Cycling time trial performance is not enhanced by either whey protein or L-alanine intake during prolonged exercise

Adam B. Schroer
James Madison University

Follow this and additional works at: https://commons.lib.jmu.edu/master201019

Part of the Kinesiology Commons

Recommended Citation
Schroer, Adam B., "Cycling time trial performance is not enhanced by either whey protein or L-alanine intake during prolonged exercise" (2013). Masters Theses. 317.
https://commons.libjmu.edu/master201019/317
Cycling Time Trial Performance is Not Enhanced by Either Whey Protein or L-Alanine Intake During Prolonged Exercise

Adam B. Schroer

A thesis submitted to the Graduate Faculty of JAMES MADISON UNIVERSITY

In Partial Fulfillment of the Requirements for the degree of Master of Science

Kinesiology

May 2013
Acknowledgements

I would first like to thank Dr. Nicholas D. Luden for serving as my thesis advisor and mentor. I truly appreciate the way you have challenged and guided me through this project and many others tasks during the past two years.

I am deeply thankful for Dr. Michael J. Saunders and Dr. Christopher J. Womack serving as members of my thesis committee. Your assistance and contribution has been very valuable.

Thanks to Dan Baur, Andrew D'Lugos and Matthew Becker for all the time you put into this study. I am grateful for the early mornings and long hours you were willing to sacrifice. This project would not have been possible or as enjoyable without your assistance.

Also, I would not have been able to accomplish all that I have without The Yuban Coffee Company®. Your quality product has made me functional throughout this project.

Finally, I would like to thank my family for their support and encouragement throughout this endeavor. You have all inspired me to strive for more than I thought possible.
# Table of Contents

Acknowledgments ..................................................................................................................... ii
List of Tables ............................................................................................................................ iv
List of Figures ............................................................................................................................ v
Abstract ...................................................................................................................................... vi

I. Introduction ............................................................................................................................. 1

II. Review of Literature .............................................................................................................. 8

   Water and Carbohydrate Supplementation ............................................................................ 8
   Carbohydrate and Protein Co-Ingestion - Performance ......................................................... 13
   Amino Acid Ingestion - Performance ..................................................................................... 18
   Protein/Amino Acid Ingestion - Physiological Effect and Proposed Mechanisms .......... 23
   Alanine Metabolism and Supplementation ............................................................................ 32
   Interleukin-6 .......................................................................................................................... 36
   Summary ................................................................................................................................. 48

III. Methodology ....................................................................................................................... 50

IV. Manuscript .......................................................................................................................... 56

V. Appendices ............................................................................................................................ 79

VI. References ............................................................................................................................ 87
List of Tables

Table 2.1: Fluid Intake ................................................................................................................10
Table 2.2: Carbohydrate Supplementation ..................................................................................12
Table 2.3: Carbohydrate and Protein Co-Ingestion - Performance ...........................................15
Table 2.4: Amino Acid Ingestion - Performance .......................................................................19
Table 2.5: Protein/Amino Acid Ingestion - Physiological Effect and Proposed Mechanisms ....26
Table 2.6: Alanine Metabolism and Supplementation ..................................................................33
Table 2.7: Interleukin-6 ................................................................................................................37
Table 4.1: Subject Characteristics ...............................................................................................73
Table 4.2: VO2, VE, RER, HR, and RPE at 20 and 120 min of fixed intensity cycling, and at 20-km during the time trial ........................................................................................................74
Table 4.3: Blood glucose and lactate at rest, 20 and 120 min of fixed intensity cycling, and at 20-km during the time trial ........................................................................................................75
Table 4.4: Individual ratings of nausea at 20 and 30-km of time trial time trial .......................76
List of Figures

Figure 2.1: Muscle Amino Acid Metabolism ................................................................. 31
Figure 2.2: Alanine-Glucose Cycle ............................................................................. 36
Figure 2.3: Interleukin-6—Effectors and Effect ......................................................... 47
Figure 3.1: Experimental Trial Protocol .................................................................... 52
Figure 4.1: 30-km time trial performance with PLA, ALA, and PRO treatments ........ 77
Figure 4.2: Concentrations of IL-6 in arterial serum before and following 120 minutes of FI
cycling with PLA, ALA, and PRO treatments ........................................................... 78
Abstract

Previous studies have reported that adding protein (PRO) to a carbohydrate (CHO) solution can enhance endurance performance. This ergogenic effect may be a function of additional calories from supplemental protein/amino acids, but this thesis has not been directly examined. Additionally, L-alanine (ALA) is readily oxidized when provided during exercise; the impact that this has on metabolism and prolonged endurance performance is unknown. The purpose of this investigation was to assess performance and various cardiovascular and metabolic outcomes during prolonged cycling, to independently gauge the efficacy of whey PRO hydrolysate and ALA supplementation. Eight trained male and female cyclists (age: 22.3±2.0 years, weight: 70.0±2.8 kg, VO\textsubscript{2\text{max}}: 59.4±1.7 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) performed 120 min of constant-load cycling (55% of peak power, 161.9±7.4 W) followed by a 30-km time trial (TT) under placebo (PLA), PRO, and ALA conditions. TT performance was not different between treatments (PLA: 57.6±1.6 min, ALA: 58.8±1.5 min, PRO: 58.8±1.8 min). VE, VO\textsubscript{2}, heart rate, rating of perceived exertion, blood glucose, blood lactate, and gastrointestinal distress were also similar across experimental conditions. Conversely, serum interleukin-6 (IL-6) levels following 120 min of cycling were elevated above rest with PLA (pre: 0.56±0.16, post: 3.14±0.84) and ALA (pre: 1.14±0.46, post: 2.62±0.71) (p<0.05), but not with PRO intake (pre: 0.81±0.25, post: 1.55±0.32). The ingestion of PRO or ALA alone does not appear to enhance performance during prolonged cycling. Thus the ergogenic effects of CHO+PRO co-ingestion reported by others are likely not the result of additional energy from protein \textit{per se}. 
Chapter One

Introduction

For centuries man has attempted to manipulate nutrition in an effort to optimize performance. Dating back to 500-400 B.C. athletes and warriors were reported to ingest deer liver and lion heart prior to muscular work believing it to impart speed, bravery, and strength (3). Well into the 20th century, ergogenic aid use was still guided more by superstition than science. In 1925, Gordon et al. reported the first scientific evidence of improved physical condition with carbohydrate intake during a marathon race (61). Although improved performance from carbohydrate intake could be inferred from these results, it was not verified until much later, in the 1980’s, when Coyle and colleagues demonstrated that carbohydrate supplementation during prolonged cycling helps maintain blood glucose levels, carbohydrate oxidation rates, and consequently performance (33). Subsequent studies have substantiated the ergogenic effect of carbohydrate intake rates ranging from 30-60 grams/hour during prolonged endurance exercise (≥120 minutes) (32, 33). While it is clear that carbohydrate provision is beneficial during prolonged exercise, scientific query into other possible ergogenic aids to this type of exercise has continued. Specifically, researchers have examined the acute performance effects of various nitrogen-based supplements since the 1990’s without a clear consensus on the efficacy of such treatments (12). Therefore, the overarching purpose of this study is to investigate the effect of two separate nitrogen-based supplements (whey protein and l-alanine) on prolonged endurance performance.

