± 6.97	Gender	0.580
		Female	11.36 ± 7.25	41.53 ± 12.77	51.54 ± 17.85	47.53 ± 23.59	45.81 ± 19.41	24.67 ± 13.39	20.32 ± 16.87	Time x Gender	0.485
		FB M	7.03 ± 3.72	56.84 ± 39.09	58.10 ± 40.62	55.34 ± 25.24	32.82 ± 20.03	18.29 ± 7.49	13.88 ± 5.75	Treatment x Gender	0.673
		FB F	8.65 ± 1.41	46.08 ± 16.81	54.59 ± 25.22	47.44 ± 35.11	40.94 ± 23.21	29.24 ± 15.71	20.65 ± 17.41	Treatment x Time x Gender	0.782
		PLA M	5.95 ± 4.92	29.46 ± 15.98	45.99 ± 19.03	54.95 ± 22.13	31.14 ± 18.06	21.71 ± 19.93	9.63 ± 7.94		
		PLA F	14.07 ± ###	36.99 ± 6.56	48.48 ± 9.11	47.62 ± 8.07	50.68 ± 16.65	20.11 ± 10.77	19.99 ± 18.99		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). In study 1, multivariate analysis revealed overall Wilks' Lambda treatment (p<0.001), time (p<0.001), gender (p=0.072), treatment x time (p=0.003), treatment x gender (p=0.554), time x gender (p=0.86), and treatment x time x gender (p=0.548). In study 2, multivariate analysis revealed overall Wilks' Lambda treatment (p<0.001), time (p=0.001), gender (p=0.001), gender (p=0.494), treatment x time (p<0.001), treatment x gender (p=0.866), time x gender (p=0.631), and treatment x time x gender (p=0.719). Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, M=male, F=female, and GIR=Glucose Insulin Ratio. † denotes p<0.05

Table 2. Pharmacokinetic Study glucose and insulin response to an oral glucose challenge. Reprinted with permission from (Grubic, 2018)
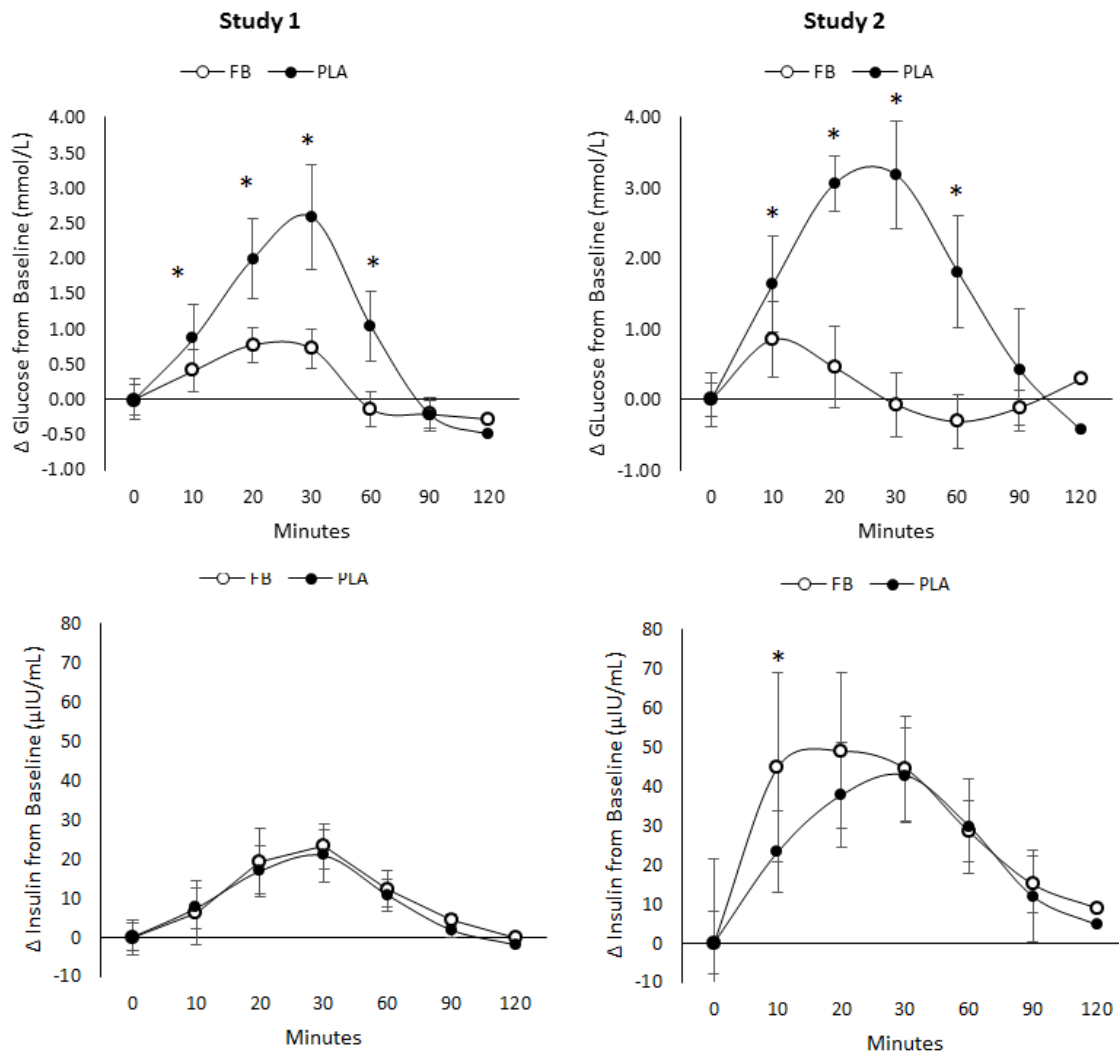
Figure 4. Mean changes with 95% CI's in glucose (top panel) and insulin (bottom panel) during Study 1 and Study 2 for the placebo (PLA) and Food Bar (FB) treatments. Confidence intervals crossing zero are statistically significant (p<0.05). * represents p<0.05 difference between PLA and FB. Reprinted with permission from (Grubic, 2018)
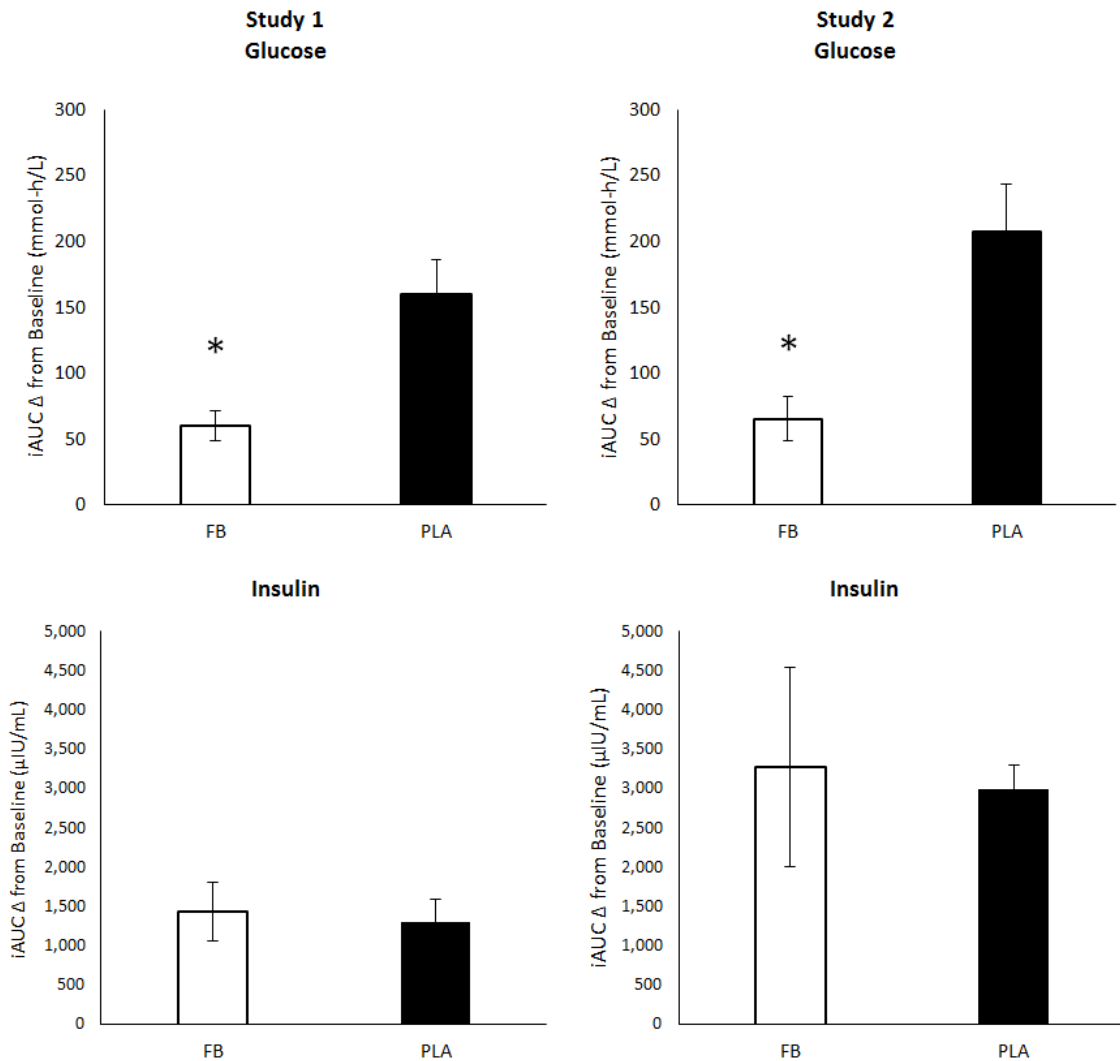
Figure 5. Integrated area under the curve (iAUC) change from baseline for glucose and insulin observed in Study 1 and Study 2 for the placebo (PLA) and Food Bar (FB) treatments. * represents p<0.05 difference between PLA and FB. Reprinted with permission from (Grubic, 2018).

Finally, Table 3 presents responses to eating satisfaction questions. In both experiments, participants reported less subjective ratings of appetite, hunger, and greater satisfaction from food and feeling of fullness. Finally, no significant time, treatment, or

time by treatment effects were observed in subjective ratings of hypoglycemia, dizziness, headache, fatigue, or stomach upset.

Discussion

There is significant interest in developing low glycemic functional foods for consumers trying to maintain healthy blood glucose levels as well as athletes who want to consume low glycemic protein bars [77-80]. However, many protein and energy bars contain large amounts of carbohydrate and/or have a relatively high glycemic index, Therefore, these products may not be not suitable for individuals who are glucose intolerant and/or diabetic [79, 81] or for athletes who may be susceptible to hypoglycemia [77, 79, 80, 82, 83]. Isomalto-oligosaccharides is a prebiotic high fiber, low calorie source of carbohydrate that has been used in functional foods primarily in Asia [205-209]. Reports indicate that IMO serve as a soluble dietary fiber and prebiotic that can promote activity of the probiotic gut flora and improve gut function thereby help manage cholesterol [205, 208, 210-212]. The purpose of this study was to determine the glycemic and insulinemic response of ingesting a whey protein food bar with IMO as the source of carbohydrate. We hypothesized that ingestion of a mixed ingredient food bar containing IMO would promote a low to moderate glycemic response and positively affect perceptions about appetite with no evidence of hypoglycemia.

Pharmacokinetic Eating Satisfaction Inventory

	Variable	Treatment	Minutes 0	60	120	Effect	p-Level
Study 1	Appetite	Time	5.77 ± 2.08	4.87 ± 1.90 †	5.40 ± 2.18 †	Time	0.013
		FB	6.40 ± 1.82	4.55 ± 1.76 †	4.65 ± 2.21 †*	Treatment	0.001
		PLA	5.15 ± 2.18	5.20 ± 2.02	6.15 ± 1.93 †	Treatment x Time	0.001
	Hunger	Time	5.63 ± 2.11	4.75 ± 2.16 †	5.77 ± 1.92	Time	0.006
		FB	5.80 ± 2.46	4.05 ± 2.06 †*	4.75 ± 1.83 †*	Treatment	0.453
		PLA	5.45 ± 1.73	5.45 ± 2.06	6.80 ± 1.40 †	Treatment x Time	0.002
	Satisfaction	Time	0.53 ± 1.99	5.07 ± 2.38 †	4.52 ± 2.08 †	Time	0.001
		FB	0.55 ± 1.76	6.50 ± 1.57 †*	5.65 ± 1.50 †*	Treatment	0.453
		PLA	0.50 ± 2.24	3.65 ± 2.21 †	3.40 ± 1.98 †	Treatment x Time	0.013
	Fulllness	Time	2.85 ± 2.08	5.05 ± 2.01 †	3.87 ± 2.10 †	Time	0.001
		FB	2.85 ± 2.11	5.85 ± 2.13 †*	5.15 ± 1.76 †*	Treatment	0.453
		PLA	2.85 ± 2.11	4.25 ± 1.55 †	2.60 ± 1.60	Treatment x Time	0.002
	Energy	Time	5.72 ± 1.71	6.03 ± 1.63	5.90 ± 1.32	Time	0.420
		FB	5.55 ± 1.85	6.30 ± 1.49	6.20 ± 1.40	Treatment	0.077
		PLA	5.90 ± 1.59	5.75 ± 1.74	5.60 ± 1.19	Treatment x Time	0.103
Study 2	Appetite	Time	5.80 ± 2.09	3.85 ± 2.32 †	5.10 ± 2.63	Time	0.009
		FB	6.00 ± 2.71	2.80 ± 2.39 †*	3.60 ± 2.22 †*	Treatment	0.001
		PLA	5.60 ± 1.35	4.90 ± 1.79	6.60 ± 2.17 †	Treatment x Time	0.020
	Hunger	Time	6.00 ± 1.97	3.75 ± 2.27 †	5.15 ± 2.87 †	Time	0.002
		FB	6.20 ± 2.30	2.50 ± 2.22 †*	3.50 ± 2.46 †*	Treatment	0.453
		PLA	5.80 ± 1.69	5.00 ± 1.56	6.80 ± 2.30 †	Treatment x Time	0.009
	Satisfaction	Time	0.60 ± 1.43	4.40 ± 2.82 †	3.90 ± 2.81	Time	0.001
		FB	0.40 ± 1.27	5.00 ± 2.91 †	4.90 ± 2.81 †	Treatment	0.453
		PLA	0.80 ± 1.62	3.80 ± 2.74 †	2.90 ± 2.56 †	Treatment x Time	0.145
	Fulllness	Time	1.90 ± 1.83	5.35 ± 0.57 †	2.62 ± 3.80 †	Time	0.001
		FB	1.50 ± 1.72	6.50 ± 0.63 †*	2.55 ± 5.10 †*	Treatment	0.453
		PLA	2.30 ± 1.95	4.20 ± 0.54 †	2.25 ± 2.50	Treatment x Time	0.020
	Energy	Time	5.85 ± 1.84	6.15 ± 2.23	6.10 ± 1.71	Time	0.632
		FB	6.40 ± 1.51	6.90 ± 1.45	6.80 ± 1.32	Treatment	0.077
		PLA	5.30 ± 2.06	5.40 ± 2.68	5.40 ± 1.84	Treatment x Time	0.799

Data are means ± standard deviations (SD) or standard error of the mean (SEM). In study 1, multivariate analysis revealed overall Wilks' Lambda treatment ($p<0.001$), time ($p=0.001$), and treatment x time ($p=0.008$). In study 2, multivariate analysis revealed overall Wilks' Lambda treatment ($p<0.122$), time ($p=0.013$), and treatment x time ($p=0.424$). Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, M=male, F=female. † denotes $p<0.05$ difference from baseline. * $p<0.05$ difference between PLA and FB.

Table 3. Pharmacokinetic Study eating satisfaction inventory. Reprinted with permission from (Grubic, 2018)

Results of this study support this contention. In this regard, we found that the glycemic and insulinemic response of ingesting one and two servings of this FB were much more favorable than ingesting equivalent amounts of reference carbohydrate.

Analysis of iAUC changes from baseline which has been suggested to be a more accurate assessment of glycemic response to ingesting food [222, 223] indicated that the FB study had a low glycemic index (34 [CI 23, 46]) and glycemic load 8.5 [CI 5.6, 11.6] [222] when normalized to the dextrose reference. Glucose levels increased less than 15% from fasting values after FB ingestion compared to an increase of up to 73% with dextrose. Additionally, although the treatments differed in caloric content and sweetness which influence perceptions about appetite, hunger, and satiety [224]; ingestion of the energy/food bar also decreased perceptions of appetite and hunger and increase feelings of fullness with no symptoms associated with hypoglycemia. These findings indicate that the food bar studied may be a good food choice for individuals on low glycemic diets and/or trying to manage weight [225-232].

Interestingly, even though glucose levels were only modestly increased following FB ingestion, insulin levels increased in both groups with values generally higher following FB ingestion. There are several possible reasons for this finding. First, there is some evidence that amino acid ingestion can modestly increase insulin levels and that ingestion of protein or amino acids with carbohydrate may promote a greater effect [233-236]. So, since the FB treatment contained 20 g of whey protein, this may have contributed to this finding. Second, although IMO is a prebiotic, it is a type of oligosaccharide that has been reported to stimulate growth of "friendly" bacteria and thereby promote activity of the probiotic gut flora and improve gut function [208, 237-239]. Therefore, it is possible that intestinal absorption of glucose was enhanced thereby serving to help maintain blood glucose levels to a greater degree while the increased

availability of amino acids served to stimulate insulin levels. Additional research should examine potential mechanisms associated with these findings.

It is also important to note that changes in blood glucose and insulin, macronutrient content of a food, portion size, perceptions about sweetness, and energy content of a food affect subjective ratings of satiety as well as secretion of appetite-related hormones [240, 241]. Generally, hypoglycemia stimulates appetite and hunger while increases in blood glucose and insulin after consuming food reduces appetite and hunger. In this study, perceptions about appetite and hunger decreased while satisfaction with food and feelings of fullness increased to a greater degree with FB treatment despite blood glucose levels increasing by less than 15%. While this may simply be related to these other factors [240], it is interesting that these findings were observed with only a modest increase in blood glucose. Additional research is needed to examine how IMO and foods using IMO as a carbohydrate source influence satiety.

The maintenance of blood glucose while observing a similar or higher increase in insulin also has some potential applications for individuals involved in exercise training. It is recommended that athletes consume low to moderate sources of carbohydrate with 10 to 20 g of high quality protein prior to intense and prolonged exercise in order to maintain blood glucose availability, prevent hypoglycemia, minimize exercise induced protein degradation during exercise, and stimulate protein synthesis [77, 78, 80, 82]. However most commercially available energy/food bars contain large amounts of high glycemic carbohydrate and/or low amounts of quality protein which may not be optimal for athletes to ingest prior to exercise. Additionally, they are typically marketed as in-

between meal snacks or meal replacements rather than to optimize nutrient availability around exercise [202]. The energy/food bar studied contains a low glycemic source of carbohydrate (IMO and plant fiber) and 20 g of high quality whey protein that would provide more than 6 g of essential amino acids (EAA). We found that this energy/food bar has a low GI, elicited only a modest increase in blood glucose levels, and promoted a similar increase in insulin as compared to a high GI carbohydrate (dextrose). Theoretically, this may serve as an optimal pre-exercise source of carbohydrate for active individuals because in can provide a more sustained release of glucose while stimulating insulin and thereby lessening exercise-induced catabolism during exercise [77, 78, 80, 82]. Additional research should evaluate whether ingestion of this energy/food bar prior to, during, and/or following intense exercise can help maintain blood glucose level, reduce markers of catabolism, and/or promote recovery.

In conclusion, using IMO as a carbohydrate source in a protein energy/food bar promoted a significantly lower glycemic response while still stimulating insulin release. The protein/food bar had a low glycemic index (34 [CI 23, 46]) and glycemic load 8.5 [CI 5.6, 11.6] [222] when normalized to the dextrose reference. It also reduced perceptions related to appetite with no effect on hypoglycemia related symptoms. Thus, this protein/food bar may serve as a low glycemic food option for individuals on a low glycemic diet or trying to maintain weight and/or athletes interested in optimizing nutrient availability around exercise. Additional research should evaluate the potential benefits of using IMO as a carbohydrate source in functional foods as well as other potential health effects of increasing dietary availability of IMO.

CHAPTER IV

EXERCISE STUDY

EFFECTS OF INGESTING A FOOD BAR CONTAINING WHEY PROTEIN AND

ISOMALTO-OLIGOSACCHARIDES ON PERFORMANCE AND RECOVERY

FROM AN ACUTE BOUT OF RESISTANCE-EXERCISE AND SPRINT

CONDITIONING*

Introduction

Ingestion of carbohydrate and protein prior to, during, and/or following exercise has been reported to enhance energy substrate availability, sustain exercise performance, and promote recovery [77, 242]. For this reason, active individuals often ingest energy drinks, gels and/or bars prior to, during, and/or following exercise [77-79, 242]. However, most commercially available energy drinks, gels, and bars have a relatively high glycemic index (GI) and therefore may not be not suitable for individuals who are glucose intolerant, diabetic, or susceptible to hypoglycemia during exercise [77, 79, 82, 83, 242]. There has been significant interest in identifying how carbohydrate protein, and/or amino acids consumption, influence exercise capacity and/or performance. Research has shown that different types of carbohydrate and protein can have varying effects on substrate availability, exercise metabolism, performance, and/or recovery.

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*This paper "Effects of ingesting a food bar containing whey protein and isomalto-oligosaccharides on performance and recovery from an acute bout of resistance-exercise and sprint conditioning" has been prepared and submitted for an invited special edition of Nutrients on "Integrated Role of Nutrition and Physical Activity for Lifelong Health".

For example, we previously reported that ingestion of moderate to low GI carbohydrate gel during prolonged cycling maintained blood glucose and insulin levels to a greater degree than a higher GI gel [83]. Additionally, adding different types of carbohydrate with low to high GI's to whey protein had differential effects on glucose and insulin responses following intense resistance-exercise [82]. Based on this type of research, it has been recommended that athletes consume low to moderate GI carbohydrate prior to and during exercise [77, 242]. Moreover, consuming whey protein and/or essential amino acids prior to, during, and/or following intense exercise can enhance protein synthesis [77, 242]

Isomalto-oligosaccharides (IMO) is a prebiotic high fiber, low calorie source of carbohydrate that has been used as a functional food and prebiotic fiber sweetener in Asia for over 3 decades [205-209]. Basic animal studies indicate that IMO's serve as a soluble dietary fiber and can stimulate activity of the probiotic gut flora, improve gut function, and help manage cholesterol in animals fed on a high fat diet [205, 208, 210-212]. Given the interest in developing food and energy bars that provide quality protein with a low to moderate glycemic profile, we previously reported that ingesting a whey protein energy bar with IMO as the source of carbohydrate had a GI of 34 and a glycemic load of 8.5 [243]. Additionally, that ingesting this energy bar increased insulin to a greater degree while maintaining blood glucose compared to a dextrose control [243]. Theoretically, ingestion of this food bar prior to, during, and/or following intense exercise could maintain blood glucose and increase insulin levels during exercise, lessen the catabolic effects of intense exercise, and/or hasten recovery.

The purpose of this study was to determine if ingesting this low-glycemic food bar prior to, during, and following intense exercise would affect glucose homeostasis, exercise performance and/or recovery. The primary outcome measure was glucose homeostasis during and following exercise. Secondary outcome measures included assessment of performance, ratings of muscle soreness, markers of catabolism and inflammation, and subjective ratings of appetite, hypoglycemia, and readiness to perform. We hypothesized that ingestion of the FB studied would better maintain glucose homeostasis than placebo, better maintain exercise capacity during intense training, and hasten recovery.

Materials and Methods

Experimental Design

This study was conducted at a university research setting with approval by an Institutional Review Board (IRB2017-0602) in compliance with the Declaration of Helsinki standards for ethical principles regarding human participant research and was registered with clinicatrials.gov (#NCT03704337). This study was conducted in a randomized, counter-balanced, crossover, and open label manner. The independent variable was nutrient intake. The primary outcome measure was glucose homeostasis as determined by assessing glucose and insulin responses. Secondary outcome measures included assessment of performance as determined by assessing resistance-exercise lifting volume, agility and sprint performance, and isokinetic strength; and, recovery as determined by assessing ratings of muscle soreness; markers of catabolism, stress, and inflammation; and, ratings of readiness to perform. Additionally, dietary energy and

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macronutrient, subjective ratings of symptoms of hypoglycemia and subjective ratings of appetite and eating satisfaction were assessed.

Participants

Twelve highly-trained men between the ages 18–35 years with a body fat percentage (BF%) less than 25%, or body mass index (BMI) less than 25 kg/m2, were recruited to participate in this study. Participants were required to have the capability to bench press their body weight and barbell squat at least 1.5 times their body weight; have been engaged in a resistance training program involving upper and lower body exercises for the last year; and, involved in sprint conditioning training for the last six months. Individuals who expressed interest in participating in the study were screened by phone to determine if they met initial eligibility to participate in this study. Qualified individuals were invited to attend a familiarization session in which participants received a written and verbal explanation of the study design, testing procedures, and read and signed informed consent statements. Those giving consent completed personal, training, and medical histories and had a physical examination by a research assistant. The research coordinator reviewed medical history forms, physical examination measurements, and determined eligibility to participate. Participants were excluded from the study if they reported: 1.) any uncontrolled metabolic disorders or cardiovascular disorder, including heart disease, a history of hypertension, diabetes, thyroid disease, hypogonadism; 2.) hepatorenal, musculoskeletal, autoimmune, or neurological disease; 3.) they were currently taking prescribed medication or dietary supplements for thyroid, hyperlipidemia, hypoglycemia, anti-hypertensive, anti-inflammatory, weight loss (e.g.

thermogenic compounds) within three months before the start of this study; 4.) had any known allergies to some of the nutrients contained in the food bar (i.e., almonds, milk, soy, peanuts, tree nuts, egg, and wheat); 5.) did not meet BF% or BMI criteria; or, 6.) did not meet bench press and/or squat one repetition maximum (1RM) criteria. Figure 6 presents a Consolidated Standards of Reporting Trials (CONSORT) diagram for the study. A total of 43 individuals passed phone screens, 17 participants gave consent to participate in the study and underwent familiarization, 12 individuals met all screening criteria and were allocated to the study with all of these participants completed the study.

Nutritional Intervention

In a placebo controlled, counterbalanced, crossover, and open label manner; participants ingested 25 grams of dextrose gel (*Valeant Pharmaceuticals North America LLC, Bridgewater, NJ, USA*) which served as a carbohydrate-matched placebo (PLA) or a commercially-available food bar (FB, *FitJoy™, Nutrabolt, Bryan TX*) containing 20 g of a whey protein, 25 g of carbohydrate as IMO plant fiber (*VitaFiber™, BioNutra North America, Inc. Edmonton, Alberta, Canada*) consisting of 13 g fiber and 4 g of sugar, and 7 g of fat (1.5 g saturated fat) prior to, during, and following intense exercise. One FB contained 220 calories while the PL contained 100 calories of carbohydrate. Participants were given as much time as need to ingest the nutrients which typically lasted less than 3-5 minutes. The rationale in using a carbohydrate matched dextrose gel rather than a iso-caloric amount of carbohydrate is that athletes typically ingest carbohydrate drinks and/or gels prior to and during exercise so efficacy of the FB would need to be established compared to common practice; the amount of carbohydrate was

consistent with recommendations of the amount of carbohydrate per hour athletes should consume (i.e., 30 – 60 g/h or carbohydrate); providing an iso-caloric amount of carbohydrate gel to match the energy intake of the FB (i.e., 3 x 55 g per servings over a 1.25 hr period of training) would have likely promoted hypoglycemia and impaired exercise performance; and, costs of manufacturing an energy bar containing all nutrients with a different source of carbohydrate for this initial exploratory study was cost prohibitive..After a 7-day washout period, participants repeated the experiment while ingesting the remaining nutritional intervention.

Testing Sequence

Figure 7 presents the general experimental design employed in this study. Participants were instructed to refrain from non-steroidal anti-inflammatory drug (NSAID) and pain relief medication for 48 h, exercise for 24 h, and fast for 10 h prior to reporting to the lab for testing. Once arriving at the lab participants completed appetite, hypoglycemia, and readiness to perform related questionnaires, and donated a fasting blood sample. Baseline ratings of pain to a standard amount of pressure applied to several locations on the thigh, isokinetic muscular strength and endurance measurements, and arterialized-venous glucose measurements from a finger were then obtained. Participants then ingested their assigned nutrient (PLA or FB) and rested passively for 30 min. Participants then completed a rigorous resistance-training exercise protocol consisting of 11 total upper and lower body exercises. Midway through the exercise session, participants ingested another serving of the PLA or FB. After the resistance-exercise was completed, participants performed three 40-yard (FYD) and

three repeated Nebraska Agility Drills (NAD) utilizing a 1:4 work to rest ratio. Arterialized-venous samples were also taken immediately before exercise, midway during resistance-exercise, following resistance-exercise, following performing the sprints, and following isokinetic testing. After completing the exercise bout, participants completed questionnaires, donated a venous blood sample, rated pain to standard pressure applied to the thigh, and performed isokinetic tests. Participants consumed a final serving of PLA or FB prior to leaving the lab and were instructed not to eat any additional food for another 2 h. Participants refrained from exercise and NSAID or pain relief medication during the 48-h recovery period. Participants then reported to the lab two days later after fasting for 10 h. Participants then donated a venous blood sample, rated pain to a standard amount of pressure applied to the thigh, and performed isokinetic testing. Participants observed a 7-day washout period and then repeated the experiment in a crossover manner while ingesting the alternative nutrient.
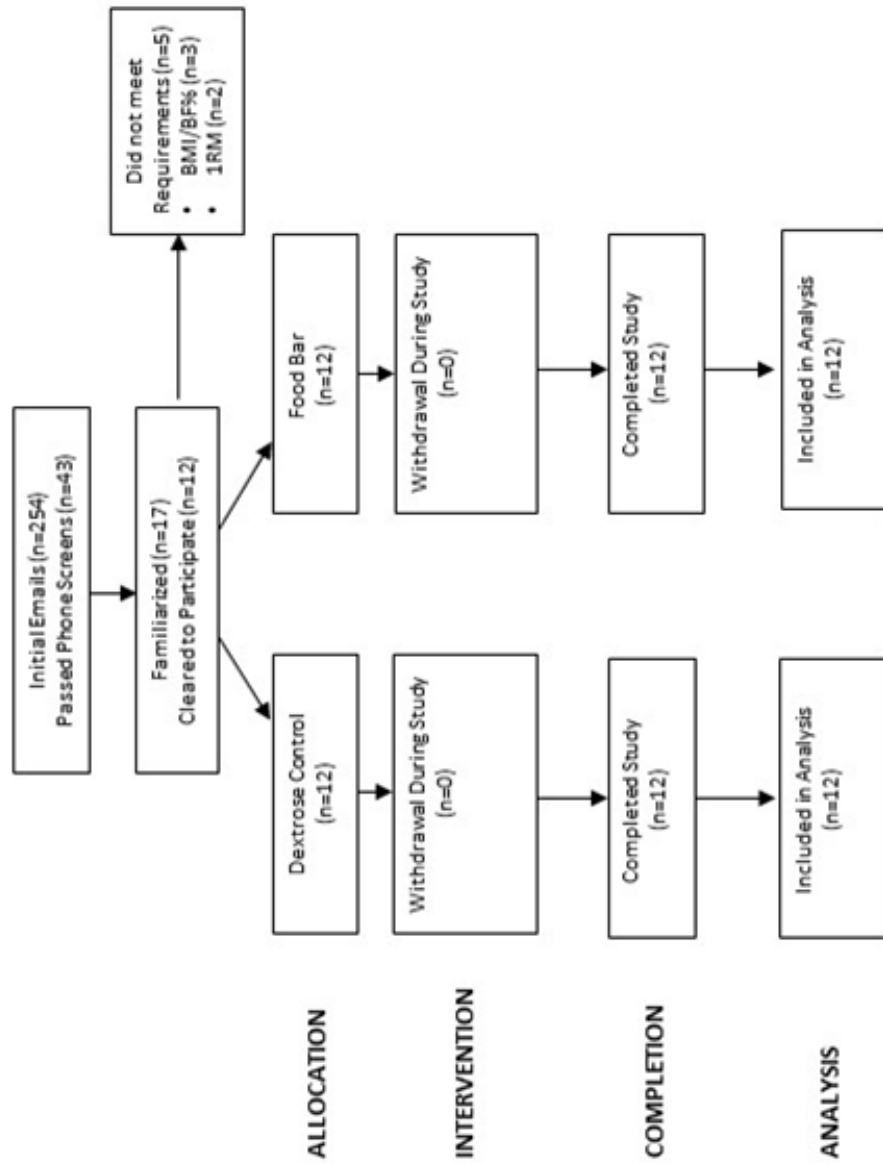
Figure 6. Exercise Study Consolidated Standards of Reporting Trials (CONSORT) diagram
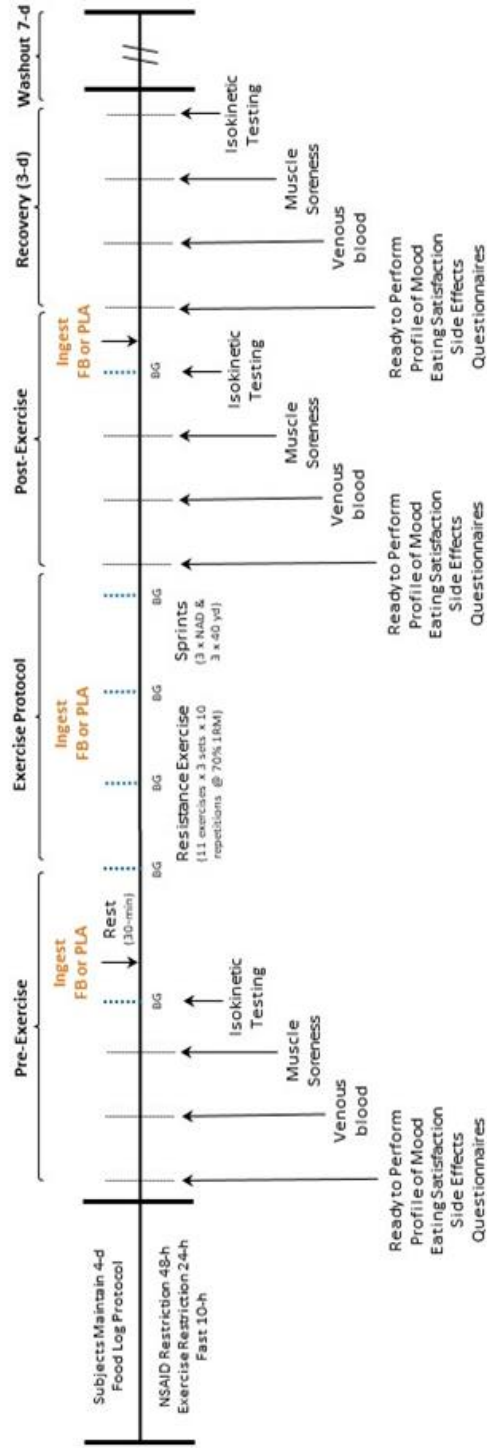
Figure 7. Timeline for testing. NSAID = non-steroidal anti-inflammatory drugs, FB = food bar, PLA = placebo, 1RM = one repetition maximum, BG = blood glucose, NAD = Nebraska Agility Drill).

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Procedures

Demographics

Body weight and height was determined on a Healthometer Professional Scale

model 500KL (Pelstar LLC, Alsip, IL, USA). Heart rate was taken at the radial artery

and systolic and diastolic blood pressure was measured using standard procedures [213].

Body composition was determined with a Hologic Discovery W Dual-Energy X-ray

Absorptiometer (DXA; Hologic Inc., Waltham, MA, USA) equipped with APEX

Software (APEX Corporation Software, Pittsburg, PA, USA). Test-retest reliability

studies performed with this DXA machine have previously yielded mean coefficients of

variation for bone mineral content and lean mass of 0.31-0.45% with a mean intra-class

correlation of 0.985 [244].

Dietary Assessment

Participants were instructed to record all food and beverage intakes each week

that they were involved within the study protocol on 4-day dietary food questionnaires (3

weekdays, 1 weekend day), which is reflective of their average dietary intake on normal

days. Food records were entered and analyzed with Food Processor Nutrition Analysis

Software Version 11.2.285 (Esha Nutrition Research, Salem, OR) and analyzed for

average energy and macronutrients by study researchers [245].

Resistance Exercise Protocol

During the familiarization testing session participants followed a protocol to

determine 1RM for chest press, barbell squat, wide-grip latissimus dorsi (lat) pull, leg

press, incline bench press, dumbbell lunges, seated row, leg extension, dumbbell curls,

triceps rope press-down, and biceps curls [246]. For exercises in which 1RM was exceeded by available weights, the Epley formula was used to predict the 1RM based on the number of repetitions performed at a given weight [247]. Rest periods between participants was not limited during 1RM determination so that the participants had sufficient opportunity to reach their true maximum weight, however participants were encouraged to try to reach their 1RM within 3-5 sets of their warmup set per agreement with NSCA 1RM testing protocols [248]. During the resistance exercise protocol, each participant performed three sets of 10 repetitions with approximately 70% of their 1RM for each of the 11 total exercises (i.e., chest press, barbell squat, wide-grip lat pull, leg press, incline bench press, lunges, seated row, leg extension, dumbbell curls, rope press-down, and preacher curls) [246]. Each set was followed by a 2-minute rest period. All lifting was performed under the supervision of researchers and a certified strength and conditioning coach. If a participant could not complete the full 10 repetitions at the 70% 1RM load, the weight was immediately reduced so that the 10 repetitions could be completed. The weight and the number of repetitions was recorded by researchers on each participant's workout card immediately following each completed set, so that total lifting volume could later be calculated. Test-to-test reliability for total lifting revealed a mean Cv of 0.012 with a mean intraclass correlation of 0.996.

Conditioning Drills

Directly following the resistance-exercise protocol, each participant performed three 40-yard sprint trials separated by about 20-seconds of rest in between, to implement a 1:4 work to rest interval ratio. When ready, the participant lined up at the

starting line and was instructed to sprint as fast as they could all the way through the finish line. Participants were also instructed to start in a static position, but had the option to start in a three point stance or standing, and had to maintain the same starting position for each time-trial. The recorded time for the 40-yard dash began on the participant's first motion forward and ended once the participant crossed the finish line at 40-yards [249-251]. The test was performed on the same gym floor for each participant with lines denoting start and stop points. Test-to-test reliability for the 40-yard dash sprint times revealed a mean Cv of 0.184 with a mean intraclass correlation of 0.916. Participants then performed three NAD agility tests. The NAD is designed to test agility and change of direction skills [252]. The test is set up using four cones. Two cones are set up in line with one another five yards apart. One set of cones are offset by one yard. Participants are asked to sprint 5-yd to the cone on the next line, change direction and sprint back to the next cone, change direction and sprint to the last cone. Timing began on the participant's first motion forward and ended once the participant crossed the last cone. Each participant completed three trials of this drill for time, implementing a 1:4 work to rest ratio. Test-to-test reliability for the NAD sprint times revealed a mean Cv of 1.128 with a mean intraclass correlation of 0.792.

Muscle Soreness Assessment

A Commander algometer (JTECH Medical, Salt Lake City, UT, USA) was used to apply a standardized amount of pressure (50 N) applied to the distal medialis (VM) at 25% of the distance from the patella to the greater trochanter near the hip and the distal vastus lateralis (DVL) at 25% and mid-lateral vastus medialis (MLVL) at 50% of the

distance between the patella to the greater trochanter. The three sites were marked with permanent ink to standardize the location of assessment. Participants were asked to sit with both legs straight on a bench while the algometer measurements were taken. Pressure was applied to each site for 3-sec [204]. Participants were asked to rate their perception of muscle soreness using a visual analog GPRS at each site. The GPRS consisted of a straight horizontal-line with no hash-markings and only wording beneath the line. The line read from left-to-right "no pain, dull ache, slight pain, more slight pain, painful, very painful, and unbearable pain". Participants were instructed to scribe one clear mark bisecting the line which represented their pain level the best for each of the three pressure application sites. A ruler was used to measure the participant's mark from the left-to-right in cm and was recorded in the data as such numerical value. Testing order (i.e., VM, DVL, MLVL) was standardized across all testing sessions for all participants. Participants recorded their perceived level of soreness on the GPRS evaluation line before moving onto the next site. Test-to-test reliability for this protocol revealed a mean intraclass correlation of 0.90 [253].