From a historical standpoint, our understanding of the role that endogenous protein and its constituent amino acids have as an energy provider during exercise shifted dramatically ~150 yrs ago. Protein was believed to be the primary substrate used by contracting skeletal muscle until 1866, when Fick and Wislicenus proved that non-nitrogenous fuels (carbohydrate and fat) yield the majority of energy (50, 86). Currently, protein is considered largely inconsequential as an energy source during exercise, with carbohydrate and fat being given the majority of credit for
ATP production. However, amino acids can be used to generate metabolites for intramuscular oxidative metabolism and serve as gluconeogenic pre-cursors in the liver to prevent hypoglycemia during prolonged exercise (38). In this manner, amino acids can supply 5-10 percent of the total ATP utilized during exercise (19). Due to the relatively small contribution of amino acids to energy production and the abundance of endogenous stores, protein was virtually ignored until recently as a potential ergogenic aid to endurance exercise.

The performance value of protein intake during endurance exercise has gained interest over the past decade. This query has been addressed by exclusively examining the performance impact of adding small doses of protein (~5-20 g/hr) to standard carbohydrate treatments. Interestingly, initial studies demonstrated that protein enhances performance to a large extent (29-36%) (69, 123). Subsequent research has been mixed, as protein co-ingestion with carbohydrate was beneficial in some studies (2, 47, 58, 69, 122–124), but not others (18, 41, 104, 116, 139, 140). Stearns and colleagues recently reviewed the literature on this topic and determined that carbohydrate-protein supplements, when matched for carbohydrate content (treatments containing protein = more calories), generated a 10.5% improvement in performance over carbohydrate supplements (58). Some reports have also indicated that protein (6-10g/hr) combined with a low dose of carbohydrate (i.e. 25g/hr) is capable of maintaining or improving the performance efficacy of a higher total calorie carbohydrate (i.e. 50g/hr) beverage (47, 92, 93). This suggests that the effects of protein intake are more potent than carbohydrate. However, whether these studies would have detected a dose-response effect between the low and high carbohydrate beverage regardless of protein inclusion is questionable. In fact, many carbohydrate studies have been unable to detect a performance dose-response (i.e. more carbohydrate = proportionally larger gains in performance) (55, 57, 95, 96). Therefore, protein co-ingestion may not have played a role in maintaining performance in the aforementioned studies.

The current literature possesses a number of important weaknesses, to which the current project is designed to address. Firstly, though the current literature points to the ergogenic
potential of protein intake during endurance exercise in some conditions (time to exhaustion protocols and iso-carbohydrate comparisons), the performance benefits of protein remain uncertain, particularly during time trial efforts. Most studies reporting a positive effect of protein utilized time to exhaustion (TTE) exercise protocols rather than time trial (TT) protocols. Although TTE protocols yield valuable insight, improvements in TTE performance do not always translate to proportional gains in TT performance. This weakens the external validity of TTE protocols (36, 70). Therefore, TT studies designed to examine the ergogenic effect of protein are needed to verify the prior positive TTE outcomes.

Secondly, the independent effects of protein remain unknown as carbohydrate co-ingestion likely influence the independent effects of protein due to competing mechanisms of each macronutrient (i.e. intestinal absorption and hepatic glucose production) (45, 72, 146).

Finally, as highlighted above, all previous work on this topic has exclusively examined the effect of small doses of protein (~5-20 g/hr). These rates are low compared to the recommended rate of carbohydrate intake (30-60 g/hr), therefore possibly failing to optimize (and making it more difficult to discern) the potential ergogenic effect of protein. Therefore, the first primary aim of this study is to determine whether a moderate dose (45 g/hr) of whey protein delivered during prolonged cycling improves performance during a simulated, pre-loaded time trial compared to a placebo beverage.

The second primary aim of this study is related to the first, but will be addressed independently, as detailed below. Alanine, an individual amino acid present in whey protein (and many other protein sources), is the key amino acid extracted by the liver for gluconeogenesis, and as a partial result, is readily oxidized/decarboxylated during endurance exercise (45, 149). Supplementation of alanine during prolonged exercise has been shown to increase amino acid oxidation. Specifically, 51 of 73 grams of alanine supplementation were oxidized during three hours of cycling (82). Exogenous alanine accounted for 10% of the total energy expenditure along with 5% from endogenous protein. Peak exogenous alanine oxidation (0.35 g/min) was
only slightly lower than exogenous carbohydrate oxidation rates when carbohydrate was ingested at a similar rate (0.43 g/min) (111). Presumably, alanine served as a fuel through conversion to glucose in the liver and direct oxidation in the muscle by conversion to pyruvate (82). Whether this metabolic effect is capable of producing an ergogenic effect during prolonged exercise is unknown. The only study to investigate the performance outcome of alanine supplementation implemented a relatively short-duration protocol (60 minutes) (79). Alanine supplementation would more likely benefit performance during events that are metabolically demanding (i.e. prolonged exercise), a scenario that has yet to be examined. Therefore, the second primary aim of this study is to determine whether a moderate dose (45 g/hr) of alanine delivered during prolonged cycling improves performance during a simulated, pre-loaded time trial compared to a placebo beverage. Alanine intake not only warrants investigation for its ergogenic potential, but it may also provide some insight into the mode of action by which protein supplementation improves performance. Although this mechanism is unproven, the oxidation of amino acids provided by protein supplementation has been raised as a possibility (140). If alanine improves performance, it will likely result from increased exogenous energy provision (sparing of endogenous fuel stores), thereby suggesting that protein-induced performance gains can be partially ascribed to amino acid oxidation/gluconeogenesis.