Isokinetic Assessment

Participants performed a maximum voluntary contraction (MVC) isokinetic knee extension and flexion protocol at a speed of 60 degrees/sec on their dominant leg using the Kin-Com 125AP Isokinetic Dynamometer (Chattanooga-DJO Global Inc., Vista, CA, USA). Body and knee positioning were pre-determined during a familiarization session, and recorded using standard procedures to accurately ensure testing was repeatable and to decrease any between-testing variability for all isokinetic tests

performed throughout the testing duration. Each participant went through a warm up protocol prior to testing by performing three sets of five repetitions of knee extension and flexion at approximately 50% of their MVC with one minute between sets. One minute after completing the final warm-up set, participants performed 3 MVC's of knee extension and flexion [204]. Test to test variability of performing this test yielded mean Cv values ranging from 0.1041 to 0.1340 with mean intraclass correlations ranging from 0.700 to 0.881 for leg extension variables and mean Cv values ranging from 0.098 to 0.1389 with intraclass correlations ranging from 0.905 to 0.963 for leg flexion variables.

Blood Collection and Analysis

Arterialized-venous blood samples were obtained from a clean and dried finger and measured for blood glucose using an Accu-Check Aviva Plus Blood Glucose Monitoring System (Roche Diagnostics, Indianapolis, IN, USA). New test strips were used each new test for each participant as per instructions described in the user manual. Additionally, approximately 20 mL of venous blood was collected in 8.5 mL BD Vacutainer® serum separation tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) using standard procedures [214, 215]. Samples were left at room temperature for 15 min prior to being centrifuged at 3,500 rpm for 10 min using a refrigerated (4°C) Thermo Scientific Heraeus MegaFuge 40R Centrifuge (Thermo Electron North America LLC, West Palm Beach, FL, USA) [216]. Serum was aliquoted into serum storage containers (Eppendorf North America, Inc., Hauppauge, NY, USA) and frozen at -80°C for subsequent analysis. Serum markers of catabolism were analyzed using a Cobas c111 (Roche Diagnostics, Basel, Switzerland) automated clinical chemistry analyzer. Quality

control was performed daily to determine whether the system calibrated to acceptable standards using two levels of controls. Serum samples were re-run if values were outside the control values or clinical normality. The test-to-test reliability of performing glucose analysis was 2.3±0.03% with a coefficient of variation (CV) of 1.1%. Serum insulin, testosterone, and cortisol were analyzed using an Immulite 2000 analyzer (Siemens Healthcare GmbH, Henkest, Erlangen, Germany). Serum inflammatory markers [interleukin-1β (IL-1β), IL-4, IL-6, IL-8, IL-13, tumor necrosis factor- α (TNF-α), interferon- γ (IFN-γ)] were measured using a MILLIPLEX Human High Sensitivity T-Cell Magnetic Bead Panel kit (EMD Millipore Corporation, St. Charles, MO, USA). Cytokine and chemokine measurements were assed using a Luminex MagPix instrument ((Luminex Corporation, Austin, TX, USA) which requires a minimum of 50 positive beads for each human sample. This instrument has been reported to be highly reliable and valid [254-257]. Controls and all samples were run in duplicate according to standard procedures to ensure validity. The CV's for these assays ranged between 0.02 to 1.73%.

Questionnaires

Participants were asked to subjectively rate appetite, hunger, satisfaction from food, feelings of fullness, and amount of energy using a 0 to 10 Likert scale where 0 was none, 2.5 was low, 5 was moderate, and 7.5 was high, and 10 was severe. Test to test variability of performing this survey yielded mean Cv's ranging from 0.372 to 0.784 with mean intraclass correlations ranging from 0.157 to 0.748 for the items on the survey. Participants were asked to rank the frequency and severity of their symptoms

(i.e., hypoglycemia, dizziness, headache, fatigue, stomach upset) using the following scale: 0 (none), 1-4 (light), 5-6 (mild), 7-9 (severe), or 10 (very severe). Test to test variability of performing this survey yielded mean Cv's ranging from 0.731 to 1.246 with mean intraclass correlations ranging from 0.507 to 0.882 for the items on the survey. Participants were also asked to rank their readiness to perform using the following scale: 1 (strongly disagree), 2 (disagree), 3 (neutral), 4 (agree), 5 (strongly agree). Test to test variability of performing this survey yielded mean Cv's ranging from 0.101 to 0.274 with mean intraclass correlations ranging from 0.026 to 0.881 for the items on the survey.

Statistical Analysis

Data were analyzed using IBM® SPSS® Version 25 software (IBM Corp., Armonk, NY, USA). The sample size was based on prior research we conducted that indicated an n-size of 10 – 20 would yield a power of 0.80 on changes in glucose and insulin in response to an oral glucose challenge [82, 83]. Baseline demographic data were analyzed using descriptive statistics. Data were analyzed using a treatment (2) x time point (3 or 6) general linear model (GLM) multivariate and univariate repeated measures analysis. Wilks' Lambda p-levels from multivariate tests are reported to describe overall time and treatment x time interaction effects of variables analyzed. Greenhouse-Geisser univariate tests were run to assess time and treatment x time interaction effects of individual variables within the multivariate model. Data were considered statistically significant when the probability of type I error was 0.05 or less. Least significant difference post-hoc comparisons were used to assess differences among

treatments. Results with p-levels close to statistical significance (i.e., $p > 0.05$ to $p < 0.10$) are reported with partial eta-squared (η^2) effect size where the magnitude of effect was defined as 0.01 = small, 0.06 = medium, 0.13 = large [219, 220]. Delta changes (post - pre values) and 95% confidence intervals (CI) were also calculated on the data. Mean changes with 95% lower and upper CI's completely above or below baseline were considered significantly different.

Results

Participant Characteristics

Table 4 presents participant demographics for the study. With the crossover design, there were no differences between baseline measures in demographic markers.

Table 4. Baseline participant demographics.

Variable	Mean
Age (y)	22.0 ± 1.8
Height (m)	1.78 ± 0.06
Weight (kg)	82.8 ± 10.4
Body Fat (%)	14.2 ± 3.8
Body Mass Index (kg/m2)	26.3 ± 3.8
HR (bpm)	61.8 ± 8.5
BP Systolic (mmHg)	119.0 ± 8.8
BP Diasystolic (mmHg)	71.8 ± 5.5
Bench 1RM (kg)	103.0 ± 18.0
Squat 1RM (kg)	139.5 ± 23.6
Relative Bench Ratio	1.24 ± 0.2
Relative Squat Ratio	1.69 ± 0.2

Data are mean ± SD.

Dietary Analysis

Table 5 presents energy and macronutrient intake data. Multivariate analysis revealed no significant overall Wilks' Lambda for time (p=0.508) or treatment x time (p=0.695). Likewise, univariate analysis revealed no statistically significant interactions among treatments.

Table 5. Energy and macronutrient intake.

Nutrients	Baseline	PLA	FB	p-Level
Calories (kcal)	2248 ± 462	2252 ± 668	2534 ± 603	0.157
Protein (g)	133 ± 35	130 ± 38	146 ± 45	0.337
Carbohydrates (g)	213 ± 68	221 ± 100	243 ± 58	0.408
Fat (g)	88.6 ± 18.7	83.6 ± 40.0	99.8 ± 38.1	0.260

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed no overall Wilks' Lambda time (p=0.508) or treatment x time (p=0.695) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar.

Glycemic and Insulinemic Response

Table 6 shows serum glucose and insulin data observed by treatment. Multivariate analysis revealed an overall Wilks' Lambda time (p<0.001) and treatment x time interaction (p=0.007) effects. Univariate analysis revealed significant time (p<0.001) but not treatment x time interactions in glucose and insulin responses. Insulin levels increased over time with no significant differences observed between treatments, although insulin was 38% higher immediately following exercise in the FB group (PLA 11.18±2.69, FB 15.49±2.6 uIU/mL, p=0.269, η^2=0.06). Univariate analysis for the

insulin to glucose ratio (IGR) showed a significant effect for time (p<0.001) and treatment x time (p=0.008). Post-hoc analysis revealed that the IGR significantly differed between treatments after exercise.

Table 6. Glucose and insulin response to an oral treatment during intense exercise.

Variable	Treatment	Fasted	Post-Exercise	48-h Recovery	Effect	p-Level
Glucose	PLA	5.12 ± 0.48	5.05 ± 1.52	5.35 ± 0.40	Time	0.161
(mmol/L)	FB	5.34 ± 0.40	4.81 ± 0.84	5.29 ± 0.47	Treatment x Time	0.447
	Time	5.23 ± 0.45	4.93 ± 1.21	5.32 ± 0.43		
Insulin	PLA	6.44 ± 3.44	11.18 ± 9.59 †	7.72 ± 3.60	Time	<0.001
(µIU/mL)	FB	6.27 ± 3.77	15.49 ± 9.05 †	6.41 ± 3.77	Treatment x Time	0.129
	Time	6.36 ± 3.53	13.33 ± 9.38 †	7.07 ± 3.67		
Insulin/	PLA	0.070 ± 0.039	0.110 ± 0.072 †	0.080 ± 0.037	Time	<0.001
Glucose	FB	0.065 ± 0.039	0.173 ± 0.085 †	0.067 ± 0.038	Treatment x Time	0.008
Ratio	Time	0.068 ± 0.038	0.142 ± 0.084 †	0.073 ± 0.037		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.007) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar. † denotes p<0.05 difference from baseline. ^ represents p>0.05 to p<0.10 difference between PLA and FB.

Figure 8 shows mean changes from baseline with 95% CI's in glucose, insulin, and IGR. Glucose levels after 48-h after recovery tended to be lower in FB (PLA 0.23 [-0.002, 0.46]; FB -0.05 [-0.28, 0.18] mmol/L, p=0.087, η^2=0.13). Insulin was significantly increased above baseline values after exercise in both groups with no differences observed between treatments (PLA 4.73 [0.33, 9.14], FB 9.22 [4.82, 13.62], p=0.149, η^2=0.09). IGR was also significantly higher in both groups post-exercise when compared to baseline, with FB being significantly higher between groups (PLA 0.04 [0.00, 0.08], FB 0.11 [0.07, 0.15], p=0.013, η2=0.25). No differences were seen between

groups in area under the curve.
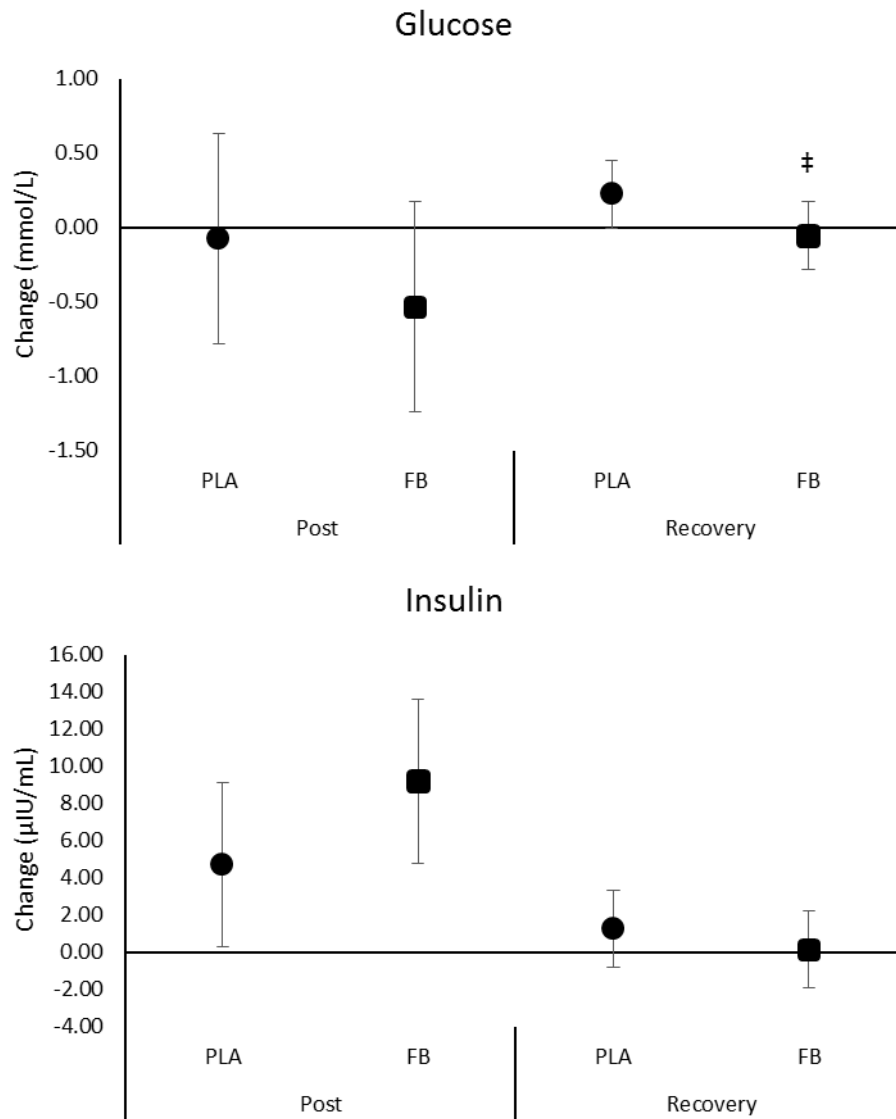
Glucose

Insulin

Figure 8. Mean changes with 95% CI in blood glucose (top panel), insulin (center panel), and the insulin to glucose ratio (bottom panel) observed in the placebo (PLA) and food bar (FB) treatments. Mean changes from baseline with 95% CI's completely above or below baseline represent a significant difference. † represents $p<0.05$ difference between treatments. ‡ represents $p>0.05$ to $p<0.10$ difference between treatments.
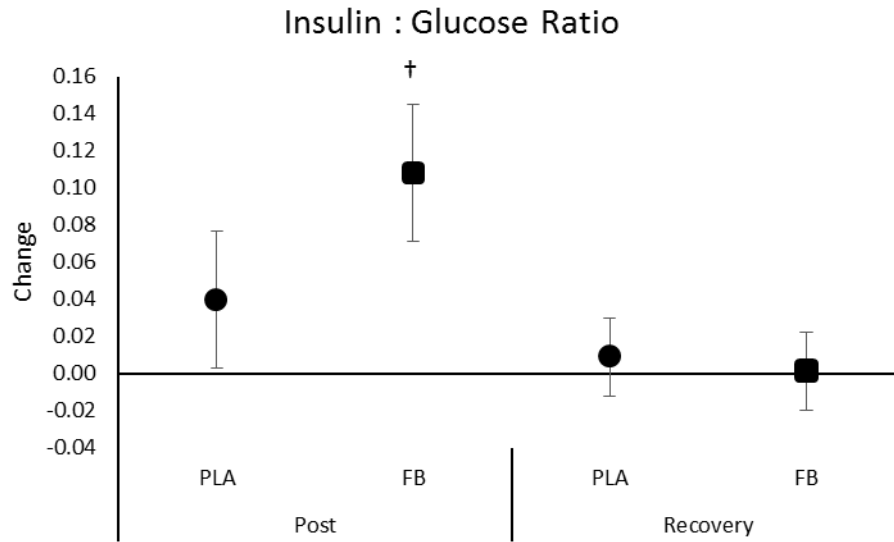
Figure 8. Continued.

Figure 9 presents mean changes with 95% CI's for glucose observed during the exercise sessions. Univariate analysis revealed significant time (p<0.001) and group x time interaction effects (p<0.001). Blood glucose generally increased to a greater degree and for a longer period of time after ingesting the PLA. Interestingly, glucose values remained within normal values (5.3±0.6 to 6.2±1.0 mmol/L) throughout the entire resistance-training and sprint protocol in the FB treatment while greater variability was seen with PLA (5.3±1.1 to 8.4±1.6 mmol/L).
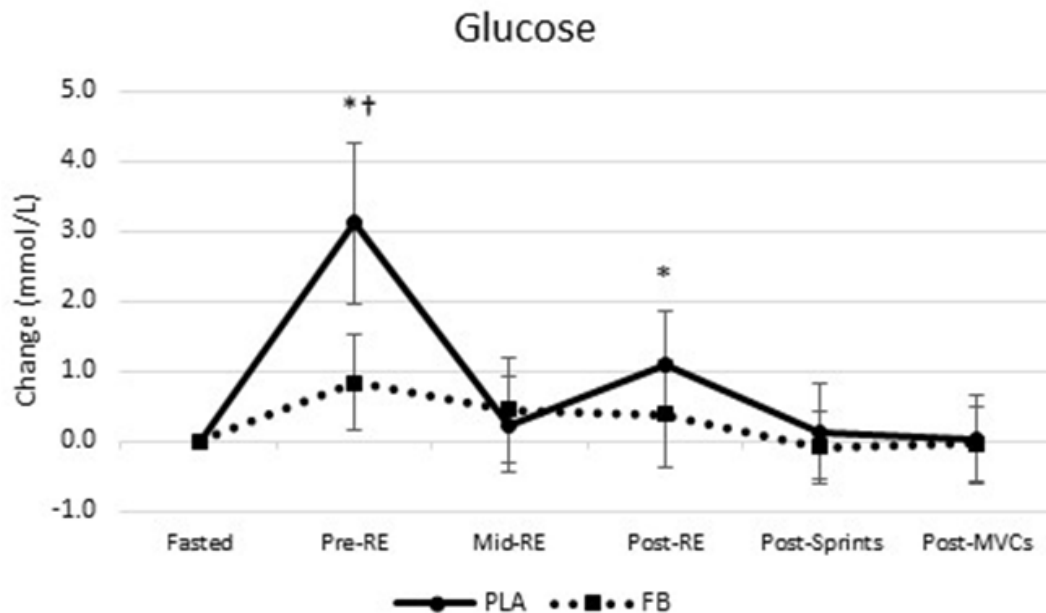
Figure 9. Mean changes with 95% CI in blood glucose observed in the placebo (PLA) and food bar (FB) treatments. RE=resistance exercise. Mean changes from baseline with 95% CI's completely above or below baseline represent a significant difference.* represents $p<0.05$ difference from baseline. † represents $p<0.05$ difference between treatments.

Resistance Exercise Performance

Table 7 presents volume of each of the upper and lower body resistance-exercises performed in the study. Multivariate analysis revealed an overall Wilks' Lambda time effect ($p<0.010$) with no treatment x time interaction effect ($p=0.808$). Univariate analysis revealed significant time effect for incline bench press ($p<0.002$), dumbbell biceps curl ($p=0.001$), and preacher curl ($p=0.032$) but no significant treatment x time interaction effects in among these exercises.

Figure 10 presents mean changes from baseline with 95% CI's for leg press and total lifting volume. Leg press volume significantly decreased from set 1 to Set 2 and Set

95

3 in the PLA treatment while participants in the FB treatment were able to maintain leg press lifting volume from Set 1 to Set 3. Post-hoc analysis revealed that leg press lifting volume tended to be lower with PLA compared to FB during set 2 (PLA -42.71 [-76.77, -8.65]; FB 0.00 [-34.06, 34.06] kg, p=0.08, η^2=0.13) and set 3 (PLA -130.79 [-235.02, -26.55]; FB -7.94 [-112.17, 96.30] kg, p=0.09, η^2=0.12) when compared to baseline. Similarly, participants maintained total lifting volume to a greater degree in Set 2 (PLA -66.9 [-111.4, -22.4], FB -28.9 [-73.4, 15.6] kg, p=0.224, η^2=0.07) and Set 3 (PLA -198.26 [-320.1, -76.4], FB -81.7 [-203.6, 40.1] kg, p=0.175, η^2=0.08) with FB treatment compared to PLA. This represented a -3.12% [-5.11, -1.14] reduction in performance in the PLA treatment compared to a -1.28% [-3.27, 0.71] reduction in performance in the FB treatment (p=0.188, η^2=0.08).

Table 7. Resistance exercise lifting volume.

Variable	Treatmer	Set 1	Set 2	Set 3	Effect	p-Level
Bench Press	PLA	718 ± 125	718 ± 125	712 ± 121	Time	0.103
(kg)	FB	718 ± 125	718 ± 125	716 ± 126	GxT	0.364
	Mean	718 ± 122	718 ± 122	714 ± 121		
Squat (kg)	PLA	979 ± 164	979 ± 164	979 ± 164	Time	1.000
	FB	979 ± 164	979 ± 164	979 ± 164	GxT	1.000
	Mean	979 ± 160	979 ± 160	979 ± 160		
Lat Pulldown	PLA	567 ± 82	567 ± 82	565 ± 84	Time	0.171
(kg)	FB	567 ± 82	567 ± 82	565 ± 84	GxT	1.000
	Mean	567 ± 80	567 ± 80	565 ± 83		
Leg Press (kg)	PLA	1916 ± 502	1874 ± 485	1786 ± 490	Time	0.064
	FB	1916 ± 502	1916 ± 502	1908 ± 500	GxT	0.101
	Mean	1916 ± 490	1895 ± 483	1847 ± 488		
Incline Bench	PLA	557 ± 113	552 ± 115	538 ± 115	Time	0.002
Press (kg)	FB	586 ± 108	576 ± 106	546 ± 120	GxT	0.291
	Mean	571 ± 109	564 ± 109	542 ± 115 †		
Dumbbell	PLA	314 ± 146	312 ± 149	312 ± 149	Time	0.401
Lunge (kg)	FB	355 ± 92	355 ± 92	363 ± 80	GxT	0.282
	Mean	335 ± 121	334 ± 123	337 ± 120		
Seated Row (kg)	PLA	612 ± 104	601 ± 104	591 ± 111	Time	0.242
	FB	633 ± 75	633 ± 75	631 ± 77	GxT	0.324
	Mean	623 ± 89	617 ± 90	611 ± 96		
Leg Extension	PLA	588 ± 244	584 ± 243	584 ± 243	Time	0.328
(kg)	FB	699 ± 138	699 ± 138	699 ± 138	GxT	0.328
	Mean	644 ± 202	642 ± 202	642 ± 202		
DB Biceps Curl	PLA	132 ± 45	130 ± 42	122 ± 42	Time	0.001
(kg)	FB	151 ± 18	140 ± 25	129 ± 32	GxT	0.199
	Mean	142 ± 35	135 ± 34 †	125 ± 37 †		
Triceps	PLA	227 ± 57	229 ± 54	228 ± 55	Time	0.413
Pressdown (kg)	FB	234 ± 46	233 ± 47	230 ± 48	GxT	0.200
	Mean	230 ± 50	231 ± 49	229 ± 51		
Biceps Curl (kg)	PLA	222 ± 55	219 ± 54	218 ± 54	Time	0.032
	FB	229 ± 45	223 ± 51	220 ± 53	GxT	0.492
	Mean	226 ± 50	221 ± 51.5 †	219 ± 53 †		
Total Lifting	PLA	6832 ± 1145	6765 ± 1162	6634 ± 1245	Time	0.012
Volume (kg)	FB	7069 ± 1103	7040 ± 1115	6987 ± 1147	GxT	0.407
	Mean	6951 ± 1106	6903 ± 1122	6811 ± 1185		

Data are means ± standard deviations (SD) in lifting volume (repetitionx x weight lifted in kg)or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p=0.010) and treatment x time (p=0.808). Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, DB=Dumb bel, GxT represents treatment x time interaction. † denotes p<0.05 difference from baseline.
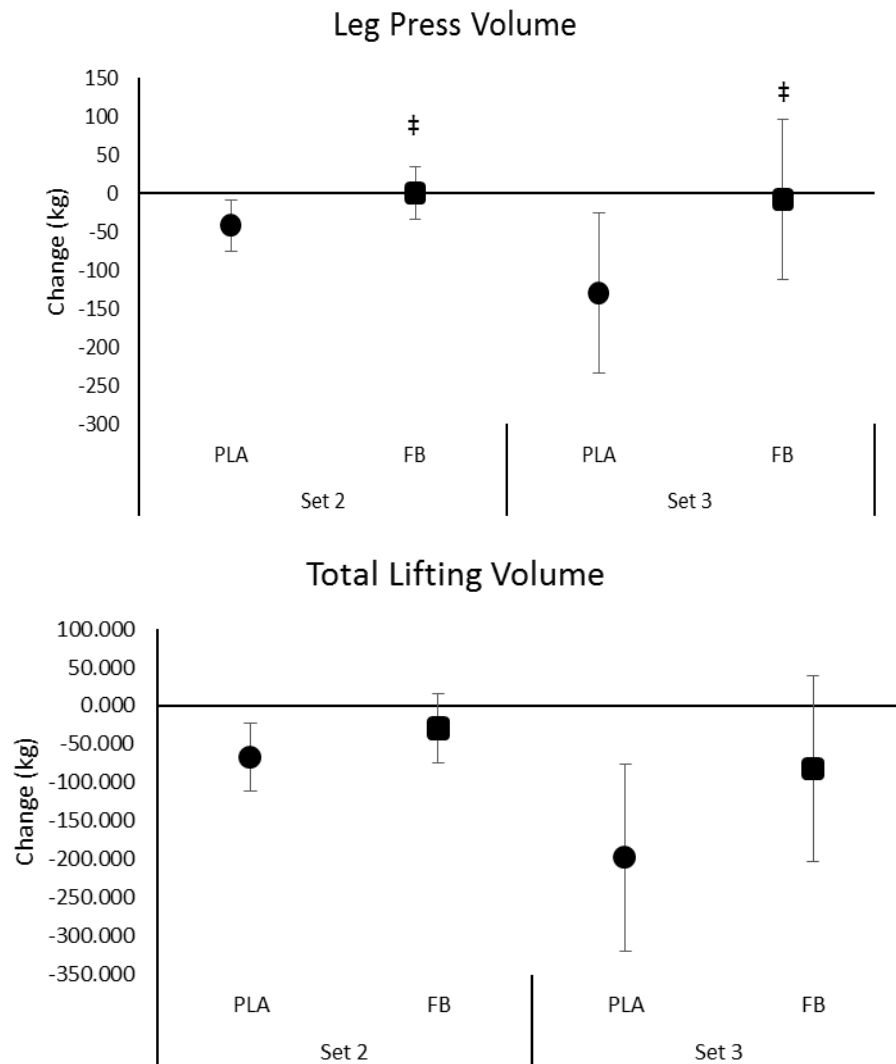
Figure 10. Mean changes with ± 95% confidence intervals in leg press volume (top panel) and total lifting volume (bottom panel) for the placebo (PLA) and food bar (FB) during exercise. Mean changes from baseline with 95% CI's completely above or below baseline represent a significant difference. ‡ represents $p > 0.05$ to $p < 0.10$ difference between treatments.

Sprint Performance

Table 8 presents performance times observed for the agility and sprint tests.

Multivariate analysis revealed a significant overall Wilks' Lambda for time (p<0.001) with no significant interaction effects (p=0.437). Univariate analysis revealed a significant time effect for agility performance (p<0.001) but not for 40-yd sprint performance (p=0.252). No significant interaction effects were seen in either agility or sprint performance. Figure 6 presents mean changes from baseline with 95% CI's for agility performance. Results revealed that agility performance in sprint 2 were significantly faster than baseline times during the FB treatment (PLA -0.13 [-0.28, 0.02]; FB -0.21 [-0.36, -0.06] sec, p=0.422, η^2=0.03) while both treatments were significantly faster than baseline values during sprint 3. No significant time or between group differences were observed for 40 yard dash results, although it should be noted that participants performed the first 40 yard dash sprint -0.15 sec faster (-2.7%) with FB treatment compared to the PLA treatment (PLA 5.50±0.38; FB 5.35±0.25 sec, p=0.251, η^2=0.06).

Table 8. Sprint performance.

Variable	Treatment	Sprint-1	Sprint-2	Sprint-3	Effect	p-Level
Nebraska Agility Drill (sec)	PLA	7.01 ± 0.68	6.89 ± 0.49	6.74 ± 0.45	Time	<0.001
	FB	7.02 ± 0.46	6.81 ± 0.43	6.71 ± 0.46	GxT	0.670
	Mean	7.01 ± 0.57	6.85 ± 0.45 †	6.73 ± 0.44 †		
Forty Yard Dash (sec)	PLA	5.50 ± 0.38	5.41 ± 0.32	5.39 ± 0.24	Time	0.252
	FB	5.35 ± 0.25	5.33 ± 0.19	5.36 ± 0.24	GxT	0.208
	Mean	5.42 ± 0.32	5.37 ± 0.26	5.37 ± 0.24		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.437) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT= treatment x time interaction. † denotes p<0.05 difference from baseline.
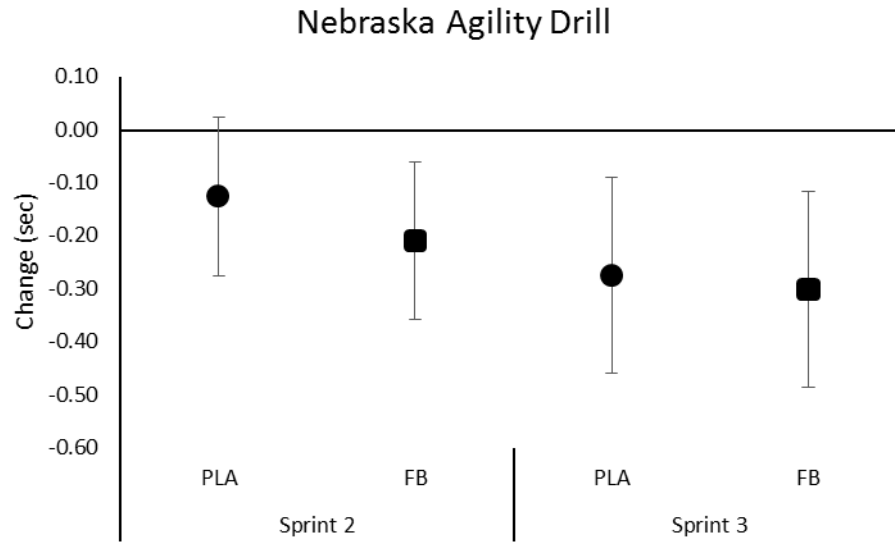
Figure 11. Mean changes with ± 95% confidence intervals in Nebraska Agility Drill performance times for the placebo (PLA) and food bar (FB) during exercise. Mean changes from baseline with 95% CI's completely above or below baseline represent a significant difference.

Isokinetic Maximal Voluntary Contraction (MVC) Performance

Table 9 displays the torque, force, power, and total work performed during the 3-repition isokinetic maximal voluntary extension/flexion contractions. Multivariate analysis revealed no significant overall Wilks' Lambda time ($p=0.352$) or treatment x time ($p=0.837$) effects. Likewise, univariate analysis did not reveal any time or treatment x time effects for extension or flexion MVC torque, force, power, or total work. Assessment of mean changes from baseline with 95% CI's did not reveal any significant changes from baseline or between treatments.

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Table 9. Isokinetic testing results.

	Variable	Treatme	Fasted	Post-Exercise	48-h Recovery	Effect	p-Level
Extension	MVC Extension Torque (Nm)	PLA	87.8 ± 13.3	85.4 ± 20.7	84.1 ± 20.1	Time	0.454
		FB	89.6 ± 21.1	85.6 ± 19.3	87.0 ± 18.5	GxT	0.882
		Mean	88.7 ± 17.3	85.5 ± 19.6	85.6 ± 19.0		
	MVC Extension Force (N)	PLA	432.8 ± 75.0	419.8 ± 107.4	412.9 ± 103.1	Time	0.442
		FB	439.2 ± 102.6	420.4 ± 96.7	425.8 ± 85.5	GxT	0.892
		Mean	436.0 ± 88.0	420.1 ± 99.9	419.4 ± 92.9		
	MVC Extension Power (W)	PLA	67.8 ± 9.6	66.3 ± 17.5	63.9 ± 15.8	Time	0.381
		FB	70.3 ± 16.8	66.2 ± 15.6	67.6 ± 15.9	GxT	0.744
		Mean	69.1 ± 13.4	66.3 ± 16.2	65.8 ± 15.6		
	MVC Extension Work (J)	PLA	71.6 ± 11.1	71.3 ± 20.8	66.8 ± 14.7	Time	0.443
		FB	75.9 ± 19.6	71.4 ± 18.2	72.8 ± 19.4	GxT	0.609
		Mean	73.7 ± 15.7	71.3 ± 19.1	69.8 ± 17.1		
Flexion	MVC Flexion Torque (Nm)	PLA	56.4 ± 15.8	59.8 ± 17.5	57.0 ± 20.7	Time	0.128
		FB	59.4 ± 20.2	61.2 ± 20.4	57.2 ± 18.8	GxT	0.690
		Mean	57.9 ± 17.8	60.5 ± 18.6	57.1 ± 19.4		
	MVC Flexion Force (N)	PLA	275.9 ± 73.7	292.5 ± 80.9	276.5 ± 88.0	Time	0.105
		FB	288.6 ± 81.3	296.7 ± 81.7	278.3 ± 76.5	GxT	0.761
		Mean	282.3 ± 76.2	294.6 ± 79.5	277.4 ± 80.7		
	MVC Flexion Power (W)	PLA	31.0 ± 9.4	32.8 ± 10.4	31.1 ± 12.6	Time	0.466
		FB	32.7 ± 12.0	33.4 ± 12.6	32.0 ± 12.1	GxT	0.887
		Mean	31.8 ± 10.6	33.1 ± 11.3	31.6 ± 12.1		
	MVC Flexion Work (J)	PLA	49.8 ± 15.4	52.9 ± 18.3	49.9 ± 19.5	Time	0.380
		FB	53.5 ± 19.0	55.0 ± 20.8	52.1 ± 19.7	GxT	0.912
		Mean	51.6 ± 17.0	53.9 ± 19.2	51.0 ± 19.2		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p=0.352) and treatment x time (p=0.837) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, MVC=Maximal Voluntary Contraction, GxT= treatment x time interaction.

Muscle Soreness Assessment

Table 10 presents subjective ratings of muscle soreness. Multivariate analysis revealed a significant overall Wilks' Lambda time effect (p<0.001) with no significant interaction effects (p=0.538). Univariate analysis showed a significant time effect for VM (p<0.001), DVL (p=0.002) and MLVL (p=0.004) with no significant interaction

effects. Figure 12 displays the mean change from baseline with 95% CI's for ratings of muscle soreness. Ratings of VM muscle soreness after the workout were higher with PLA (PLA 1.88 [0.60, 3.17]; FB 0.29 [-0.99, 1.57] cm, p=0.083, η^2=0.13). Additionally, ratings of muscle soreness at the DVL (PLA 2.13 [0.45, 3.80]; FB 1.45 [-0.22, 3.12] cm, p=0.560, η^2=0.02) and MLVL (PLA 2.32 [0.51, 4.12]; FB 1.53 [-0.28, 3.33] cm, p=0.527, η^2=0.02) sites remained above baseline values after 48 h recovery with PLA treatment while ratings with FB treatment were not significantly different from baseline values.

Table 10. Perception of quadricep muscle soreness.

Variable	Treatment	Pre-Exercise	Post-Exercise	48-hr Post	Effect	p-Level
Distal Vastus Medialis (cm)	PLA	3.31 ± 2.51	5.19 ± 3.39	6.64 ± 2.78	Time	<0.001
	FB	4.09 ± 2.50	4.38 ± 2.76	6.28 ± 2.50	GxT	0.340
	Mean	3.70 ± 2.48	4.79 ± 3.05 †	6.46 ± 2.60 †		
Distal Vastus Lateralis (cm)	PLA	3.64 ± 2.59	3.32 ± 2.89	5.77 ± 3.33	Time	0.002
	FB	2.35 ± 1.99	2.51 ± 2.32	3.80 ± 3.59	GxT	0.489
	Mean	3.00 ± 2.35	2.91 ± 2.60	4.78 ± 3.53 †		
Mid-Lateral Vastus Lateralis (cm)	PLA	2.47 ± 2.49	3.00 ± 3.22	4.78 ± 3.34	Time	0.004
	FB	1.93 ± 2.41	2.77 ± 2.58	3.46 ± 3.10	GxT	0.493
	ean	2.20 ± 2.41	2.88 ± 2.85	4.12 ± 3.22 †		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.538) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT= treatment x time interaction. † denotes p<0.05 difference from baseline.
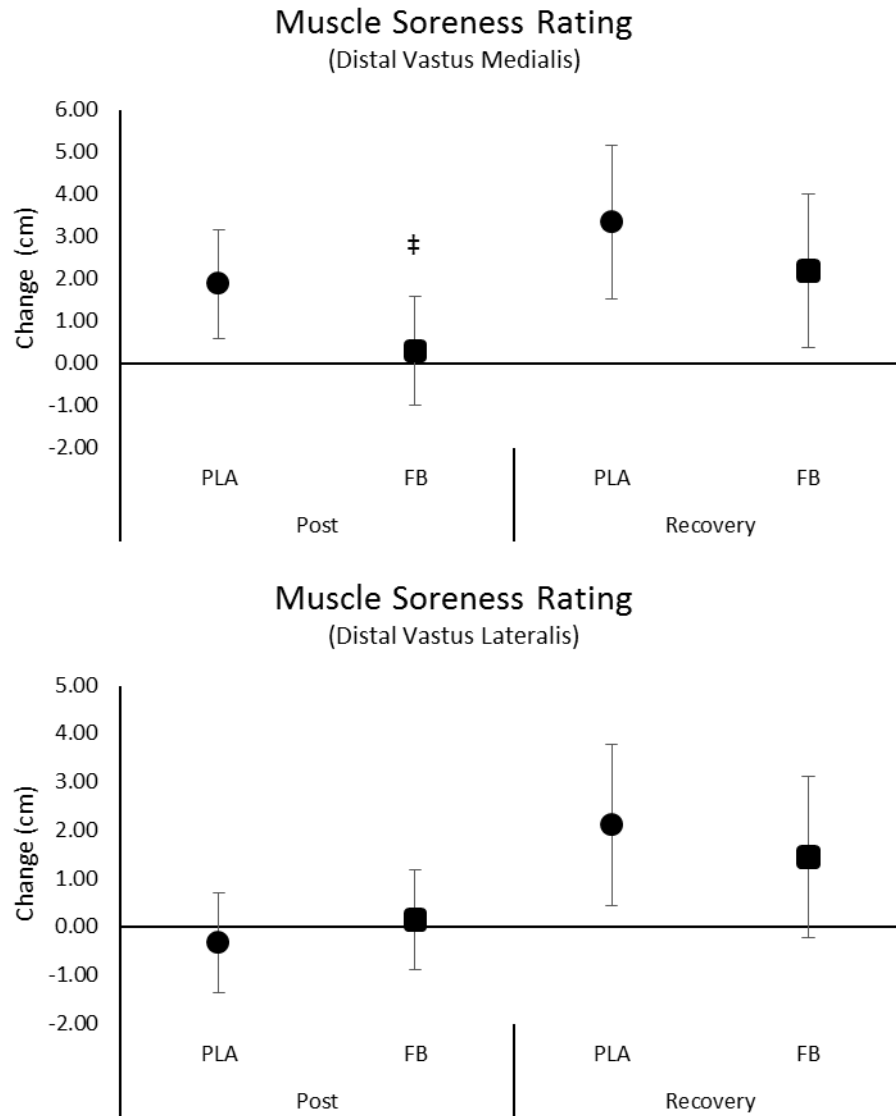
Figure 12. Mean changes from baseline with 95% confidence intervals in ratings of muscle soreness for the distal vastus medialis (top panel), distal vastus lateralis (center panel), and mid-lateral vastus lateralis (bottom panel) for the placebo (PLA) and food bar (FB) treatments. Mean changes from baseline with 95% CI's completely above or below baseline represent a significant difference. ‡ represents p>0.05 to p<0.10 difference between treatments.
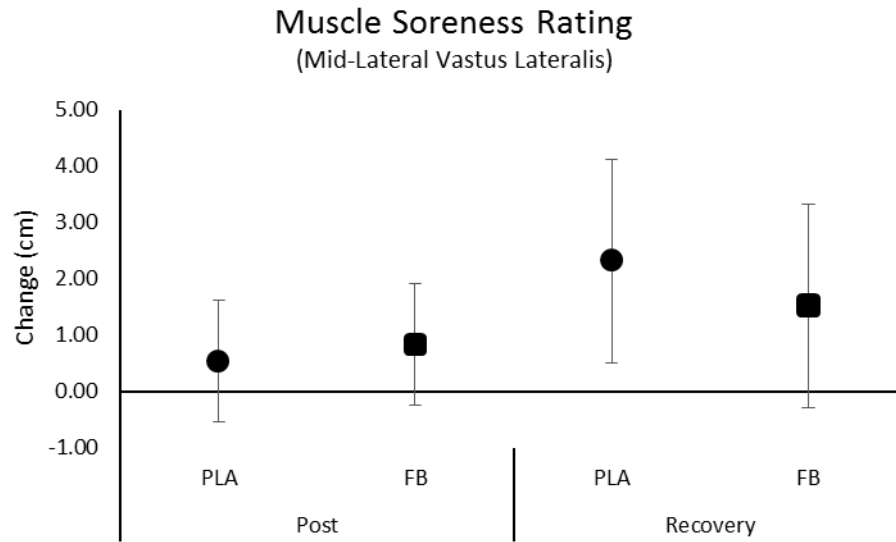
Figure 12. Continued.

Markers of catabolism

Table 11 presents the serum markers of catabolism. Multivariate analysis revealed a significant overall Wilks' Lambda time effect ($p<0.001$) with no significant interaction effects ($p=0.360$). Univariate analysis demonstrated significant effects over time for blood urea nitrogen ($p<0.001$), creatinine ($p<0.001$), lactate dehydrogenase ($p<0.001$), creatine kinase ($p=0.038$), and the blood urea nitrogen to creatinine ratio ($p=0.001$). However, no significant univariate treatment x time interaction effects were observed.