On a more exploratory note, the impact of protein and alanine supplementation on plasma interleukin-6 (IL-6) will be investigated. Plasma IL-6 levels will be measured during cycling and will be used to provide insight into the impact of protein and alanine feedings on exercise metabolism. IL-6 is a myokine released from active skeletal muscle and is capable of impacting metabolism (6, 23, 56, 60, 77). The magnitude of the exercise-induced plasma IL-6 response is mediated by intensity and, more importantly, duration (24, 105, 106, 115, 132). Muscle glycogen depletion potentiates (75, 89, 130) and CHO supplementation attenuates (44, 99, 101, 128) the increase in plasma IL-6 observed with exercise. In this way, IL-6 serves as an energy sensor in the muscle that subsequently affects metabolism in accordance with need. IL-6 can augment
AMPK activity (60, 77, 78, 89), which is likely how IL-6 increases free fatty acid oxidation and GLUT-4 translocation (and increasing skeletal muscle glucose uptake). Significant to the current study, IL-6 also enhances hepatic amino acid uptake, gluconeogenic gene mRNA abundance (e.g. PEPCK, G6P, and PGC-1α), and consequently gluconeogenesis (6, 52, 56, 88, 147). In this way, the exercise-induced increase in plasma IL-6 stimulates endogenous glucose production to maintain glucose homeostasis late in exercise. Interestingly, glutamine supplementation appears to augment the rise in plasma IL-6 during 120 minutes of cycling (67). Authors speculated that the increased plasma glutamine concentration, being a gluconeogenic pre-cursor, likely initiated a rise in plasma IL-6 to facilitate hepatic amino acid uptake. Regardless of the mechanism by which glutamine potentiates the exercise-induced plasma IL-6 response; it is capable of producing a favorable metabolic effect during prolonged exercise. Thus, the effect of both protein and alanine supplementation on plasma IL-6 will provide general information about how these nutrients influence the metabolic environment during prolonged exercise. Thus a secondary aim of this study is to determine whether protein or alanine supplementation during prolonged cycling influences plasma IL-6 following 120 minutes of cycling compared to a placebo beverage.

The purpose of the current study is to gain a more complete understanding of the potential for both protein and alanine intake to improve prolonged endurance performance and to alter the metabolic environment as indicated by plasma IL-6 levels.
**Aims and Hypotheses**

Primary Aim I: To determine whether protein supplementation during prolonged cycling improves performance on a simulated, pre-loaded time trial compared to a placebo beverage.

Primary Hypothesis I: Protein supplementation will improve cycling time trial performance compared to a placebo beverage.

Primary Aim II: To determine whether alanine supplementation during prolonged cycling improves performance on a simulated, pre-loaded time trial compared to a placebo beverage.

Primary Hypothesis II: Alanine supplementation will improve cycling time trial performance compared to a placebo beverage.

Secondary Aim: To determine whether protein or alanine supplementation during prolonged cycling influences plasma IL-6 following 120 minutes of cycling compared to a placebo beverage.

Secondary Hypothesis: Both protein and alanine supplementation will elicit higher plasma IL-6 levels following 120 minutes of cycling compared to a placebo beverage, with alanine supplementation augmenting plasma IL-6 to a larger extent than protein supplementation.
**Significance**

Several studies have demonstrated that carbohydrate-protein supplementation improves performance compared to an iso-carbohydrate beverage. However, a nearly equal number of studies have refuted this with null findings. Importantly, no studies have investigated the influence of a moderate dose of protein. Intake rates equivalent to those recommended for carbohydrate supplementation (30-60 g/hr) may be necessary to amplify/maximize the effect of protein. Nor has any study isolated the effect of protein on prolonged cycling performance by utilizing a protein-only beverage. Furthermore, ingested alanine appears to be oxidized at rates similar to supplemental carbohydrate (when ingested at like rates): thus indicating the potential for alanine supplementation to exhibit an ergogenic effect on prolonged cycling. However, the effect of alanine supplementation on prolonged cycling performance has yet to be examined. IL-6 plays a significant role in metabolism during prolonged exercise. IL-6 increases gluconeogenesis (6, 52, 147), free fatty acid oxidation (23), and skeletal muscle glucose uptake (60). Also, glutamine supplementation has been found to augment the plasma IL-6 response to exercise (67). If alanine and/or protein supplementation are also capable of augmenting plasma IL-6 during prolonged exercise it can point to a favorable metabolic effect of these treatments. Therefore, this study will determine the performance outcome as well as the potential for an IL-6 mediated effect on metabolism with both protein and alanine supplementation.
Chapter Two

Review of the Literature

Objectives

The objectives of this chapter are to provide an overview of: 1) the representative literature on standard hydration and nutrition strategies for prolonged exercise, 2) the performance effect of a small dose of protein in a carbohydrate-based sports supplement, 3) the performance effect of amino acid consumption during exercise, 4) the physiological effect and proposed mechanisms for an amino acid based ergogenic effect, 5) alanine metabolism and the effect of supplementation, and 6) the factors that affect interleukin-6 and it’s metabolic impact.

Water and Carbohydrate Supplementation

Prolonged exercise (≥120 minutes) is associated with marked physiological stress. For instance, endurance exercise necessitates a sweat response to avoid dangerous elevations in body temperature. However, sweat loss is capable of impairing performance if dehydration (loss of >2% body weight from fluids) is not avoided (125). Dehydration can impair performance by increasing heart rate and core temperature, and compromising stroke volume and cardiac output (7, 64). Fluid intake during prolonged exercise can help maintain plasma volume and consequently sweat rate, body temperature, stroke volume, heart rate, and cardiac output, thereby preserving performance capacity (7, 8, 64, 112). Thus, fluid replacement is prescribed at amounts necessary to avoid >2% loss of body weight from fluid while remaining at a tolerable rate to avoid gastrointestinal distress (125). In a previous position stand on the fluid replacement requirements during exercise, the American College of Sports Medicine recommended that fluid be ingested at a rate of ~600-1200 ml/hr during intense exercise lasting longer than one hour; an individualized amount that should not exceed sweat rates (31).

Similarly, prolonged exercise creates a challenging metabolic environment. In resting conditions and at low exercise intensities fat oxidation generates the majority of ATP. Higher
intensities consequently require more ATP and carbohydrate oxidation becomes the predominate source of this energy (117). Therefore, to sustain high levels of exercise intensity it is necessary to maintain high rates of carbohydrate oxidation. However, endogenous carbohydrate supply (blood glucose, muscle glycogen, and liver glycogen) is inherently limited and depleted stores have been shown to impair exercise performance (73, 85). Carbohydrate supplementation during prolonged exercise provides it’s ergogenic effect by delivering substrate for exogenous carbohydrate oxidation; thereby preventing a drop in blood glucose concentrations (33, 51), sparing liver glycogen (71, 72), and maintaining high rates of carbohydrate oxidation late in exercise (32). Recommendations for carbohydrate intake during exercise range from 30 to 60 g/hr (114). Recently, however, a dose-response effect has been found with carbohydrate supplementation up to 60 g/hr (126). Therefore, intake towards the upper end of this traditional range is recommended.
### Table 2.1: Fluid Intake

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitts 1944</td>
<td>6 Males</td>
<td>240-360 min marching @ 3.5mph up a grade of 2.5%</td>
<td>• No Water</td>
<td>• ↑ HR and $T_{re}$; ↓ sweat rate and mechanical efficiency; earlier exhaustion due to dehydration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Water (ad libitum)</td>
<td>• ↑ $T_{re}$ late in exercise, but finished feeling fairly fresh</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Water (matched BW loss)</td>
<td>• $T_{re}$ remained very low and felt capable of working all day</td>
</tr>
<tr>
<td>Maron 1977</td>
<td>2 Male</td>
<td>Santa Barbara Marathon (~18°C)</td>
<td>• Subjects permitted commercially available beverage ad libitum (1594 and 735ml)</td>
<td>• $T_{re}$ ↑ then plateaued. Late exercise ↑ (peak = 41.9°C &amp; 39.6°C).</td>
</tr>
<tr>
<td></td>
<td>Marathon Runners</td>
<td></td>
<td></td>
<td>• ↑ in $T_{re}$ late in exercise was result of ↓ evaporative heat loss (caused by 2.7 and 3.1% loss of BW)</td>
</tr>
<tr>
<td>Barr 1991</td>
<td>5 Male and 3 Female Cyclists</td>
<td>360 min @ 55% $VO_{2max}$ w/ intermittent breaks for measurements</td>
<td>• No Fluid</td>
<td>• Higher HR, $T_{re}$, plasma Na, and RPE. Terminated exercise ~90 min early w/ 6.4% loss of BW.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Water (Matched BW loss)</td>
<td>• ↔ between saline and water</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Saline (Water+25mmol/l NaCl)</td>
<td></td>
</tr>
<tr>
<td>Hamilton 1991</td>
<td>10 Male Cyclists</td>
<td>120 min @ 70% $VO_{2max}$</td>
<td>• No Fluid</td>
<td>From min 20 to 120:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Fluid (1.17 L/hr)</td>
<td>• 15% ↓ SV; 7% ↓ CO; 10% ↑ HR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 5% ↑ HR; 0.6°C ↓ $T_{re}$</td>
</tr>
<tr>
<td>Below 1995</td>
<td>8 Male Cyclists</td>
<td>50 min @ 80% $VO_{2max}$; ~10 min TT</td>
<td>• Fluid (1330ml)</td>
<td>TT: 10.22min; HR 4 bpm ↓ and $T_{re}$ 0.33°C ↓ after 50 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• PLA (200ml)</td>
<td>TT: 10.93min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fluid improved performance 6.5%</td>
</tr>
<tr>
<td>McConnell 1997</td>
<td>7 Male Cyclists</td>
<td>120 min @ 69% $VO_{2max}$; TTE @ 90% $VO_{2max}$</td>
<td>• High Fluid (1.16 L/hr)</td>
<td>TTE: 328 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Low Fluid (0.58 L/hr)</td>
<td>TTE: 248 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No Fluid</td>
<td>TTE: 171 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High fluid ↑ performance compared to no fluid. Low fluid ↔ from either.</td>
</tr>
</tbody>
</table>
Table 2.1: Fluid Intake (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dugas 2009 (39)</td>
<td>6 Male Cyclists</td>
<td>80km TT</td>
<td>• LO (0 and 426 ml/hr)</td>
<td>• TT: 129 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• HI [670 ml/hr \textit{(ad libitum)}; 865 ml/hr; 1324 ml/hr]</td>
<td>• TT: 125 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Fluid below ad libitum impairs performance; above does not impart greater benefits</td>
</tr>
</tbody>
</table>

HR = Heart Rate; Na = Sodium; RPE = Rating of Perceived Exertion; BW = Body Weight; SV = Stroke Volume; CO = Cardiac Output; TTE = Time to Exhaustion; TT = Time Trial; bpm = beats per minute; $T_{re}$ = Rectal Temperature
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Coyle 1983   | 9 Male and 1 Female Cyclists | TTE @ 74% VO$_{2\text{max}}$ | • PLA  
• CHO (~70g @ 20 min; ~17.5g @ 60, 90, & 120 min) | • TTE: 134min  
• TTE: 157min; ↑ blood glucose concentrations |
| Fielding 1985| 9 Males                | 240 min cycling @ 50% VO$_{2\text{max}}$ w/ intense intermittent intervals; TTE @ 100% VO$_{2\text{max}}$ | • PLA  
• CHO: 21.5g every 60min  
• CHO: 10.75g every 30min | • TTE: 81 sec; ↓ blood glucose  
• TTE: 111 sec; ↔ plasma glucose; ↑ rate of CHO oxidation  
• TTE: 121 sec; ↔ plasma glucose; ↑ rate of CHO oxidation  
• CHO every 30 min ↑ TTE compared to PLA  
• CHO every 60 min ↔ TTE either treatment |
| Coyle 1986   | 7 Male Cyclists         | TTE @ 71% VO$_{2\text{max}}$ | • PLA  
• CHO (~134g @ 20 min; ~27g every 20 min) | • TTE: 181min; ↓ plasma glucose  
• TTE: 241min; ↔ plasma glucose; ↑ rate of CHO oxidation  
• Equal muscle glycogen utilization |
| Jeukendrup 1999 | 6 Cyclists         | 120 min @ 50% VO$_{2\text{max}}$ | • PLA  
• LoCHO (35.5g/hr)  
• HiCHO (177g/hr) | Partially suppressed HGP  
• Completely suppressed HGP  
• Muscle glycogen oxidation ↔ by glucose feedings |
| Smith 2010   | 12 Male Cyclists        | 120 min @ 77% VO$_{2\text{max}}$, 20km TT | • PLA  
• 15g CHO/hr  
• 30g CHO/hr  
• 60g CHO/hr | • TT: 36.4 min  
• TT: 35.2 min  
• TT: 35.0 min  
• TT: 34.7 min  
• Performance: 60g/hr > 30g/hr = 15g/hr > PLA  
• Associated with a small ↑ in CHO oxidation  
• 30 and 60g/hr ↓ liver glucose production |

PLA = Placebo; CHO = Carbohydrate; TTE = Time to Exhaustion; TT = Time Trial; HGP = Hepatic Glucose Production
Carbohydrate and Protein Co-Ingestion – Performance