Table 11. Markers of catabolism.

Variable	Treatment	Fasted	Post-Exercise	48-hr Post	Effect	p-Level
Urea/BUN	PLA	6.60 ± 1.79	6.39 ± 1.54	5.38 ± 1.76	Time	<0.001
(mmol/L)	FB	6.61 ± 1.97	6.77 ± 1.71	5.72 ± 1.25	GxT	0.564
	Mean	6.61 ± 1.84	6.58 ± 1.60	5.55 ± 1.50 †		
Creatinine	PLA	94.7 ± 14.9	112.1 ± 22.2	97.8 ± 10.9	Time	<0.001
(umol/L)	FB	95.1 ± 15.6	108.7 ± 18.0	91.8 ± 15.2	GxT	0.382
	Mean	94.9 ± 14.9	110.4 ± 19.8 †	94.8 ± 13.3		
LDH	PLA	150 ± 21	172 ± 31	153 ± 23	Time	<0.001
(U/L)	FB	149 ± 15	176 ± 30	153 ± 18	GxT	0.675
	Mean	149 ± 18	174 ± 30 †	153 ± 20		
Creatine Kinase	PLA	289 ± 229	446 ± 232	480 ± 644	Time	0.038
(U/L)	FB	221 ± 104	396 ± 144	428 ± 374	GxT	0.940
	Mean	255 ± 177	421 ± 191 †	454 ± 516 †		
BUN/Creatinine	PLA	14.35 ± 3.72	11.95 ± 3.42	11.26 ± 3.62	Time	0.001
Ratio	FB	14.25 ± 3.92	12.96 ± 3.58	13.10 ± 3.98	GxT	0.166
	Mean	14.30 ± 3.73	12.46 ± 3.46 †	12.18 ± 3.84 †		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.360) effects. Greenhouse-Geisser univariate p-levels are presented for each vareiable. GxT represents group x time interaction. † denotes p<0.05 difference from baseline.

Stress and Sex Hormones

Table 12 displays the serum stress and sex hormones. Multivariate analysis revealed an overall Wilks' Lambda time effecc (p<0.001) with no significant treatment x time interaction effects were observed (p=0.914). Univariate analysis revealed a significant time effect for testosterone (p<0.001) with no other time or interaction effects observed. Assessment of mean changes from baseline with 95% CI's revealed that cortisol levels tended to be lower with FB treatment compared to the PLA at 48-h recovery (PLA 0.35 [-1.18, 1.88]; FB -1.38 [-2.90, 0.15] ug/dL, p=0.111, η^2=0.11). No significant differences were observed in changes in testosterone or the cortisol to testosterone ratio between treatments.

Table 12. Stress and sex hormone response.

Variable	Treatment	Fasted	Post-Exercise	48-hr Post	Effect	p-Level
Cortisol (ug/dL)	PLA	15.5 ± 2.0	14.2 ± 5.7	15.9 ± 2.5	Time	0.403
	FB	16.6 ± 1.8	14.9 ± 6.3	15.3 ± 2.4	GxT	0.644
	Mean	16.1 ± 1.9	14.6 ± 5.9	15.6 ± 2.4		
Testosterone (ng/mL)	PLA	495 ± 161	401 ± 192	466 ± 163	Time	<0.001
	FB	503 ± 187	376 ± 186	458 ± 194	GxT	0.635
	Mean	499 ± 171	389 ± 185 †	462 ± 175		
Cortisol/ Testosterone Ratio	PLA	28.0 ± 11.5	33.6 ± 20.2	29.4 ± 8.8	Time	0.112
	FB	28.7 ± 8.0	36.2 ± 16.8	29.0 ± 8.9	GxT	0.819
	Mean	28.3 ± 9.7	34.9 ± 18.2	29.2 ± 8.7		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p<0.001) and treatment x time (p=0.914) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar. GxT represents group x time interaction. † denotes p<0.05 difference from baseline.

Inflammatory Marker Response

Table 13 presents the serum inflammatory markers analyzed. Multivariate analysis revealed a significant overall Wilks' Lambda for time (p=0.037) but not for treatment x time (p=0.985). Univariate analysis revealed a time effect for IL-8 (p=0.001) and TNFα (p=0.044) with no significant interaction effects observed. Assessment of mean changes from baseline with 95% CI's revealed that IL-8 was higher than baseline values following exercise with FB treatment (PLA 0.54 [-0.07, 1.15]; FB 0.67 [0.06, 1.28] pg/mL, p=0.761, η^2=0.01) with no differences observed between treatments. No other differences from baseline or between treatments were observed among markers of inflammation.

Table 13. Inflammatory marker panel.

Variable	Treatment	Fasted	Post-Exercise	48-hr Post	Effect	p-Level
IFNy (pg/mL)	PLA	13.6 ± 7.7	13.4 ± 7.4	13.3 ± 7.1	Time	0.460
	FB	13.3 ± 7.1	13.6 ± 7.2	12.8 ± 6.5	GxT	0.569
	Mean	13.4 ± 7.2	13.5 ± 7.1	13.1 ± 6.7		
IL-13 (pg/mL)	PLA	10.5 ± 13.7	10.3 ± 13.6	10.5 ± 14.9	Time	0.584
	FB	11.2 ± 16.6	10.9 ± 16.1	10.7 ± 15.4	GxT	0.563
	Mean	10.8 ± 14.9	10.6 ± 14.6	10.6 ± 14.8		
IL-1ß (pg/mL)	PLA	1.25 ± 0.81	1.19 ± 0.73	1.22 ± 0.79	Time	0.268
	FB	1.24 ± 0.83	1.21 ± 0.77	1.17 ± 0.78	GxT	0.472
	Mean	1.24 ± 0.80	1.20 ± 0.74	1.19 ± 0.77		
IL-4 (pg/mL)	PLA	26.5 ± 15.1	25.5 ± 14.2	26.2 ± 14.6	Time	0.176
	FB	27.2 ± 13.5	26.3 ± 13.8	25.6 ± 13.7	GxT	0.371
	Mean	26.9 ± 14.0	25.9 ± 13.7	25.9 ± 13.9		
IL-6 (pg/mL)	PLA	2.96 ± 1.34	3.22 ± 1.29	3.16 ± 1.30	Time	0.128
	FB	3.02 ± 1.25	3.26 ± 1.29	2.95 ± 1.23	GxT	0.437
	Mean	2.99 ± 1.27	3.24 ± 1.26	3.05 ± 1.24		
IL-8 (pg/mL)	PLA	3.58 ± 0.95	4.12 ± 1.19	3.57 ± 1.19	Time	0.001
	FB	3.69 ± 0.94	4.36 ± 1.41	3.42 ± 1.17	GxT	0.571
	Mean	3.64 ± 0.92	4.24 ± 1.28 †	3.49 ± 1.16		
TNFα (pg/mL)	PLA	5.30 ± 2.10	5.43 ± 2.16	5.26 ± 2.09	Time	0.044
	FB	5.31 ± 1.93	5.65 ± 2.34	4.92 ± 2.14	GxT	0.261
	Mean	5.30 ± 1.97	5.54 ± 2.2 ‡	5.09 ± 2.08		

Data are means ± standard deviations (SD). A multivariate analysis revealed overall Wilks' Lambda time (p=0.037), treatment x time (p=0.985) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT represents group x time interaction. † denotes p<0.05 difference from baseline. ‡ denotes p<0.05 difference from 48-hr post-exercise.

Appetite, Hypoglycemia, and Readiness to Perform Assessment

Tables 14-16 present appetite and eating satisfaction, symptoms of hypoglycemia, and readiness to perform survey results, respectively. Multivariate analysis of responses to the eating satisfaction inventory questions revealed significant time (p=0.007) with no significant interaction effects (p=0.152). Univariate analysis revealed that ratings of

appetite and hunger declined while feelings of fullness increased over time. A significant

interaction effect was observed in feeling of fullness with food (p=0.032) while ratings

of hunger (p=0.094) and satisfaction (p=0.085) tended to differ among treatments.

Assessment of mean changes from baseline with 95% CI's revealed that hunger

decreased below baseline values with FB treatment at the midway point of exercise

(PLA -1.17 [-2.65, 0.31]; FB -3.33 [-4.81, -1.85] p=0.043, η^2=0.17) and after exercise

(PLA -0.75 [-2.32, 0.82]; FB -2.42 [-3.99, -0.85] p=0.134, η^2=0.10). Ratings of appetite

were than baseline values with FB treatment after exercise (PLA -0.67 [-2.19, 0.85]; FB

-1.92 [-3.44, -0.40] p=0.240, η^2=0.06). In terms of symptoms of hypoglycemia, a

significant overall Wilks' Lambda time effect (p<0.001) was observed with no

significant interaction effect (p=0.269). Univariate analysis revealed a time effect for

hypoglycemia (p=0.001), dizziness (p=0.001), fatigue (p<0.001), and stomach upset

(p=0.004). However, no significant interaction effects were observed in ratings of

symptoms of hypoglycemia, dizziness, headache, fatigue, or stomach upset. Finally,

analysis of responses to the readiness to perform questionnaire revealed an overall

Wilks' Lambda time effect (p=0.001) with no significant interaction effects (p=0.186).

Univariate analysis revealed a significant time effects for feelings of vigor and energy

(p=0.004), appetite (p=0.035), and muscle soreness (p=0.007) with no significant

treatment x time interactions observed. Assessment of mean changes from baseline with

95% CI's revealed that response to the question "*I have little muscle soreness*" were

significantly decreased below baseline values with PLA treatment (PLA -1.00 [-1.80, -

0.20]; FB -0.50 [-1.30, 0.30] p=0.368, η^2=0.04) as well as after 48 h of recovery (PLA -

1.00 [-1.91, -0.10]; FB -0.75 [-1.66, 0.16] p=0.689, η^2=0.01) suggesting a greater

perception of muscle soreness.

Table 14. Appetited and Eating Satisfaction Inventory

Variable	Treatment	Fasted	Mid-Exercise	Post-Exercise	Effect	p-Level
Appetite	PLA	6.08 ± 3.09	4.00 ± 3.54	5.42 ± 3.26	Time	<0.001
	FB	6.92 ± 1.93	4.50 ± 1.73	5.00 ± 1.91	GxT	0.430
	Mean	6.50 ± 2.55	4.25 ± 2.74 *	5.21 ± 2.62 *		
Hunger	PLA	6.00 ± 3.13	4.83 ± 3.13	5.25 ± 3.33	Time	<0.001
	FB	7.17 ± 2.21	3.83 ± 2.44	4.75 ± 1.86	GxT	0.094
	Mean	6.58 ± 2.72	4.33 ± 2.79 *	5.00 ± 2.65 *		
Satisfaction	PLA	3.58 ± 2.81	2.00 ± 1.65	2.33 ± 1.92	Time	0.656
	FB	2.58 ± 3.32	3.83 ± 3.01	4.67 ± 2.93	GxT	0.085
	Mean	3.08 ± 3.05	2.92 ± 2.55	3.50 ± 2.70		
Fulllness	PLA	2.33 ± 2.57	2.42 ± 2.31	2.50 ± 2.71	Time	0.017
	FB	2.17 ± 2.33	4.83 ± 2.59 *†	5.08 ± 2.07 *†	GxT	0.032
	Mean	2.25 ± 2.40	3.63 ± 2.70 *	3.79 ± 2.70 *		
Energy	PLA	5.33 ± 1.72	4.17 ± 1.70	5.33 ± 1.61	Time	0.192
	FB	5.50 ± 2.07	5.58 ± 1.62	6.00 ± 2.13	GxT	0.357
	Mean	5.42 ± 1.86	4.88 ± 1.78	5.67 ± 1.88		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p=0.007) and treatment x time (p=0.152) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT represents group x time interaction. * denotes p<0.05 difference from baseline. † p<0.05 difference between PLA and FB.

Table 15. Symptoms Hypoglycemia Inventory

Variable	Treatment	Fasted	Mid-Exercise	Post-Exercise	Effect	p-Level
Hypoglycemia	PLA	0.83 ± 2.04	1.67 ± 1.87	1.00 ± 1.76	Time	0.001
	FB	0.58 ± 0.90	2.08 ± 2.02	0.92 ± 1.56	GxT	0.465
	Mean	0.71 ± 1.55	1.88 ± 1.92 *	0.96 ± 1.63		
Dizziness	PLA	0.83 ± 2.04	1.25 ± 1.66	0.50 ± 1.24	Time	0.001
	FB	0.33 ± 0.78	2.00 ± 2.04	0.58 ± 1.24	GxT	0.095
	Mean	0.58 ± 1.53	1.63 ± 1.86 *	0.54 ± 1.22		
Headache	PLA	0.33 ± 0.65	0.67 ± 1.15	0.50 ± 0.90	Time	0.384
	FB	0.17 ± 0.39	0.33 ± 0.89	0.33 ± 0.89	GxT	0.804
	Mean	0.25 ± 0.53	0.50 ± 1.02	0.42 ± 0.88		
Fatigue	PLA	1.75 ± 2.22	4.25 ± 2.56	3.67 ± 2.67	Time	<0.001
	FB	1.25 ± 1.29	4.00 ± 2.86	3.17 ± 2.29	GxT	0.964
	Mean	1.50 ± 1.79	4.13 ± 2.66 *	3.42 ± 2.45 *		
Stomach Upset	PLA	0.50 ± 1.00	1.17 ± 1.53	0.83 ± 1.64	Time	0.004
	FB	0.50 ± 0.80	2.33 ± 2.81	0.50 ± 1.00	GxT	0.104
	Mean	0.50 ± 0.88	1.75 ± 2.29 *	0.67 ± 1.34		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time ($p<0.001$) and treatment x time ($p=0.269$) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT represents group x time interaction. * denotes $p<0.05$ difference from baseline.

Table 16. Readiness to Perform Questionnaire

Variable	Treatment	Fasted	Post-Exercise	48-hr Post	Effect	p-Level
I slept well last night	PLA	3.58 ± 0.79	3.58 ± 0.79	3.83 ± 0.83	Time	0.591
	FB	3.67 ± 0.49	3.67 ± 0.78	3.76 ± 1.15	GxT	0.591
	Mean	3.63 ± 0.65	3.63 ± 0.77	3.75 ± 0.99		
Looking forward to today's workout	PLA	3.83 ± 1.11	3.58 ± 1.00	4.08 ± 1.00	Time	0.065
	FB	3.92 ± 0.90	3.42 ± 1.24	3.83 ± 0.94	GxT	0.689
	Mean	3.88 ± 0.99	3.50 ± 1.10	3.96 ± 0.95 ^		
Optimistic about future performance	PLA	3.83 ± 0.83	4.00 ± 1.04	4.17 ± 0.94	Time	0.250
	FB	4.08 ± 0.90	3.67 ± 1.30	4.00 ± 0.85	GxT	0.150
	Mean	3.96 ± 0.86	3.83 ± 1.17	4.08 ± 0.88		
I feel vigorous & energetic	PLA	3.08 ± 0.67	3.50 ± 0.67	3.75 ± 0.97	Time	0.004
	FB	3.33 ± 1.07	3.17 ± 1.11	3.92 ± 1.00	GxT	0.237
	Mean	3.21 ± 0.88	3.33 ± 0.92	3.83 ± 0.96 *^		
My appetite is great	PLA	3.83 ± 0.94	3.92 ± 0.79	4.08 ± 0.67	Time	0.035
	FB	3.83 ± 1.03	3.33 ± 1.07	4.17 ± 0.94	GxT	0.159
	Mean	3.83 ± 0.96	3.63 ± 0.97	4.13 ± 0.80 ^		
I have little muscle soreness	PLA	4.08 ± 0.67	3.08 ± 1.00	3.08 ± 1.08	Time	0.007
	FB	4.00 ± 0.43	3.50 ± 0.90	3.25 ± 1.36	GxT	0.655
	Mean	4.04 ± 0.55	3.29 ± 0.95 †	3.17 ± 1.20 *		

Data are means ± standard deviations (SD) or standard error of the mean (SEM). A multivariate analysis revealed overall Wilks' Lambda time (p=0.001), treatment x time (p=0.186) effects. Greenhouse-Geisser univariate p-levels are presented for each variable. PLA=Placebo, FB=Food Bar, GxT represents group x time interaction. * denotes $p<0.05$ difference from baseline. ^ denotes $p<0.05$ difference from 48-hr post-exercise.

Discussion and Conclusion

We previously reported that ingesting a whey protein energy bar with IMO as the source of carbohydrate had a GI of 34 and a glycemic load of 8.5 [243]. Additionally, that ingesting this energy bar increased insulin to a greater degree while maintaining blood glucose to a better degree compared to a dextrose control [243]. Theoretically, ingestion of this food bar prior to, during, and/or following exercise could serve as a low-glycemic source of carbohydrate and lessen the catabolic effects of intense exercise.

The purpose of this study was to examine the effects of ingesting a commercially available low-glycemic whey protein energy/food bar with IMO as the source of carbohydrate prior to, during, and following exercise affects exercise capacity and/or recovery from intense-exercise. We hypothesized that ingestion this whey protein food bar containing IMO would promote a low to moderate glycemic response with a similar insulin response during exercise, help athletes maintain exercise performance capacity during an intense training session, and hasten recovery. Based on results observed, we accept our hypotheses. The following assesses the impact of ingesting this energy/food bar prior to, during, and following intense exercise on primary and secondary outcomes.

Primary Outcome – Glucose Homeostasis

Results of this study found that the glycemic and insulinemic response of ingesting the food bar prior to, during, and following intense exercise was more favorable in maintaining euglycemia than ingesting equivalent amounts of reference carbohydrate (dextrose). In this regard, blood glucose levels never increased outside of normal values after FB ingestion compared to an increase of up to 58% with dextrose. Blood glucose levels were significantly higher than baseline prior to and following exercise in the PLA treatment. Additionally, pre-exercise blood glucose levels in the PLA treatment were significantly higher than FB blood glucose values. Interestingly, even though glucose levels were only modestly increased following FB ingestion, insulin concentration and the GIR were significantly higher than baseline values in both treatments and the GIR following exercise was significantly higher with FB ingestion compared to the dextrose placebo. These findings indicate that FB ingestion promoted a more favorable glucose

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homeostasis and anti-catabolic hormonal environment. These results support our initial

findings that ingestion of this FB promotes a mild increase in blood glucose while

serving to increase insulin levels to a greater degree than dextrose [243]. It also provides

rationale as to why consumption of this FB may lessen exercise-induced catabolism

and/or promote recovery from intense exercise.

There are several possible reasons for these findings. First, amino acid ingestion

has been reported to modestly increase insulin levels [233, 258, 259] and co-ingestion of

protein or amino acids with carbohydrate has been reported to promote a greater effect

on insulin [233-236, 258, 260, 261]. The FB studied contained 25 g of IMO with 20 g of

whey protein. Thus, it is possible that co-ingestion of IMO and whey protein promoted a

greater increase in insulin than the dextrose placebo. Second, the FB was high in fiber

and only contained 4 g of digestible carbohydrate (sugar) which would have likely

promoted a more gradual release of glucose into the blood thereby facilitating a more

sustained increase in insulin. There is evidence that consuming whey protein with fiber

affects the glycemic response of co-ingested carbohydrates [262-264]. So it is possible

that co-ingesting whey protein with a high fiber carbohydrate may have augmented

insulin response. Third, although IMO is a prebiotic, it is classified as a type of

oligosaccharide that has been reported to stimulate growth of "friendly" bacteria which

improve gut function through the promotion of activity of the probiotic gut flora [208,

237-239]. Therefore, it could be possible that intestinal absorption of glucose was

enhanced thereby serving to help maintain blood glucose levels to a greater degree while

the increased availability of amino acids served to stimulate insulin levels. Additional

research should examine the potential mechanisms associated with these findings.

Secondary Outcome – Exercise Performance & Recovery

Since we previously found that ingesting this FB promoted a modest and more sustained increase in blood glucose, we hypothesized that ingesting this FB prior to and during intense exercise may help athletes maintain performance over time. Results of this study provides some support for this hypothesis. In this regard, we observed that total lifting volume from Set 1 to Set 2 and Set 3 was maintained to a greater degree during the FB treatment while significantly decreasing below baseline values with PLA. While it's understandable that athletes/experienced lifters may not be able to maintain 70% of 1RM for each exercise during an intense workout due to fatigue, this finding suggests that ingestion of the FB helped maintain the quality of the resistance-exercise training session. We also found that agility performance was improved from Sprint 1 to Sprint 2 in the FB treatment while being unchanged in the PLA treatment and that the participants performed the first 40 yard sprint -0.15 sec faster with FB compared to PLA. While this latter finding was not statistically significant, it represents a meaningful performance difference from an applied standpoint.

We also hypothesized that since the FB we previously investigated increased insulin to a greater degree than dextrose and insulin serves as an anticatabolic hormone, ingesting this FB around an intense exercise bout may lessen exercise-induced catabolism and/or perceptions of delayed onset of muscle soreness (DOMS) [77, 78, 82, 242]. Results of this study found evidence that ingesting this FB may lessen perceptions of muscle soreness but it had limited effects on markers of catabolism or inflammation. In this

regard, participants rated the pain response to a standard amount of pressure applied to several locations on the thigh to be significantly higher than baseline values after exercise (VM site) and after 48 h of recovery (DVL and MVL) with PLA treatment while ratings in the FB treatment were unchanged. Ratings at the VM site also tended to be lower in the FB treatment compared to PLA after exercise. Additionally, participants did not respond as positively to the statement *"I have little muscle soreness"*. These findings support prior reports that whey protein supplementation can affect recovery and/or perceptions of muscle soreness in response to intense training [265-267]. However, there was less evidence indicating that ingestion of this FB prior to, during, and following intense exercise lessened markers of catabolism. In this regard, we found no significant effects on markers of whole body catabolism, muscle enzyme efflux, anabolic and catabolic hormones, or inflammatory markers. These findings support results of other studies that reported limited to no effects of consuming whey protein prior to and/or during exercise on markers of catabolism or inflammation [268-270]. Additional research is necessary to explore the impact of consuming whey protein with different forms of carbohydrate on markers of recovery from intense exercise.

Finally, analysis of subjective ratings of symptoms revealed that ingestion of PLA and FB prior to, during and following exercise were well tolerated and had minimal effects on ratings of hypoglycemia, dizziness, headache, fatigue, and stomach upset. These findings indicated that both nutritional interventions were well tolerated. Moreover, while the treatments differed in caloric content and sweetness which could influence perceptions about appetite, hunger [224]; ingestion of the FB was associated

with a greater increase in feeling of fullness with some evidence of less hunger and greater satisfaction from food ratings. Finally, we did not observe significant differences between treatments in questions related to readiness to perform. Collectively, these findings indicate that the food bar studied serve as a good low-glycemic food choice for active individuals to consume prior to, during, and/or following intense exercise training.

Limitations

Although this study employed a randomized, crossover experimental design and assess the effects of consuming these nutritional interventions around an intense training consistent with the type of training many athletes perform, there are some limitations to the study that should be noted. First, the dextrose placebo was matched in CHO g (25 g), and was a reference carbohydrate for determining the GI and GL of the food source. However, it was provided as a gel and the placebo was not matched for total calories. This could have influence some of the differences observed in performance and/or perception of soreness. Second, while the study was sufficiently powered and a number of outcome variables were statistically significant, we found borderline significant levels with moderate to large effect sizes suggesting that having a larger n-size may have revealed more significant findings. Third, given we were trying to assess a normal training bout of exercise, we limited venous blood assessment data points and therefore may have missed some of the effects of the nutritional interventions on blood markers. Finally, we chose to have participants record and try to replicate nutritional intake during each treatment. While there were no significant differences in dietary records and participants fasted and refrained from exercise training and NSAID use prior to reporting

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to the lab, it is possible that differences in diet, hydration, and/or rest between treatments may have influence results. With that said, the major strengths of this study were the randomized and crossover experimental design and assessment of a typical intense training bout used in the strength and conditioning of athletes. Additionally, the practical assessment of whether having athletes ingest a energy/food bar prior to, during, and/or following exercise has any influence on exercise training performance and/or recovery.

Conclusion

Results of this study demonstrated that ingestion of a whey protein with IMO as the source of carbohydrate prior to, during, and following intense resistance-exercise and sprint conditioning maintained blood glucose and increased insulin to a greater degree than consuming a carbohydrate matched dextrose placebo. Additionally, FB ingestion helped maintain resistance and sprint exercise performance. However, markers of catabolism and inflammation were not affected. Results indicate that this FB can serve as a good low glycemic food option for individuals to take prior to, during, and/or following intense exercise. Additional research should evaluate the potential benefits of using IMO as a carbohydrate source in functional foods as well as other potential health/exercise effects of increasing dietary availability of IMO, as well as in longer study protocols over weeks to months.

CHAPTER V

SUMMARY OF CONCLUSIONS

Discussion

Ingestion of nutrients prior to exercise contributes to fuel availability which may reduce catabolism during exercise and promote recovery. Favorable performance benefits to training have been reported with low GI carbohydrate and protein supplementation in close proximity to exercise bouts, in particular if less than adequate amounts of carbohydrate are consumed beforehand. Athletes commonly consume glucose/electrolyte solutions (GES), gels, and energy bars prior ro, during, and/or following exercise in order to increase carbohydrate and protein availability, sustain performance, and enhance recovery. Many commercially available GES, gels, and energy bars have moderate to high GI which increases the likelihood of hypoglycemia during exercise.

We sought to determine whether using a high fiber (isomalto-oligosaccharide, IMO) in a whey protein energy/food bar would serve as an effective source of carbohydrate and protein for athletes. Previous research has demonstrated that IMOs could present an advantage on blood glucose and insulin in healthy adults. Isomalto-oligosaccharides have also proven their enhancing effects on metabolism, bifidogenic flora, bowel functions, and the immune system. In this context, IMOs could theoretically serve as a low glycemic food option for individuals on a low glycemic diet and/or athletes. It was therefore theorized that the implementation of a low GI carbohydrate and protein food bar with IMO ingested prior to and during exercise might be an effective

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nutrient strategy to positively affect glucose homeostasis as well as enhance performance and recovery.

The purpose of this study was to determine if consuming a food/energy bar containing whey protein and IMO as the source of carbohydrate would serve as an effective pre-, mid-, or post-exercise nutritional source for trained athletes. This was accomplished by conducting two studies. The first study determined the GI and GL of consuming one and two servings of this FB. The second study examined the effects of ingesting this FB prior to, during, and following exercise on glucose and insulinemic responses, exercise performance and/recovery.

In the Pharmacokinetic Study, we hypothesized that ingestion of a mixed ingredient FB with IMO would promote a moderate glycemic response and positively affect perceptions about appetite and satisfaction from food with no evidence of hypoglycemia. The results of this study supported the contention. The FB examined demonstrated a glycemic and insulinemic response with one and two servings of this FB which were much more favorable than ingesting equivalent amounts of reference carbohydrate. The glycemic index (GI) and glycemic load (GL) of the studied FB had a low GI of 34 and GL of 8.5 while promoting a similar insulin response to a high GI carbohydrate (dextrose) [203]. Perhaps the most interesting find stemming from the Pharmacokinetic Study was the fact that there was a similar insulin response to the reference carbohydrate (25 g of dextrose), despite blood glucose values remaining in the normal reference value range. This maintained glucose homeostasis combined with and similar insulin response to a reference carbohydrate PLA led to a significantly higher

insulin to glucose ratio (GIR) for the FB in comparison to the carbohydrate. The maintenance of blood glucose while observing a similar or greater insulin response has some potential applications for individuals involved in exercise programs. It is also highly beneficial for a FB to maintain blood glucose while reducing perceptions related to appetite with no effect of hypoglycemia symptoms. In this regard, additional research needed to be performed to evaluate the FB surrounding exercise.

The insulin and glucose responses found in the Pharmacokinetic Study led us to then examine the acute effects of ingesting this commercially available whey protein food bar with IMO on exercise capacity and recovery when taken prior, during, and after a single resistance-exercise and sprint-conditioning bout. The purpose of this study was to examine how this FB would affect exercise capacity and/or recovery from intense-exercise. We hypothesized that ingestion of this whey protein FB containing IMO would promote a low to moderate glycemic response with a similar insulin response during exercise, help athletes maintain exercise performance capacity during an intense training session, and hasten recovery. Based on results observed, we accept our hypotheses.

Results of this study found that the glycemic and insulinemic response of ingesting the FB prior to, during, and following intense exercise was more favorable in maintaining euglycemia than ingesting equivalent amounts of reference carbohydrate (dextrose). In this regard, blood glucose levels never increased outside of normal resting values after FB ingestion compared to an increase of up to 58% with dextrose. In agreement with the Pharmacokinetic study, the insulin response was again higher than the dextrose PLA despite no significant changes in blood glucose. This led to an

increased GIR following exercise for the FB compared to the PLA, suggesting a more favorable glucose homeostasis and anti-catabolic environment. This provides rationale as to why consumption of this FB may lessen exercise-induced catabolism and/or promote recovery from intense exercise.

The food bar also provided evidence to support an enhanced ability to maintain exercise-workloads and promote recovery. In this regard, we observed that total lifting volume from Set 1 to Set 2 and Set 3 was maintained to a greater degree during the FB treatment while significantly decreasing below baseline values with PLA. We also found that agility performance was improved from Sprint 1 to Sprint 2 in the FB treatment while being unchanged in the PLA treatment and that the participants performed the first 40 yard sprint -0.15 sec faster with FB compared to PLA. Results of this study also found evidence that ingesting this FB may lessen perceptions of muscle soreness but it had limited effects on markers of catabolism or inflammation. These findings support results of other studies that reported limited to no effects of consuming whey protein prior to and/or during exercise on markers of catabolism or inflammation. Finally, subjective ratings to hunger, appetite, and hypoglycemia were well tolerated by this food bar. Collectively, these findings indicate that the FB studied serve as a good low GI food choice for active individuals to consume prior to, during, and following intense exercise training.

Future Research

Future studies should examine the potential mechanisms involved with the glucose homeostasis and improved insulinemic responses observed in this study.

Additional research is also needed to examine how IMO and foods using IMO as a carbohydrate source influence signs and symptoms of satiety. Research should also evaluate the potential benefits of using IMO as a carbohydrate in functional foods as well as other potential health effects of increasing dietary availability of IMO. In regard to performance, future studies should repeat this study design to examine if results observed with this FB on performance and recovery are consistently reproduced. Additionally, the practical assessment of whether having athletes ingest an energy/food bar prior to, during, and/or following exercise has any influence on exercise training performance and/or recovery. It would also be prudent to test this FB on different populations (e.g. females, obese, diabetic, etc.). Additional research should evaluate the potential benefits of using IMO as a carbohydrate source in functional foods as well as other potential health/exercise effects of increasing dietary availability of IMO, as well as in longer study protocols over weeks to months. Lastly, future research should also evaluate the long-term chronic effects of IMO ingestion with exercise tolerance and performance.

Conclusion

In conclusion, the current studies demonstrated that ingestion of a whey protein with IMO maintained blood glucose and increased insulin to a greater degree than consuming a carbohydrate matched dextrose placebo. Moreover, this FB ingested prior to, during, and following a single bout of high volume resistance-training and sprint conditioning appears to be an effective dietary food in maintaining performance and reducing perceived muscle soreness. However, markers of catabolism and inflammation

were not affected. Results indicate that this FB can serve as a good low glycemic food

option for individuals to take prior to, during, and/or following intense exercise.

REFERENCES

1.   Kerksick, C.M., et al., *International society of sports nutrition position stand: nutrient timing.* Journal of the International Society of Sports Nutrition, 2017. **14**(1): p. 33.

2.   Haff, G.G., et al., *Carbohydrate supplementation attenuates muscle glycogen loss during acute bouts of resistance exercise.* International journal of sport nutrition and exercise metabolism, 2000. **10**(3): p. 326-339.

3.   Karlsson, J. and B. Saltin, *Diet, muscle glycogen, and endurance performance.* Journal of Applied Physiology, 1971. **31**(2): p. 203-206.

4.   Sherman, W., et al., *Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance.* International journal of sports medicine, 1981. **2**(02): p. 114-118.

5.   Nieman, D.C., et al., *Muscle cytokine mRNA changes after 2.5 h of cycling: influence of carbohydrate.* Medicine & Science in Sports & Exercise, 2005. **37**(8): p. 1283-1290.

6.   Warren, G.L., et al., *Excitation-contraction uncoupling: major role in contraction-induced muscle injury.* Exercise and sport sciences reviews, 2001. **29**(2): p. 82-87.

7.   Schoenfeld, B.J., A.A. Aragon, and J.W. Krieger, *The effect of protein timing on muscle strength and hypertrophy: a meta-analysis.* Journal of the International Society of Sports Nutrition, 2013. **10**(1): p. 53.

8.   Shimomura, Y., et al., *Nutraceutical effects of branched-chain amino acids on skeletal muscle.* The Journal of nutrition, 2006. **136**(2): p. 529S-532S.

9.   Van Hall, G., et al., *Mechanisms of activation of muscle branched‑chain alpha‑ keto acid dehydrogenase during exercise in man.* The Journal of physiology, 1996. **494**(3): p. 899-905.

10.   Fallon, K.E., *The acute phase response and exercise: the ultramarathon as prototype exercise.* Clinical Journal of Sport Medicine, 2001. **11**(1): p. 38-43.

11.   Vider, J., et al., *Acute immune response in respect to exercise-induced oxidative stress.* Pathophysiology, 2001. **7**(4): p. 263-270.

12.   Mastaloudis, A., et al., *Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners.* Free Radical Biology and Medicine, 2004. **36**(10): p. 1329-1341.

13.   Wynne, H.A. and M. Campbell, *Pharmacoeconomics of nonsteroidal anti-inflammatory drugs (NSAIDs).* Pharmacoeconomics, 1993. **3**(2): p. 107-123.

14.   Mauvais-Jarvis, F., et al., *Glucose response to intense aerobic exercise in type 1 diabetes: maintenance of near euglycemia despite a drastic decrease in insulin dose.* Diabetes Care, 2003. **26**(4): p. 1316-1317.

15.   Febbraio, M.A., et al., *Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance.* Journal of Applied Physiology, 2000. **89**(6): p. 2220-2226.

16.   Colberg, S.R., et al., *Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement.* Diabetes care, 2010. **33**(12): p. e147-e167.

17.   Björck, I., et al., *Food properties affecting the digestion and absorption of carbohydrates.* The American journal of clinical nutrition, 1994. **59**(3): p. 699S-705S.

18.   Kreider, R.B., et al., *Exercise & Sport Nutrition: Principles, Promises, Science & Recommendations*. 2009: Fitness Technologies Press Santa Barbara, CA.

19.   Williams, C. and I. Rollo, *Carbohydrate nutrition and team sport performance.* Sports Medicine, 2015. **45**(1): p. 13-22.

20.   Hawley, J.A. and J.J. Leckey, *Carbohydrate dependence during prolonged, intense endurance exercise.* Sports Medicine, 2015. **45**(1): p. 5-12.

21.   Cannon, J.G. and J.B. Blumberg, *Acute phase immune responses in exercise.* Handbook of oxidants and antioxidants in exercise, 2000. **177**.

22.   Nieman, D.C., *Immune response to heavy exertion.* Journal of applied physiology, 1997. **82**(5): p. 1385-1394.

23.   Urso, M.L., *Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment?* Journal of applied physiology, 2013. **115**(6): p. 920-928.

24.   Janssen, G., et al., *Plasma activity of muscle enzymes: quantification of skeletal muscle damage and relationship with metabolic variables.* International journal of sports medicine, 1989. **10**(S 3): p. S160-S168.

25. Proske, U. and T.J. Allen, *Damage to skeletal muscle from eccentric exercise.* Exercise and sport sciences reviews, 2005. **33**(2): p. 98-104.

26. Smith, L.L., *Acute inflammation: the underlying mechanism in delayed onset muscle soreness?* Medicine and science in sports and exercise, 1991. **23**(5): p. 542-551.

27. Walsh, L., et al., *Human forearm position sense after fatigue of elbow flexor muscles.* The Journal of physiology, 2004. **558**(2): p. 705-715.

28. Louis, E., et al., *Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle.* Journal of applied physiology, 2007. **103**(5): p. 1744-1751.

29. Bourgeois, J., et al., *Naproxen does not alter indices of muscle damage in resistance-exercise trained men.* Medicine and science in sports and exercise, 1999. **31**(1): p. 4-9.

30. Dudley, G.A., et al., *Efficacy of naproxen sodium for exercise-induced dysfunction muscle injury and soreness.* Clinical journal of sport medicine: official journal of the Canadian Academy of Sport Medicine, 1997. **7**(1): p. 3-10.

31. Pizza, F., et al., *Anti-inflammatory doses of ibuprofen: effect on neutrophils and exercise-induced muscle injury.* International journal of sports medicine, 1999. **20**(02): p. 98-102.

32. Tokmakidis, S.P., et al., *The effects of ibuprofen on delayed muscle soreness and muscular performance after eccentric exercise.* The Journal of Strength & Conditioning Research, 2003. **17**(1): p. 53-59.

33. Trappe, T.A., et al., *Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis.* American Journal of Physiology-Endocrinology and Metabolism, 2002. **282**(3): p. E551-E556.

34. Burd, N.A., et al., *Effect of a cyclooxygenase-2 inhibitor on postexercise muscle protein synthesis in humans.* American Journal of Physiology-Endocrinology and Metabolism, 2010. **298**(2): p. E354-E361.

35. Mikkelsen, U., et al., *Local NSAID infusion does not affect protein synthesis and gene expression in human muscle after eccentric exercise.* Scandinavian journal of medicine & science in sports, 2011. **21**(5): p. 630-644.

36. Petersen, S.G., et al., *Exercise and NSAIDs: effect on muscle protein synthesis in patients with knee osteoarthritis.* Medicine and science in sports and exercise, 2011. **43**(3): p. 425-431.

37. Armstrong, R., *Mechanisms of exercise-induced delayed onset muscular soreness: a brief review.* Medicine and science in sports and exercise, 1984. **16**(6): p. 529-538.

38. Cheung, K., P.A. Hume, and L. Maxwell, *Delayed onset muscle soreness.* Sports Medicine, 2003. **33**(2): p. 145-164.

39. Gulick, D.T. and I.F. Kimura, *Delayed onset muscle soreness: what is it and how do we treat it?* Journal of Sport Rehabilitation, 1996. **5**(3): p. 234-243.

40. Newham, D., et al., *Muscle pain and tenderness after exercise.* Australian Journal of Sports Medicine and Exercise Science, 1982. **14**: p. 129-131.

41.   Armstrong, R., *Initial events in exercise-induced muscular injury.* Medicine and science in sports and exercise, 1990. **22**(4): p. 429-435.

42.   Francis, K. and T. Hoobler, *Effects of aspirin on delayed muscle soreness.* The Journal of sports medicine and physical fitness, 1987. **27**(3): p. 333-337.

43.   Asmussen, E., *Observations on experimental muscular soreness.* Acta Rheumatologica Scandinavica, 1956. **2**(1-4): p. 109-116.

44.   Schwane, J.A., et al., *Is lactic acid related to delayed-onset muscle soreness?* The Physician and sportsmedicine, 1983. **11**(3): p. 124-131.

45.   Rogatzki, M.J., et al., *Lactate is always the end product of glycolysis.* Frontiers in neuroscience, 2015. **9**.

46.   Feron, O., *Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells.* Radiotherapy and oncology, 2009. **92**(3): p. 329-333.

47.   Cleak, M. and R. Eston, *Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise.* British journal of sports medicine, 1992. **26**(4): p. 267-272.

48.   Stauber, V., *T.(1989). Eccentric action of muscles: Physiology, injury, and adaptation.* Exercise and Sport Sciences Reviews: p. 157-185.

49.   Hough, T., *Ergographic studies in muscular soreness.* American Physical Education Review, 1902. **7**(1): p. 1-17.

50.   Sydney-Smith, M. and B.M. Quigley, *Delayed Onset Muscle Soreness: Evidence of Connective Tissue Damage, Lipid Peroxidation and Altered Renal Function After Exercise*. 1992: National Sports Research Centre.

51.   Evans, W., et al., *Metabolic changes following eccentric exercise in trained and untrained men.* Journal of Applied Physiology, 1986. **61**(5): p. 1864-1868.

52.   Hasson, S.M., et al., *Effect of ibuprofen use on muscle soreness, damage, and performance: a preliminary investigation.* Medicine and science in sports and exercise, 1993. **25**(1): p. 9-17.

53.   Smith, M. and C. Jackson, *199 Delayed Onset Muscle Soreness (doms), Serum Creatine Kinase (sck), And Creatine Kinase-mb (ck-mb) Related To Performance Measurements In Football.* Medicine & Science in Sports & Exercise, 1990. **22**(2): p. S34.

54.   Friden, J., P. Sfakianos, and A. Hargens, *Muscle soreness and intramuscular fluid pressure: comparison between eccentric and concentric load.* Journal of Applied Physiology, 1986. **61**(6): p. 2175-2179.