In contrast to carbohydrate and water supplementation, it was not until recently that researchers began testing the efficacy of adding a small amount of protein (5-20 g/hr) to a standard carbohydrate-based sports drink during prolonged exercise. Initial rationale for this research question was based on the notion that protein could potentiate the insulin response to carbohydrate supplementation during exercise, thereby exhausting a smaller amount of muscle glycogen. However, this theory has been disproven as supplemental protein does not modulate the insulin response to carbohydrate intake during prolonged exercise (69). Nonetheless, initial findings were positive, as the addition of protein appears to confer a large performance effect (29-36% improvement) compared to an iso-carbohydrate beverage (69, 123). However, subsequent data have been equivocal with some supporting an ergogenic effect of protein co-ingestion (2, 47, 58, 93, 122, 124), while others have indicated no improvement (18, 41, 104, 116, 139, 140). A 2010 meta-analysis of these studies established a significant improvement in performance when supplements were matched for carbohydrate content, but not when matched for calories (129). Some studies have also found that protein added to a small dose of carbohydrate is capable of maintaining the ergogenic effect of a higher total calorie carbohydrate beverage (47, 92, 93). This suggests protein inclusion in a sports drink may be capable of providing an equal ergogenic effect to a carbohydrate-only beverage while reducing the amount of calories that an athlete must intake. Furthermore, the literature has recently expanded beyond typical endurance exercise to include soccer. Protein and carbohydrate co-ingestion improved high intensity running capacity following a simulated soccer game compared to a carbohydrate-only beverage (2), suggesting that protein may be capable of exerting an ergogenic effect during other forms of non-continuous high intensity exercise (i.e. basketball, field hockey, etc.). However, skepticism over recommending protein as an ergogenic aid persists, presumably due to methodological limitations in the current literature. For example, most studies reporting a positive effect utilized time to exhaustion exercise protocols rather than time trial protocols, which is of greater external validity. Typically,
athletes do not compete in events that require maintaining a fixed power output as long as possible, as occurs in time to exhaustion protocols (36).
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ivy 2003</strong></td>
<td>9 Male Cyclists</td>
<td>180 min varied intensity cycling (45-75% VO$<em>{2peak}$); TTE @ 85% VO$</em>{2peak}$</td>
<td>• PLA&lt;br&gt;• CHO (47g/hr)&lt;br&gt;• CHO+PRO (47+12g/hr)</td>
<td>• TTE: 12.6 min&lt;br&gt;• TTE: 19.7 min&lt;br&gt;• TTE: 26.9 min&lt;br&gt;• PRO ↑ ergogenic effect of iso-CHO beverage</td>
</tr>
<tr>
<td>(69)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Saunders 2004</strong></td>
<td>15 Male Cyclists</td>
<td>TTE @ 75% VO$_{2peak}$</td>
<td>• CHO (37.2g/hr)&lt;br&gt;• CHO+PRO (37+9g/hr)</td>
<td>• TTE: 82.3 min&lt;br&gt;• TTE: 106.3 min&lt;br&gt;• PRO ↑ ergogenic effect of iso-CHO beverage</td>
</tr>
<tr>
<td>(123)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Van Essen 2006</strong></td>
<td>10 Male Cyclists</td>
<td>80 km Time Trial (TT)</td>
<td>• PLA&lt;br&gt;• CHO (60g/hr)&lt;br&gt;• CHO+PRO (60+20g/hr)</td>
<td>• TT: 141 min&lt;br&gt;• TT: 135 min&lt;br&gt;• TT: 135 min&lt;br&gt;With optimal dose of CHO (60g/hr), PRO had no effect on performance</td>
</tr>
<tr>
<td>(41)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Romano-Ely 2006</strong></td>
<td>14 Male Cyclists</td>
<td>TTE @ 70% VO$_{2peak}$</td>
<td>• CHO (60g/hr)&lt;br&gt;• CHO+PRO (45+12g/hr)</td>
<td>• TTE: 138 min&lt;br&gt;• TTE: 141 min&lt;br&gt;Iso-caloric beverages had = effect on performance</td>
</tr>
<tr>
<td>(116)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Saunders 2007</strong></td>
<td>8 Male and 5 Female Cyclists</td>
<td>TTE @ 75%</td>
<td>• CHO (40.9g/hr)&lt;br&gt;• CHO+PRO (41+10g/hr)&lt;br&gt;• Gel Supplements</td>
<td>• TTE: 102.8 min&lt;br&gt;• TTE: 116.6 min&lt;br&gt;PRO ↑ ergogenic effect of iso-CHO equally in men and women</td>
</tr>
<tr>
<td>(122)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Osterberg 2008</strong></td>
<td>13 Male Cyclists</td>
<td>120 min @ 5% below OBLA; Time to complete set amount of work (7kJ/kg)</td>
<td>• PLA&lt;br&gt;• CHO (60g/hr)&lt;br&gt;• CHO+PRO (75+16g/hr)</td>
<td>• TT: 39.7 min&lt;br&gt;• TT: 37.1 min&lt;br&gt;• TT: 38.8 min&lt;br&gt;CHO ↑ over PLA only; ↔ between CHO+PRO and either treatment&lt;br&gt;↑ CHO and caloric content in CHO+PRO may have led to GI discomfort</td>
</tr>
<tr>
<td>(104)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Exercise</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>--------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Valentine 2008</td>
<td>11 Male Cyclists</td>
<td>TTE @ 75% VO_{peak}</td>
<td>• PLA</td>
<td>• TTE: 107.1 min</td>
</tr>
<tr>
<td>(140)</td>
<td></td>
<td></td>
<td>• CHO (78g/hr)</td>
<td>• TTE: 117.5 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• CHO+CHO (97g/hr)</td>
<td>• TTE: 121.3 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• CHO+PRO (78+19g/hr)</td>
<td>• TTE: 126.2 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• CHO+CHO and CHO+PRO ↑ over PLA, with ↔ between CHO and any treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• At least some of the additive ergogenic effect of PRO is caused by ↑ calories</td>
</tr>
<tr>
<td>Saunders 2009</td>
<td>13 Male Cyclists</td>
<td>60 km TT</td>
<td>• CHO (60g/hr)</td>
<td>• TT: 135.0 min; Final 20km: 45.0 min</td>
</tr>
<tr>
<td>(124)</td>
<td></td>
<td></td>
<td>• CHO+PRO (60+18g/hr)</td>
<td>• TT: 134.4 min; Final 20km: 44.3min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• With optimal dose of CHO, PRO did not affect total time, but improved late exercise TT performance</td>
</tr>
<tr>
<td>Breen 2010</td>
<td>12 Male Cyclists</td>
<td>120 min @ 55% W_{max}; ~1 hr TT (~880kJ)</td>
<td>• CHO (65g/hr)</td>
<td>• TT: 60:13</td>
</tr>
<tr>
<td>(18)</td>
<td></td>
<td></td>
<td>• CHO+PRO (65+19g/hr)</td>
<td>• TT: 60:51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• With optimal dose of CHO, PRO had no effect on performance</td>
</tr>
<tr>
<td>Ghosh 2010</td>
<td>8 Male Recreational Cyclists</td>
<td>60 min @ 60% VO_{peak}; TTE @ 90% VO_{peak}</td>
<td>• PLA</td>
<td>• TTE: 4.09 min</td>
</tr>
<tr>
<td>(58)</td>
<td></td>
<td></td>
<td>• Sago (60g CHO)</td>
<td>• TTE: 5.49 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Sago+Soy (53+15g PRO)</td>
<td>• TTE: 7.53 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Soy delayed fatigue during high intensity cycling</td>
</tr>
<tr>
<td>Toone 2010</td>
<td>12 Male Cyclists</td>
<td>45 min variable intensity cycling; 6 km TT</td>
<td>• CHO (95g)</td>
<td>• TT: 433 sec</td>
</tr>
<tr>
<td>(139)</td>
<td></td>
<td></td>
<td>• CHO+PRO (72+22g)</td>
<td>• TT: 438 sec (p=0.048)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Replacing some CHO with PRO resulted in 1% decrement in high intensity performance</td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Exercise</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Martinez-Lagunas 2010</td>
<td>7 Male and 5 Female Cyclists</td>
<td>150 min varied intensity cycling (55-75% VO_{2peak}); TTE @ 80% VO_{2peak}</td>
<td>PLA, CHO (45g/hr), LCHO+PRO (23+6g/hr), HCHO+PRO (34+9g/hr)</td>
<td>TTE: 14.7 min, TTE: 26.9 min, TTE: 28.9 min, TTE: 30.5 min. Each treatment delayed fatigue compared to PLA; ↔ between others. PRO maintained efficacy with nearly half the calories.</td>
</tr>
<tr>
<td>Ferguson-Stegall 2010</td>
<td>8 Male and 7 Female Cyclists</td>
<td>180 min varied intensity cycling (45-70% VO_{2peak}); TTE @ 74-85% VO_{2peak}</td>
<td>CHO (50g dextrose/hr), CHO+PRO (25g of dextrose-maltodextrin-fructose mix+10g PRO/hr)</td>
<td>TTE: 26 min, TTE: 31 min (p=0.064). TTE in cyclists @ or below VT: CHO-35.5 min; CHO+PRO-45.6 min (p=0.006). Below VT PRO delayed fatigue even with 50% ↓ CHO and 30% ↓ calories.</td>
</tr>
<tr>
<td>McCleave 2011</td>
<td>14 Female Cyclists</td>
<td>180 min varied intensity cycling (54-70% VO_{2peak}); TTE @ VT (~75.06% VO_{2peak})</td>
<td>CHO (50g dextrose/hr), CHO+PRO (25g of dextrose-maltodextrin-fructose mix+10g PRO/hr)</td>
<td>TTE: 42 min, TTE: 50 min. PRO co-ingestion delayed fatigue in women with 50% less CHO and 30% fewer calories. Ergogenic affect likely the result of PRO inclusion and use of mixture of CHO sources.</td>
</tr>
<tr>
<td>Alghannam 2011</td>
<td>6 Male Football (soccer) Players</td>
<td>5x15 min intermittent exercise designed to simulate soccer game with 15 min half-time; RTF @ 80% VO_{2peak}</td>
<td>PLA, CHO (70.8g), CHO+PRO (49.6+21.2g), Given prior to testing and during half-time</td>
<td>RTF: 11.0 min, RTF: 16.5 min, RTF: 23.0 min. CHO+PRO delayed fatigue compared to CHO and PLA. PRO can exert ergogenic benefit during intermittent activity.</td>
</tr>
</tbody>
</table>