55.   Lee, E.C., et al., *Biomarkers in Sports and Exercise: Tracking Health, Performance, and Recovery in Athletes.* Journal of strength and conditioning research, 2017. **31**(10): p. 2920.

56.   Gillespie, C. and V. Edgerton, *The role of testosterone in exercise-induced glycogen supercompensation.* Hormone and metabolic research, 1970. **2**(06): p. 364-366.

57.   Cevada, T., et al., *Salivary cortisol levels in athletes and nonathletes: a systematic review.* Hormone and Metabolic Research, 2014. **46**(13): p. 905-910.

58.   Urhausen, A., H. Gabriel, and W. Kindermann, *Blood hormones as markers of training stress and overtraining.* Sports medicine, 1995. **20**(4): p. 251-276.

59.   Vervoorn, C., et al., *The behaviour of the plasma free testosterone/cortisol ratio during a season of elite rowing training.* International journal of sports medicine, 1991. **12**(03): p. 257-263.

60.   Banfi, G., et al., *Usefulness of free testosterone/cortisol ratio during a season of elite speed skating athletes.* International journal of sports medicine, 1993. **14**(07): p. 373-379.

61.   Biolo, G., et al., *An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein.* American Journal of Physiology-Endocrinology And Metabolism, 1997. **273**(1): p. E122-E129.

62.   Wolfe, R.R., *Effects of insulin on muscle tissue.* Current Opinion in Clinical Nutrition & Metabolic Care, 2000. **3**(1): p. 67-71.

63.   Kraemer, W.J. and N.A. Ratamess, *Hormonal responses and adaptations to resistance exercise and training.* Sports medicine, 2005. **35**(4): p. 339-361.

64.   Chandler, R., et al., *Dietary supplements affect the anabolic hormones after weight-training exercise.* Journal of applied physiology, 1994. **76**(2): p. 839-845.

65.   Kraemer, W.J., et al., *Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation.* Journal of Applied Physiology, 1998. **85**(4): p. 1544-1555.

66. Raastad, T., T. Bjøro, and J. Hallen, *Hormonal responses to high-and moderate-intensity strength exercise.* European journal of applied physiology, 2000. **82**(1-2): p. 121-128.

67. Koch, A., R. Pereira, and M. Machado, *The creatine kinase response to resistance exercise.* J Musculoskelet Neuronal Interact, 2014. **14**(1): p. 68-77.

68. Mougios, V., *Reference intervals for serum creatine kinase in athletes.* British journal of sports medicine, 2007. **41**(10): p. 674-678.

69. Hong, C.-Z. and I. Lien, *Metabolic effects of exhaustive training of athletes.* Archives of physical medicine and rehabilitation, 1984. **65**(7): p. 362-365.

70. Karlsson, J., B. Diamant, and B. Saltin, *Lactate dehydrogenase activity in muscle after prolonged severe exercise in man.* Journal of applied physiology, 1968. **25**(1): p. 88-91.

71. Smith, L.L., *Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress?* Medicine & Science in Sports & Exercise, 2000. **32**(2): p. 317.

72. Margonis, K., et al., *Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis.* Free Radical Biology and Medicine, 2007. **43**(6): p. 901-910.

73. Suzuki, K., et al., *Systemic inflammatory response to exhaustive exercise. Cytokine kinetics.* Exercise immunology review, 2002. **8**: p. 6-48.

74. Brenner, I., et al., *Impact of three different types of exercise on components of the inflammatory response.* European journal of applied physiology and occupational physiology, 1999. **80**(5): p. 452-460.

75. Toumi, H. and T. Best, *The inflammatory response: friend or enemy for muscle injury?* British journal of sports medicine, 2003. **37**(4): p. 284-286.

76. Gleeson, M., *Biochemical and immunological markers of over-training.* Journal of sports science & medicine, 2002. **1**(2): p. 31.

77. Kerksick, C.M., et al., *International society of sports nutrition position stand: nutrient timing.* J Int Soc Sports Nutr, 2017. **14**: p. 33.

78. Jager, R., et al., *International Society of Sports Nutrition Position Stand: protein and exercise.* J Int Soc Sports Nutr, 2017. **14**: p. 20.

79. Campbell, B., et al., *International Society of Sports Nutrition position stand: energy drinks.* J Int Soc Sports Nutr, 2013. **10**(1): p. 1.

80. Kreider, R.B., et al., *ISSN exercise & sport nutrition review: research & recommendations.* J Int Soc Sports Nutr, 2010. **7**: p. 7.

81. Hertzler, S., *Glycemic index of "energy" snack bars in normal volunteers.* J Am Diet Assoc, 2000. **100**(1): p. 97-100.

82. Kreider, R.B., et al., *Effects of ingesting protein with various forms of carbohydrate following resistance-exercise on substrate availability and markers of anabolism, catabolism, and immunity.* J Int Soc Sports Nutr, 2007. **4**: p. 18.

83. Earnest, C.P., et al., *Low vs. high glycemic index carbohydrate gel ingestion during simulated 64-km cycling time trial performance.* J Strength Cond Res, 2004. **18**(3): p. 466-72.

84. Macdougall, J.D., et al., *Muscle Substrate Utilization And Lactate Production.* Can J Appl Physiol, 1999. **24**.

85. Pascoe, D.D., et al., *Glycogen Resynthesis In Skeletal Muscle Following Resistive Exercise.* Med Sci Sports Exerc, 1993. **25**.

86. Robergs, R.A., et al., *Muscle Glycogenolysis During Differing Intensities Of Weight-Resistance Exercise.* J Appl Physiol, 1991. **70**.

87. Tesch, P.A., E.B. Colliander, and P. Kaiser, *Muscle metabolism during intense, heavy-resistance exercise.* European journal of applied physiology and occupational physiology, 1986. **55**(4): p. 362-366.

88. Haff, G.G., et al., *The Effect Of Carbohydrate Supplementation On Multiple Sessions And Bouts Of Resistance Exercise.* J Strength Cond Res, 1999. **13**.

89. Dalton, R.A., et al., *Acute Carbohydrate Consumption Does Not Influence Resistance Exercise Performance During Energy Restriction.* Int J Sport Nutr, 1999. **9**.

90. Kulik, J.R., et al., *Supplemental Carbohydrate Ingestion Does Not Improve Performance Of High-Intensity Resistance Exercise.* J Strength Cond Res, 2008. **22**.

91. Burke, L.M., et al., *Carbohydrates for training and competition.* Journal of sports sciences, 2011. **29**(sup1): p. S17-S27.

92. Atkinson, F.S., K. Foster-Powell, and J.C. Brand-Miller, *International tables of glycemic index and glycemic load values: 2008.* Diabetes care, 2008.

93. Thomas, D., J. Brotherhood, and J. Brand, *Carbohydrate feeding before exercise: effect of glycemic index.* Int J Sports Med, 1991. **12**(2): p. 180-6.

94. Wong, S.H., et al., *Effect of the glycaemic index of pre-exercise carbohydrate meals on running performance.* European Journal of Sport Science, 2008. **8**(1): p. 23-33.

95. Wu, C.-L. and C. Williams, *A low glycemic index meal before exercise improves endurance running capacity in men.* International journal of sport nutrition and exercise metabolism, 2006. **16**(5): p. 510-527.

96. Wee, S.-l., et al., *Influence of high and low glycemic index meals on endurance running capacity.* Medicine and Science in Sports and Exercise, 1999. **31**(3): p. 393-399.

97. Maiorana, A., et al., *Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes.* Diabetes research and clinical practice, 2002. **56**(2): p. 115-123.

98. Solomon, T.P., et al., *A low–glycemic index diet combined with exercise reduces insulin resistance, postprandial hyperinsulinemia, and glucose-dependent insulinotropic polypeptide responses in obese, prediabetic humans–.* The American journal of clinical nutrition, 2010. **92**(6): p. 1359-1368.

99. Castaneda, C., et al., *A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes.* Diabetes care, 2002. **25**(12): p. 2335-2341.

100. Goffin, D., et al., *Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of*

knowledge on these prebiotics. Critical reviews in food science and nutrition, 2011. **51**(5): p. 394-409.

101.   Mookiah, S., et al., *Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens.* Journal of the Science of Food and Agriculture, 2014. **94**(2): p. 341-348.

102.   Singh, D., et al., *A novel cobiotic-based preventive approach against high-fat diet-induced adiposity, nonalcoholic fatty liver and gut derangement in mice.* International Journal of Obesity, 2016. **40**(3): p. 487-496.

103.   Singh, D.P., et al., *Isomalto-oligosaccharides, a prebiotic, functionally augment green tea effects against high fat diet-induced metabolic alterations via preventing gut dysbacteriosis in mice.* Pharmacological Research, 2017. **123**: p. 103-113.

104.   Wang, W., et al., *Isomalto-oligosaccharides ameliorate visceral hyperalgesia with repair damage of ileal epithelial ultrastructure in rats.* PloS one, 2017. **12**(4): p. e0175276.

105.   Playne, M. and R. Crittenden, *Part II Biotechnology strategies for producing specific food ingridents.* Bioprocesses and Biotechnology for Functional Foods and Nutraceuticals, 2004: p. 120-121.

106.   Kothari, D. and A. Goyal, *Enzyme‑resistant isomalto‑oligosaccharides produced from Leuconostoc mesenteroides NRRL B‑1426 dextran hydrolysis for functional*

food application. Biotechnology and applied biochemistry, 2016. **63**(4): p. 581-589.

107. Ojha, S., S. Mishra, and S. Chand, *Production of isomalto-oligosaccharides by cell bound α-glucosidase of Microbacterium sp.* LWT-Food Science and Technology, 2015. **60**(1): p. 486-494.

108. Pazur, J.H. and D. French, *The action of transglucosidase of Aspergillus oryzae on maltose.* J. biol. Chem, 1952. **196**(1): p. 265-272.

109. McCleary, B.V., et al., *Purification, properties, and industrial significance of transglucosidase from Aspergillus niger.* Carbohydrate research, 1989. **185**(1): p. 147-162.

110. Nakakuki, T., *Present status and future prospects of functional oligosaccharide development in Japan.* Journal of Applied Glycoscience, 2005. **52**(3): p. 267-271.

111. Kalra, P.A., *Introducing iron isomaltoside 1000 (Monofer®)—development rationale and clinical experience.* NDT plus, 2011. **4**(suppl_1): p. i10-i13.

112. Singh, D.P., et al., *Coadministration of isomalto-oligosaccharides augments metabolic health benefits of cinnamaldehyde in high fat diet fed mice.* BioFactors, 2017.

113. Singh, D.P., et al., *Co-supplementation of isomalto-oligosaccharides potentiates metabolic health benefits of polyphenol-rich cranberry extract in high fat diet-fed mice via enhanced gut butyrate production.* European Journal of Nutrition, 2017: p. 1-15.

114. Fooks, L.J., R. Fuller, and G.R. Gibson, *Prebiotics, probiotics and human gut microbiology.* International dairy journal, 1999. **9**(1): p. 53-61.

115. Kolida, S., K. Tuohy, and G.R. Gibson, *The human gut flora in nutrition and approaches for its dietary modulation.* Nutrition Bulletin, 2000. **25**(3): p. 223-231.

116. Rastall, R.A. and V. Maitin, *Prebiotics and synbiotics: towards the next generation.* Current Opinion in Biotechnology, 2002. **13**(5): p. 490-496.

117. Macfarlane, S., G. Macfarlane, and J.t. Cummings, *Prebiotics in the gastrointestinal tract.* Alimentary pharmacology & therapeutics, 2006. **24**(5): p. 701-714.

118. Roberfroid, M.B., *Functional foods: concepts and application to inulin and oligofructose.* British Journal of Nutrition, 2002. **87**(S2): p. S139-S143.

119. Tuohy, K., et al., *Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy.* Current pharmaceutical design, 2005. **11**(1): p. 75-90.

120. Gibson, G.R. and M.B. Roberfroid, *Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics.* The Journal of nutrition, 1995. **125**(6): p. 1401.

121. Van Loo, J., A. Franck, and M. Roberfroid, *Functional food properties of non-digestible oligosaccharides–reply by Van Loo et al.* British Journal of Nutrition, 1999. **82**(4): p. 329-329.

122. Yatake, T., *Anomalously linked oligosaccharides.* Oligosaccharides. Japanese Technology Reviews, 1993. **3**(2): p. 79-89.

123. Hamada, S. and M. Torii, *Interaction of glucosyltransferase from Streptococcus mutans with various glucans.* Microbiology, 1980. **116**(1): p. 51-59.

124. Delzenne, N.M. and C.M. Williams, *Prebiotics and lipid metabolism.* Current opinion in lipidology, 2002. **13**(1): p. 61-67.

125. Lee, M., S. Ly, and K. Lee, *Improving Effects of Fructooligosaccharide and Isomaltooligosaccharide Contained in Sponge Cakes on the Constipation of Femal College Students.* Journal of the Korean Society of Food Science and Nutrition, 2003.

126. Cockburn, E., et al., *Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage.* European journal of applied physiology, 2012. **112**(9): p. 3187-3194.

127. Gilson, S.F., et al., *Effects of chocolate milk consumption on markers of muscle recovery following soccer training: a randomized cross-over study.* Journal of the International Society of Sports Nutrition, 2010. **7**(1): p. 19.

128. Slater, G. and S.M. Phillips, *Nutrition guidelines for strength sports: sprinting, weightlifting, throwing events, and bodybuilding.* Journal of sports sciences, 2011. **29**(sup1): p. S67-S77.

129. Haff, G.G., et al., *The Effect of Carbohydrate Supplementation on Multiple Sessions and Bouts of Resistance Exercise.* The Journal of Strength & Conditioning Research, 1999. **13**(2): p. 111-117.

130. Karlsson, J. and B. Saltin, *Diet, Muscle Glycogen, And Endurance Performance.* J Appl Physiol, 1971. **31**.

131. Sherman, W.M., et al., *Effect Of Exercise-Diet Manipulation On Muscle Glycogen And Its Subsequent Utilization During Performance.* Int J Sports Med, 1981. **2**.

132. Ivy, J.L., et al., *Muscle Glycogen Synthesis After Exercise: Effect Of Time Of Carbohydrate Ingestion.* J Appl Physiol, 1988. **64**.

133. Coyle, E.F., et al., *Muscle Glycogen Utilization During Prolonged Strenuous Exercise When Fed Carbohydrate.* J Appl Physiol, 1986. **61**.

134. Coyle, E.F., et al., *Substrate Usage During Prolonged Exercise Following A Preexercise Meal.* J Appl Physiol, 1985. **59**.

135. Kerksick, C.M., et al., *International society of sports nutrition position stand: nutrient timing.* Journal of the International Society of Sports Nutrition, 2017. **14**(1): p. 33.

136. Gleeson, M., D.C. Nieman, and B.K. Pedersen, *Exercise, Nutrition And Immune Function.* J Sports Sci, 2004. **22**.

137. Rodriguez, N.R., N.M. Marco, and S. Langley, *American College Of Sports Medicine Position Stand. Nutrition And Athletic Performance.* Med Sci Sports Exerc, 2009. **41**.

138. Howarth, K.R., et al., *Coingestion Of Protein With Carbohydrate During Recovery From Endurance Exercise Stimulates Skeletal Muscle Protein Synthesis In Humans.* J Appl Physiol, 2009. **106**.

139.   Van Hall, G., S.M. Shirreffs, and J.A. Calbet, *Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion.* Journal of Applied Physiology, 2000. **88**(5): p. 1631-1636.

140.   van Loon, L.J., et al., *Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures.* The American journal of clinical nutrition, 2000. **72**(1): p. 106-111.

141.   Carter, S.L., C. Rennie, and M.A. Tarnopolsky, *Substrate Utilization During Endurance Exercise In Men And Women After Endurance Training.* Am J Physiol Endocrinol Metab, 2001. **280**.

142.   Devries, M.C., et al., *Menstrual Cycle Phase And Sex Influence Muscle Glycogen Utilization And Glucose Turnover During Moderate-Intensity Endurance Exercise.* Am J Phys Regul Integr Comp Phys, 2006. **291**.

143.   Riddell, M.C., et al., *Substrate Utilization During Exercise Performed With And Without Glucose Ingestion In Female And Male Endurance Trained Athletes.* Int J Sport Nutr Exerc Metab, 2003. **13**.

144.   Wismann, J. and D. Willoughby, *Gender Differences In Carbohydrate Metabolism And Carbohydrate Loading.* J Int Soc Sports Nutr, 2006. **3**.

145.   Rennie, M.J., *Control of muscle protein synthesis as a result of contractile activity and amino acid availability: implications for protein requirements.* International journal of sport nutrition and exercise metabolism, 2001. **11**(s1): p. S170-S176.

146. Zawadzki, K., B. Yaspelkis 3rd, and J. Ivy, *Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise.* Journal of Applied Physiology, 1992. **72**(5): p. 1854-1859.

147. Biolo, G., et al., *Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans.* American Journal of Physiology-Endocrinology And Metabolism, 1995. **268**(3): p. E514-E520.

148. Tipton, K.D., et al., *Postexercise net protein synthesis in human muscle from orally administered amino acids.* American Journal of Physiology-Endocrinology And Metabolism, 1999. **276**(4): p. E628-E634.

149. Tipton, K., et al., *Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers.* The Journal of nutritional biochemistry, 1999. **10**(2): p. 89-95.

150. Børsheim, E., et al., *Essential amino acids and muscle protein recovery from resistance exercise.* American Journal of Physiology-Endocrinology And Metabolism, 2002. **283**(4): p. E648-E657.

151. Tipton, K.D., et al., *Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise.* American Journal of Physiology-Endocrinology And Metabolism, 2001. **281**(2): p. E197-E206.

152. Volek, J.S., *Influence of nutrition on responses to resistance training.* Medicine & Science in Sports & Exercise, 2004. **36**(4): p. 689-696.

153.   Farnfield, M.M., et al., *Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion.* Applied Physiology, Nutrition, and Metabolism, 2011. **37**(1): p. 21-30.

154.   Tang, J.E., et al., *Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men.* Applied Physiology, Nutrition, and Metabolism, 2007. **32**(6): p. 1132-1138.

155.   Tipton, K.D., *Role of protein and hydrolysates before exercise.* International journal of sport nutrition and exercise metabolism, 2007. **17**(s1): p. S77-S86.

156.   Hulmi, J.J., et al., *Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression.* Amino acids, 2009. **37**(2): p. 297-308.

157.   Coffey, V.G., et al., *Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints.* European journal of applied physiology, 2011. **111**(7): p. 1473-1483.

158.   Breen, L., et al., *The influence of carbohydrate–protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis.* The Journal of physiology, 2011. **589**(16): p. 4011-4025.

159.   Ferguson-Stegall, L., et al., *Postexercise carbohydrate–protein supplementation improves subsequent exercise performance and intracellular signaling for protein synthesis.* The Journal of Strength & Conditioning Research, 2011. **25**(5): p. 1210-1224.

160.   Ivy, J., et al., *Post exercise carbohydrate–protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation.* Amino acids, 2008. **35**(1): p. 89-97.

161.   Saunders, M.J., N.D. Luden, and J.E. Herrick, *Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage.* Journal of Strength and Conditioning Research, 2007. **21**(3): p. 678.

162.   Morifuji, M., et al., *Dietary whey protein increases liver and skeletal muscle glycogen levels in exercise-trained rats.* British journal of nutrition, 2005. **93**(4): p. 439-445.

163.   Jäger, R., et al., *International society of sports nutrition position stand: protein and exercise.* Journal of the International Society of Sports Nutrition, 2017. **14**(1): p. 20.

164.   Tipton, K.D., et al., *Stimulation Of Net Muscle Protein Synthesis By Whey Protein Ingestion Before And After Exercise.* Am J Physiol Endocrinol Metab, 2007. **292**.

165.   Andersen, L.L., et al., *The Effect Of Resistance Training Combined With Timed Ingestion Of Protein On Muscle Fiber Size And Muscle Strength.* Metab Clin Exp, 2005. **54**.

166.   Hoffman, J.R., et al., *Effect Of Protein-Supplement Timing On Strength, Power, And Body-Composition Changes In Resistance-Trained Men.* Int J Sport Nutr Exerc Metab, 2009. **19**.

167. Delmonico, M.J., et al., *Can Dual Energy X-Ray Absorptiometry Provide A Valid Assessment Of Changes In Thigh Muscle Mass With Strength Training In Older Adults?* Eur J Clin Nutr, 2008. **62**.

168. Schoenfeld, B.J., et al., *Pre- Versus Post-Exercise Protein Intake Has Similar Effects On Muscular Adaptations.* Peerj, 2017. **5**.

169. Ayers, K., M. Pazmino-Cevallos, and C. Dobose, *The 20-Hour Rule: Student-Athletes Time Commitment To Athletics And Academics.* Vahperd Journal, 2012. **33**.

170. Bird, S.P., K.M. Tarpenning, and F.E. Marino, *Effects Of Liquid Carbohydrate/Essential Amino Acid Ingestion On Acute Hormonal Response During A Single Bout Of Resistance Exercise In Untrained Men.* Nutrition, 2006. **22**.

171. Bird, S.P., K.M. Tarpenning, and F.E. Marino, *Liquid Carbohydrate/Essential Amino Acid Ingestion During A Short-Term Bout Of Resistance Exercise Suppresses Myofibrillar Protein Degradation.* Metab Clin Exp, 2006. **55**.

172. Bird, S.P., K.M. Tarpenning, and F.E. Marino, *Independent And Combined Effects Of Liquid Carbohydrate/Essential Amino Acid Ingestion On Hormonal And Muscular Adaptations Following Resistance Training In Untrained Men.* Eur J Appl Physiol, 2006. **97**.

173. Bohe, J., et al., *Latency And Duration Of Stimulation Of Human Muscle Protein Synthesis During Continuous Infusion Of Amino Acids.* J Physiol, 2001. **532**.

174.   Burd, N.A., et al., *Enhanced Amino Acid Sensitivity Of Myofibrillar Protein Synthesis Persists For Up To 24 H After Resistance Exercise In Young Men.* J Nutr, 2011. **141**.

175.   Dreyer, H.C., et al., *Leucine-Enriched Essential Amino Acid And Carbohydrate Ingestion Following Resistance Exercise Enhances Mtor Signaling And Protein Synthesis In Human Muscle.* Am J Physiol Endocrinol Metab, 2008. **294**.

176.   Fujita, S., et al., *Nutrient Signalling In The Regulation Of Human Muscle Protein Synthesis.* J Physiol, 2007. **582**.

177.   West, D.W., et al., *Rapid Aminoacidemia Enhances Myofibrillar Protein Synthesis And Anabolic Intramuscular Signaling Responses After Resistance Exercise.* Am J Clin Nutr, 2011. **94**.

178.   Mitchell, C.J., et al., *Acute Post-Exercise Myofibrillar Protein Synthesis Is Not Correlated With Resistance Training-Induced Muscle Hypertrophy In Young Men.* PLoS One, 2014. **9**.

179.   Cribb, P.J. and A. Hayes, *Effects Of Supplement Timing And Resistance Exercise On Skeletal Muscle Hypertrophy.* Med Sci Sports Exerc, 2006. **38**.

180.   Willoughby, D.S., J.R. Stout, and C.D. Wilborn, *Effects Of Resistance Training And Protein Plus Amino Acid Supplementation On Muscle Anabolism, Mass. And Strength Amino Acids, 2007.* **32**.

181.   Tipton, K.D., et al., *Timing Of Amino Acid-Carbohydrate Ingestion Alters Anabolic Response Of Muscle To Resistance Exercise.* Am J Physiol Endocrinol Metab, 2001. **281**.

182.   Esmarck, B., et al., *Timing Of Postexercise Protein Intake Is Important For Muscle Hypertrophy With Resistance Training In Elderly Humans.* J Physiol, 2001. **535**.

183.   Borde, R., T. Hortobagyi, and U. Granacher, *Dose-Response Relationships Of Resistance Training In Healthy Old Adults: A Systematic Review And Meta-Analysis.* Sports Med, 2015. **45**.

184.   Cermak, N.M., et al., *Protein Supplementation Augments The Adaptive Response Of Skeletal Muscle To Resistance-Type Exercise Training: A Meta-Analysis.* Am J Clin Nutr, 2012. **96**.

185.   Candow, D.G., et al., *Protein Supplementation Before And After Resistance Training In Older Men.* Eur J Appl Physiol, 2006. **97**.

186.   Aragon, A.A. and B.J. Schoenfeld, *Nutrient Timing Revisited: Is There A Post-Exercise Anabolic Window?* J Int Soc Sports Nutr, 2013. **10**.

187.   Schoenfeld, B.J., A.A. Aragon, and J.W. Krieger, *The Effect Of Protein Timing On Muscle Strength And Hypertrophy: A Meta-Analysis.* J Int Soc Sports Nutr, 2013. **10**.

188.   Macnaughton, L.S., et al., *The Response Of Muscle Protein Synthesis Following Whole-Body Resistance Exercise Is Greater Following 40 G Than 20 G Of Ingested Whey Protein.* Phys Rep, 2016. **4**.

189.   Kraemer, W.J., et al., *Effects Of A Multi-Nutrient Supplement On Exercise Performance And Hormonal Responses To Resistance Exercise.* Eur J Appl Physiol, 2007. **101**.

190. Baty, J.J., et al., *The Effect Of A Carbohydrate And Protein Supplement On Resistance Exercise Performance, Hormonal Response, And Muscle Damage.* J Strength Cond Res, 2007. **21**.

191. White, J.P., et al., *Effect Of Carbohydrate-Protein Supplement Timing On Acute Exercise-Induced Muscle Damage.* J Int Soc Sports Nutr, 2008. **5**.

192. Beelen, M., et al., *Protein Coingestion Stimulates Muscle Protein Synthesis During Resistance-Type Exercise.* Am J Physiol Endocrinol Metab, 2008. **295**.

193. Bird, S.P., et al., *Triphasic Multinutrient Supplementation During Acute Resistance Exercise Improves Session Volume Load And Reduces Muscle Damage In Strength-Trained Athletes.* Nutr Res, 2013. **33**.

194. Abdulla, H., et al., *Role Of Insulin In The Regulation Of Human Skeletal Muscle Protein Synthesis And Breakdown: A Systematic Review And Meta-Analysis.* Diabetologia, 2016. **59**.

195. Kimball, S.R., P.A. Farrell, and L.S. Jefferson, *Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise.* Journal of Applied Physiology, 2002. **93**(3): p. 1168-1180.

196. Hulmi, J.J., et al., *The Effects Of Whey Protein With Or Without Carbohydrates On Resistance Training Adaptations.* J Int Soc Sports Nutr, 2015. **12**.

197. Power, O., A. Hallihan, and P. Jakeman, *Human Insulinotropic Response To Oral Ingestion Of Native And Hydrolysed Whey Protein.* Amino Acids, 2009. **37**.

198. Staples, A.W., et al., *Carbohydrate Does Not Augment Exercise-Induced Protein Accretion Versus Protein Alone.* Med Sci Sports Exerc, 2011. **43**.

199. Rasmussen, B.B., et al., *An Oral Essential Amino Acid-Carbohydrate Supplement Enhances Muscle Protein Anabolism After Resistance Exercise.* J Appl Physiol, 2000. **88**.

200. MacKinnon, L.T., *Overtraining effects on immunity and performance in athletes.* Immunology & Cell Biology, 2000. **78**(5): p. 502-509.

201. Kreider, R.B., et al., *ISSN exercise & sport nutrition review: research & recommendations.* Journal of the International Society of Sports Nutrition, 2010. **7**(1): p. 7.

202. Painter, J.E. and V.I. Prisecaru, *The effects of various protein and carbohydrate ingredients in energy bars on blood glucose levels in humans.* Cereal foods world, 2002. **47**(6): p. 236.

203. Grubic TJ, S.R., Dalton R, Collins PB, Reyes AG, Favot CJ, Rasmussen C, Greenwood M, Murano PS, Earnest CP and Kreider RB, *Glycemic and Insulinemic Response to Ingestion of a Novel Food Bar Containing Whey Protein and Isomalto-Oligosaccharides.* Austin Journal of Nutrition and Food Sciences, 2018. **6**(1).

204. Levers, K., et al., *Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals.* Journal of the International Society of Sports Nutrition, 2016. **13**(1): p. 22.

205. Goffin, D., et al., *Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of knowledge on these prebiotics.* Crit Rev Food Sci Nutr, 2011. **51**(5): p. 394-409.

206.   Wang, W., et al., *Isomalto-oligosaccharides ameliorate visceral hyperalgesia with repair damage of ileal epithelial ultrastructure in rats.* PLoS One, 2017. **12**(4): p. e0175276.

207.   Singh, D.P., et al., *Isomalto-oligosaccharides, a prebiotic, functionally augment green tea effects against high fat diet-induced metabolic alterations via preventing gut dysbacteriosis in mice.* Pharmacol Res, 2017. **123**: p. 103-113.

208.   Singh, D.P., et al., *A novel cobiotic-based preventive approach against high-fat diet-induced adiposity, nonalcoholic fatty liver and gut derangement in mice.* Int J Obes (Lond), 2016. **40**(3): p. 487-96.

209.   Mookiah, S., et al., *Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens.* J Sci Food Agric, 2014. **94**(2): p. 341-8.

210.   Singh, D.P., et al., *Co-supplementation of isomalto-oligosaccharides potentiates metabolic health benefits of polyphenol-rich cranberry extract in high fat diet-fed mice via enhanced gut butyrate production.* Eur J Nutr, 2017.

211.   Singh, D.P., et al., *Coadministration of isomalto-oligosaccharides augments metabolic health benefits of cinnamaldehyde in high fat diet fed mice.* Biofactors, 2017.

212.   Kalra, P.A., *Introducing iron isomaltoside 1000 (Monofer(R))-development rationale and clinical experience.* NDT Plus, 2011. **4**(Suppl 1): p. i10-i13.

213.   Medicine, A.C.o.S., *ACSM's guidelines for exercise testing and prescription.* 2013: Lippincott Williams & Wilkins.

214. Bowling, J.L. and A. Katayev, *An evaluation of the Roche Cobas c 111.* Laboratory Medicine, 2010. **41**(7): p. 398-402.

215. Jenkins, D., et al., *Glycemic index of foods: a physiological basis for carbohydrate exchange.* The American journal of clinical nutrition, 1981. **34**(3): p. 362-366.

216. Bush, V.J., et al., *Comparison of BD Vacutainer SST™ plus tubes with BD SST™ II plus tubes for common analytes.* Clinica chimica acta, 2001. **306**(1): p. 139-143.

217. Brand-Miller, J. and S. Holt, *Testing the glycaemic index of foods: in vivo, not in vitro.* Eur J Clin Nutr, 2004. **58**(4): p. 700-1.

218. Foster-Powell, K., S.H. Holt, and J.C. Brand-Miller, *International table of glycemic index and glycemic load values: 2002.* Am J Clin Nutr, 2002. **76**(1): p. 5-56.

219. Zhang, Y., et al., *PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel.* Comput Methods Programs Biomed, 2010. **99**(3): p. 306-14.

220. Pruessner, J.C., et al., *Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change.* Psychoneuroendocrinology, 2003. **28**(7): p. 916-31.

221. Page, P., *Beyond statistical significance: clinical interpretation of rehabilitation research literature.* Int J Sports Phys Ther, 2014. **9**(5): p. 726-36.

222. Wallace, A.J., et al., *A glucose reference curve is the optimum method to determine the glycemic glucose equivalent values of foods in humans.* Nutr Res, 2008. **28**(11): p. 753-9.

223. Wolever, T.M., *Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values.* Br J Nutr, 2004. **91**(2): p. 295-301.

224. Sorensen, L.B., et al., *Effect of sensory perception of foods on appetite and food intake: a review of studies on humans.* Int J Obes Relat Metab Disord, 2003. **27**(10): p. 1152-66.

225. Barazzoni, R., et al., *Carbohydrates and insulin resistance in clinical nutrition: Recommendations from the ESPEN expert group.* Clin Nutr, 2017. **36**(2): p. 355-363.

226. Sandouk, Z. and M.C. Lansang, *Diabetes with obesity--Is there an ideal diet?* Cleve Clin J Med, 2017. **84**(7 Suppl 1): p. S4-S14.

227. Baetge, C., et al., *Efficacy of a randomized trial examining commercial weight loss programs and exercise on metabolic syndrome in overweight and obese women.* Appl Physiol Nutr Metab, 2017. **42**(2): p. 216-227.

228. Kerksick, C.M., et al., *Changes in weight loss, body composition and cardiovascular disease risk after altering macronutrient distributions during a regular exercise program in obese women.* Nutr J, 2010. **9**: p. 59.

229. Kreider, R.B., et al., *A carbohydrate-restricted diet during resistance training promotes more favorable changes in body composition and markers of health in*

obese women with and without insulin resistance. Phys Sportsmed, 2011. **39**(2): p. 27-40.

230.   Kreider, R.B., et al., *A structured diet and exercise program promotes favorable changes in weight loss, body composition, and weight maintenance.* J Am Diet Assoc, 2011. **111**(6): p. 828-43.

231.   Lockard, B., et al., *Retrospective Analysis of Protein- and Carbohydrate-Focused Diets Combined with Exercise on Metabolic Syndrome Prevalence in Overweight and Obese Women.* Metab Syndr Relat Disord, 2016. **14**(4): p. 228-37.

232.   Wilborn, C., et al., *Obesity: prevalence, theories, medical consequences, management, and research directions.* J Int Soc Sports Nutr, 2005. **2**: p. 4-31.

233.   Gunnerud, U.J., E.M. Ostman, and I.M. Bjorck, *Effects of whey proteins on glycaemia and insulinaemia to an oral glucose load in healthy adults; a dose-response study.* Eur J Clin Nutr, 2013. **67**(7): p. 749-53.

234.   Notomi, T., et al., *Insulinogenic sucrose+amino acid mixture ingestion immediately after resistance exercise has an anabolic effect on bone compared with non-insulinogenic fructose+amino acid mixture in growing rats.* Bone, 2014. **65**: p. 42-8.

235.   Salehi, A., et al., *The insulinogenic effect of whey protein is partially mediated by a direct effect of amino acids and GIP on beta-cells.* Nutr Metab (Lond), 2012. **9**(1): p. 48.

236. Schmid, R., et al., *Role of amino acids in stimulation of postprandial insulin, glucagon, and pancreatic polypeptide in humans.* Pancreas, 1989. **4**(3): p. 305-14.

237. Mizubuchi, H., et al., *Isomalto-oligosaccharides polarize Th1-like responses in intestinal and systemic immunity in mice.* J Nutr, 2005. **135**(12): p. 2857-61.

238. Kihara, M. and T. Sakata, *Production of short-chain fatty acids and gas from various oligosaccharides by gut microbes of carp (Cyprinus carpio L.) in micro-scale batch culture.* Comp Biochem Physiol A Mol Integr Physiol, 2002. **132**(2): p. 333-40.

239. Rycroft, C.E., et al., *A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides.* J Appl Microbiol, 2001. **91**(5): p. 878-87.

240. Reynolds, R.C., et al., *Effect of the glycemic index of carbohydrates on day-long (10 h) profiles of plasma glucose, insulin, cholecystokinin and ghrelin.* Eur J Clin Nutr, 2009. **63**(7): p. 872-8.

241. Speechly, D.P., G.G. Rogers, and R. Buffenstein, *Acute appetite reduction associated with an increased frequency of eating in obese males.* Int J Obes Relat Metab Disord, 1999. **23**(11): p. 1151-9.

242. Kerksick, C.M., et al., *ISSN exercise & sports nutrition review update: research & recommendations.* J Int Soc Sports Nutr, 2018. **15**(1): p. 38.

243. Grubic, T., et al., *Glycemic and insulinemic response to ingestion of a novel food bar containing whey protein and isomalto-oligosaccharides.* Austin Journal of Nutrition and Food Sciences, 2018. **6**(1).

244. Magrans-Courtney, T., et al., *Effects of diet type and supplementation of glucosamine, chondroitin, and MSM on body composition, functional status, and markers of health in women with knee osteoarthritis initiating a resistance-based exercise and weight loss program.* J Int Soc Sports Nutr, 2011. **8**(1): p. 8.

245. Bazzano, L.A., et al., *Agreement on nutrient intake between the databases of the First National Health and Nutrition Examination Survey and the ESHA Food Processor.* American journal of epidemiology, 2002. **156**(1): p. 78-85.

246. Kreider, R.B., et al., *Effects of ingesting protein with various forms of carbohydrate following resistance-exercise on substrate availability and markers of anabolism, catabolism, and immunity.* Journal of the International Society of Sports Nutrition, 2007. **4**(1): p. 18.

247. Mayhew, J.L., et al., *Muscular endurance repetitions to predict bench press strength in men of different training levels.* The Journal of sports medicine and physical fitness, 1995. **35**(2): p. 108-113.

248. Haff, G.G. and N.T. Triplett, *Essentials of Strength Training and Conditioning 4th Edition*. 2015: Human kinetics.

249. Brewer, J. and J. Davis, *A physiological comparison of English professional and semi-professional soccer players.* J. Sports Sci, 1992. **10**(2): p. 146.

250. Helgerud, J., et al., *Aerobic endurance training improves soccer performance.* Medicine & Science in Sports & Exercise, 2001. **33**(11): p. 1925-1931.

251. Kollath, E. and K. Quade, *Measurement of sprinting speed of professional and amateur soccer players.* Science and Football II. London: E&FN Spon, 1993: p. 31-36.

252. Sheppard, J.M. and W.B. Young, *Agility literature review: classifications, training and testing.* Journal of sports sciences, 2006. **24**(9): p. 919-932.

253. Levers, K., et al., *Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals.* J Int Soc Sports Nutr, 2016. **13**: p. 22.

254. Dunbar, S.A. and M.R. Hoffmeyer, *Microsphere-Based Multiplex 2.9 Immunoassays: Development and Applications Using Luminex® xMAP® Technology.* The Immunoassay Handbook: Theory and applications of ligand binding, ELISA and related techniques, 2013. **157**.

255. Litteljohn, D. and S. Hayley, *Cytokines as potential biomarkers for Parkinson's disease: a multiplex approach.* Psychoneuroimmunology: Methods and Protocols, 2012: p. 121-144.

256. Wang, K., et al., *Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants.* Journal of reproductive immunology, 2005. **66**(2): p. 175-191.

257.   Jacobson, J.W., et al., *Analysis of individual data from bead-based assays ("bead arrays").* Cytometry Part A, 2006. **69**(5): p. 384-390.

258.   Soong, Y.Y., et al., *Effect of co-ingestion of amino acids with rice on glycaemic and insulinaemic response.* Br J Nutr, 2015. **114**(11): p. 1845-51.

259.   Suzuki, M., *Glycemic carbohydrates consumed with amino acids or protein right after exercise enhance muscle formation.* Nutr Rev, 2003. **61**(5 Pt 2): p. S88-94.

260.   Frid, A.H., et al., *Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects.* Am J Clin Nutr, 2005. **82**(1): p. 69-75.

261.   Gunnerud, U., et al., *The glycemic, insulinemic and plasma amino acid responses to equi-carbohydrate milk meals, a pilot- study of bovine and human milk.* Nutr J, 2012. **11**: p. 83.

262.   Brand-Miller, J., et al., *Cocoa powder increases postprandial insulinemia in lean young adults.* J Nutr, 2003. **133**(10): p. 3149-52.

263.   Pantophlet, A.J., et al., *Metabolic Profiling Reveals Differences in Plasma Concentrations of Arabinose and Xylose after Consumption of Fiber-Rich Pasta and Wheat Bread with Differential Rates of Systemic Appearance of Exogenous Glucose in Healthy Men.* J Nutr, 2017. **147**(2): p. 152-160.

264.   Weickert, M.O. and A.F.H. Pfeiffer, *Impact of Dietary Fiber Consumption on Insulin Resistance and the Prevention of Type 2 Diabetes.* J Nutr, 2018. **148**(1): p. 7-12.

265.   Brown, M.A., E.J. Stevenson, and G. Howatson, *Whey protein hydrolysate supplementation accelerates recovery from exercise-induced muscle damage in females.* Appl Physiol Nutr Metab, 2018. **43**(4): p. 324-330.

266.   Buckley, J.D., et al., *Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise.* J Sci Med Sport, 2010. **13**(1): p. 178-81.

267.   Farup, J., et al., *Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise.* Amino Acids, 2014. **46**(11): p. 2503-16.

268.   Kim, J., C. Lee, and J. Lee, *Effect of timing of whey protein supplement on muscle damage markers after eccentric exercise.* J Exerc Rehabil, 2017. **13**(4): p. 436-440.

269.   Rahbek, S.K., et al., *No differential effects of divergent isocaloric supplements on signaling for muscle protein turnover during recovery from muscle-damaging eccentric exercise.* Amino Acids, 2015. **47**(4): p. 767-78.

270.   Roberts, J., et al., *The short-term effect of high versus moderate protein intake on recovery after strength training in resistance-trained individuals.* J Int Soc Sports Nutr, 2017. **14**: p. 44.