PRO = Protein; CHO = Carbohydrate; GI = Gastrointestinal; VT = Ventilatory Threshold; RTF = Running To Fatigue; TTE = Time to Exhastion; TT = Time Trial
Amino Acid Ingestion – Performance

In addition to whole protein sources (i.e. soy and whey), the performance effect of various amino acids [branch-chain amino acids (BCAA), tyrosine, mixed amino acids, and alanine] has also been examined. Of these amino acids, BCAA supplementation has been the only type of amino acid to exert an ergogenic effect. BCAA intake before and during prolonged exercise can decrease perceived exertion and improve physical as well as mental performance in humans and animals (12, 13, 21, 22, 97). These effects are thought to be due to diminished central fatigue (100). However, a number of other studies have contradicted these results with null findings (11, 27, 63, 90, 135, 142, 148). Another amino acid potentially capable of effecting performance through reduced central fatigue is tyrosine. However, it has been largely ineffective for performance (28, 135, 136). This may have been due to major weaknesses in methods of these studies. Specifically, Struder et al. and Sutton et al. provided tyrosine early, but not late in exercise (135, 136). Thus, the plasma tyrosine concentration was possibly unchanged from the placebo trial late in exercise, which would fail to exert an ergogenic effect. Chinevere et al. utilized tyrosine supplementation throughout exercise, but the statistical power was too weak to draw conclusions about its ergogenic effect. Another individual amino acid, alanine, has been investigated as an ergogenic aid due to its ability to provide exogenous energy (82). However, the only study to investigate alanine supplementation subjected cyclists to an exercise protocol of insufficient duration (60 minutes) to stress endogenous fuel stores (79). Therefore, further research investigating the ergogenic effect of alanine supplementation throughout prolonged exercise is necessary before conclusions can be drawn. As is shown above, various amino acids have the potential to impart an ergogenic effect during prolonged exercise through multiple mechanisms. However, further research is required to fully elucidate the efficacy of these treatments.
Table 2.4: Amino Acid Ingestion – Performance

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blomstrand 1991</td>
<td>96 Experimental and 97 Control Runners</td>
<td>Marathon (&lt;3:30 min)</td>
<td>PLA, BCAA (16g Total)</td>
<td>Performance: BCAA ↑ performance for slower (3.05-3.30 hr) but not faster (&lt;3.05 hr) marathon runners</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subjects were permitted any other drinks <em>ad libitum.</em></td>
<td>Mental performance was ↑ post-exercise with BCAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCAA ingestion ↑ plasma concentration of these amino acids</td>
</tr>
<tr>
<td>Blomstrand 1991a</td>
<td>6 Female Soccer Players</td>
<td>90 min Soccer Game</td>
<td>CHO (78g), CHO+BCAA (78g + 9.75g)</td>
<td>BCAA intake improved mental performance post-exercise</td>
</tr>
<tr>
<td>Verger 1994</td>
<td>34 Rats</td>
<td>Moderate intensity treadmill running to exhaustion</td>
<td>PLA, CHO, BCAA Ingested before/during exercise</td>
<td>TTE: 191min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TTE: 208min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TTE: 179min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHO delayed fatigue compared to BCAA</td>
</tr>
<tr>
<td>Van Hall 1995</td>
<td>10 Male Cyclists</td>
<td>TTE @ 70-75% P_max</td>
<td>CHO (78g sucrose), Tryptophan (3.9g+78g sucrose), L-BCAA (7.8g+78g sucrose), H-BCAA (23.4g+78g sucrose)</td>
<td>TTE: ↔ between any trial (~122 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Believed to ↑ brain tryptophan levels 7- to 20-fold</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Believed to ↓ brain tryptophan levels 8-12%</td>
</tr>
<tr>
<td>Madsen 1996</td>
<td>9 Male Cyclists</td>
<td>100 km TT</td>
<td>PLA, CHO (87.5g maltodextrin+87.5g glucose), CHO+BCAA (CHO+18g BCAA)</td>
<td>TT: 159.8min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT: 160.1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT: 157.2min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↔ between any treatment</td>
</tr>
<tr>
<td>Blomstrand 1997</td>
<td>7 Male Cyclists</td>
<td>Glycogen depleting exercise prior to trials. 60 min @ ~70% VO2max, 20 min TT</td>
<td>PLA, BCAA (6.7g)</td>
<td>TT: 250kJ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT: 275kJ; ↑ cognitive performance; ↓ RPE and mental fatigue.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↔ in performance</td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Exercise</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Calders 1997</td>
<td>Rats</td>
<td>Treadmill run to exhaustion</td>
<td>PLA</td>
<td>TTE: 76min</td>
</tr>
<tr>
<td>(22)</td>
<td></td>
<td></td>
<td>BCAA Injected prior to exercise</td>
<td>TTE: 99min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCAA extended time to exhaustion.</td>
</tr>
<tr>
<td>Mittleman 1998</td>
<td>7 Males and 6 Females</td>
<td>Temp-34.4°C 120 min rest TTE @ 40% VO₂peak</td>
<td>PLA</td>
<td>TTE: 137min</td>
</tr>
<tr>
<td>(97)</td>
<td></td>
<td></td>
<td>BCAA (Males-15.8g; Females-9.4g)</td>
<td>TTE: 153min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCAA supplementation delayed the onset of fatigue in the heat</td>
</tr>
<tr>
<td>Struder 1998</td>
<td>10 Male Cyclists</td>
<td>TTE @ workload that corresponds to blood lactate level of 2.0mmol/L</td>
<td>PLA</td>
<td>TTE: 157 min</td>
</tr>
<tr>
<td>(135)</td>
<td></td>
<td></td>
<td>Paroxetine (20mg; serotonin re-uptake inhibitor)</td>
<td>TTE: 131 min; augmented brain serotonergic activity and fatigue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BCAA (14g; 7g @ 60min)</td>
<td>TTE: 152 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L-Tyrosine (10g; 10g @ 60min)</td>
<td>TTE: 150 min</td>
</tr>
<tr>
<td>Calders 1999</td>
<td>Rats</td>
<td>Treadmill run to exhaustion</td>
<td>PLA</td>
<td>TTE: 118min</td>
</tr>
<tr>
<td>(21)</td>
<td></td>
<td></td>
<td>CHO</td>
<td>TTE: 179min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BCAA</td>
<td>TTE: 158min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CHO+BCAA</td>
<td>TTE: 171min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCAA ↓ fatigue compared to PLA; ↔ compared to CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCAA has no additional effect when supplemented with CHO</td>
</tr>
</tbody>
</table>

Table 2.4: Amino Acid Ingestion – Performance (Continued)
Table 2.4: Amino Acid Ingestion – Performance (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Chinevere 2002 | 9 Cyclists | 90 min @ 70% VO$_{2\text{peak}}$; TT (amount of work that would be completed in 30 min @ 70% VO$_{2\text{peak}}$) | • PLA  
• CHO (51g/hr)  
• Tyrosine+CHO (3.65g+51g/hr)  
• Tyrosine (3.65g/hr) | • TT: 34.4 min  
• TT: 27.2 min  
• TT: 26.1 min  
• TT: 32.6 min  
• Statistical power for differences between CHO & Tyrosine+CHO (0.11) and PLA & Tyrosine (0.08) were too small to make conclusions |
| Watson 2004    | 8 Males  | Glycogen depleted state Temp-30°C TTE @ 50% VO$_{2\text{peak}}$ | • PLA  
• BCAA (6g/hr during 2 hr rest; 7.2g/hr during exercise) | • TTE: 103.9 min  
• TTE: 111.0 min  
• ↔ between treatments |
| Cheuvront 2004 | 7 Heat-Acclimated Males | Glycogen depleted and hypohydrated Temp-40°C 60 min @ 50% VO$_{2\text{peak}}$; 30 min TT | • CHO (70g)  
• CHO+BCAA (60g CHO+10g BCAA) | • Mean power output: 79.5W  
• Mean power output: 91.7W  
• ↔ in performance  
• ↔ in cognitive performance, mood, or perceived exertion |
| Sutton 2005    | 20 Males | 120 min (or until volitional exhaustion) treadmill w/ weighted backpack @ 70% VO$_{2\text{max}}$; handgrip, pull-ups, and stair-stepping tests | 30 min prior to exercise:  
• PLA  
• L-Tyrosine (12.1g) | • 119.2 min  
• 118.9 min  
• ↔ in treadmill time, muscle strength, or muscle endurance |
| Klein 2009     | 9 Male and 1 Female Cyclist | 45 min @ 75% VO$_{2\text{peak}}$; 15 min performance trial (amount of work completed in 15 min) | • PLA  
• CHO (30g prior; 45g during)  
• ALA (30g prior; 45g during)  
• CHO+ALA (30+30g prior; 45+45g during) | • Work completed: 229 kJ  
• Work completed: 222kJ  
• Work completed: 219kJ  
• Work completed: 218kJ  
• ↔ in performance |
### Table 2.4: Amino Acid Ingestion – Performance (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Knechtle 2011 | 28 Male Ultra-Runners (14 per group) | 100km Ultra-Marathon Race | • PLA  
• Amino Acid Pills (52.5g Total)  
Subjects were allowed food and drinks *ad libitum.* | • Performance: 698 min  
• Performance: 624 min (p=0.033)  
• When adjusted for personal best time in previous 100km races, difference was no longer significant |

PLA = Placebo; CHO = Carbohydrate; PRO = Protein; ALA = Alanine; BCAA = Branched-Chain Amino Acids; TT = Time Trial; TTE = Time to Exhausion; RPE = Rating of Perceived Exertion
**Protein/Amino Acid Ingestion – Physiological Effect and Proposed Mechanisms**

**Central Fatigue**

Though a number of mechanisms have been proposed, a clear mechanistic explanation for how a small dose of protein can enhance endurance performance remains elusive. Traditionally, peripheral mechanisms (muscular fatigue) are credited with fatigue during endurance exercise, but central means have also been linked to fatigue during endurance performance (4). The most widely researched mechanism to date is the effect of amino acid provision on central nervous system fatigue. Brain concentration of tryptophan is a pre-cursor for serotonin, which has been linked to lethargy in humans (10, 48). Plasma free-tryptophan competes with other plasma neutral amino acids (i.e. tyrosine, alanine, phenylalanine, leucine, and valine) for transport across the blood-brain barrier (49, 107, 151). Therefore, the plasma free-tryptophan/neutral amino acid ratio is the main determinate of brain serotonin levels. During prolonged exercise plasma free-tryptophan increases and plasma neutral amino acids decreases causing elevated brain tryptophan (10, 15). Therefore, neutral amino acid supplementation increases the concentration of plasma neutral amino acids and inhibits the uptake of plasma tryptophan by the brain (49, 63). This subsequently decreases brain serotonin synthesis, thus theoretically attenuating central fatigue. However, whether this actually attenuates central fatigue in practice is unknown. Tyrosine supplementation is thought to not only attenuate brain uptake of tryptophan, but also potentiate uptake of tyrosine and consequently increase synthesis of dopamine (26). However, as stated previously, it has not been established that tyrosine increases endurance performance (28, 135, 136). However, some studies have found neutral amino acid supplementation (BCAA) to diminish perceived exertion, improve performance, and increase cognitive function during endurance exercise (11–13, 21, 22, 97), but others have shown no effect (27, 63, 90, 135, 142, 148). Due to equivocal results, firm conclusions about the effect of neutral amino acid supplementation on central fatigue during prolonged exercise cannot yet be drawn.
Tricarboxylic Acid Cycle Intermediates

Another potential mechanistic explanation for the acute performance benefit of protein/amino acid supplementations is an increase in tricarboxylic acid cycle intermediate (TCAI) pool size. TCAI levels rapidly increase at the onset of exercise and then slowly decreases with exercise duration (59, 121). It was previously surmised that depletion of TCAI contributes to fatigue by impairing aerobic energy production (121), and that protein intake could maintain TCAI pool size and sustain aerobic energy provision (69). However, it now appears that TCAI pool size is inconsequential for oxidative metabolism (17). Moreover, researchers recently reported that protein co-ingestion with carbohydrate has no effect on TCAI pool size compared to carbohydrate supplementation alone during exercise (25).

Fluid, Sodium, and Carbohydrate Delivery

Certain amino acids (arginine, glutamine, and alanine) have been found to increase intestinal absorption of sodium, fluid, and glucose in vitro (29, 146), all of which could favorably impact performance. Neutral amino acids are absorbed from the intestines at a comparable rate to that of carbohydrate (37) and co-transported with sodium across the intestines via glucose independent transporters (29). Due to increased sodium absorption with neutral amino acids, more fluid can be absorbed from the intestines via solvent drag (87). Therefore, amino acid supplementation is capable of improving performance through increased fluid replacement and, when carbohydrate is co-ingested, exogenous carbohydrate oxidation. Arginine (0.44g/hr), but not glutamine, co-ingestion with carbohydrate was reported to increase exogenous carbohydrate oxidation by 12% (118). However, milk protein concentrate co-ingestion (~0.41g arginine/hr) with carbohydrate did not increase exogenous carbohydrate oxidation (120). Therefore, it is unknown whether protein supplementation can improve sodium, fluid, or glucose absorption in vitro.
Exogenous Amino Acid Oxidation

The final avenue by which protein/amino acid ingestion has been proposed to enhance performance is through exogenous energy provision, thereby slowing endogenous substrate utilization. Endogenous protein is typically considered a poor source of ATP, as it supplies only 5-10% of the total energy cost of exercise. Nonetheless, amino acids are oxidized in skeletal muscle (Figure 2.1) and provide gluconeogenic pre-cursors to the liver late in exercise in an attempt to maintain blood glucose concentrations (143). Exogenous amino acids have been shown to be oxidized during exercise in this manner (30, 81, 82). Protein inclusion (18 g/hr) in a carbohydrate beverage results in a two-fold increase in amino acid oxidation during prolonged exercise (81). Furthermore, 50.6 g of the 73.7 g supplemented alanine was oxidized during three hours of cycling (82). This proposed mechanism has a considerable amount of evidence, as most studies reporting performance gains with protein co-ingestion have been compared to carbohydrate matched treatments rather than calorically-matched treatments, which points to an energy provision effect of protein/amino acid supplementation. However, most studies have utilized rates of protein/amino acid intake well below the rate of carbohydrate intake known to produce a clear metabolic effect (30-60 g/hr). Additional research with protein/amino acid intake rates equivalent to those recommended for carbohydrate are necessary to examine the maximum effect of exogenous energy provision.
Table 2.5: Protein/Amino Acid Ingestion – Physiological Effect and Proposed Mechanisms

<table>
<thead>
<tr>
<th>Study</th>
<th>Mechanism</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fernstrom 1971 (48)</td>
<td>Central Fatigue</td>
<td>Normal physiological dose of tryptophan given to rats</td>
<td>• Plasma and brain tryptophan ↑ 10 to 60 minutes after injection</td>
<td>• Plasma and brain tryptophan ↑ 10 to 60 minutes after injection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Brain serotonin concentrations were ↑ 60 minutes after injection</td>
<td>• Brain serotonin concentrations were ↑ 60 minutes after injection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Tryptophan is rate limiting for serotonin synthesis in the brain</td>
<td>• Tryptophan is rate limiting for serotonin synthesis in the brain</td>
</tr>
<tr>
<td>Fernstrom 1972 (49)</td>
<td>Central Fatigue</td>
<td></td>
<td>• ↑ plasma tryptophan leads to ↑ brain tryptophan and brain serotonin</td>
<td>• ↑ plasma tryptophan leads to ↑ brain tryptophan and brain serotonin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Even when plasma tryptophan is ↑, brain serotonin does not ↑ when other plasma neutral amino acids are elevated</td>
<td>• Even when plasma tryptophan is ↑, brain serotonin does not ↑ when other plasma neutral amino acids are elevated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• The ratio of tryptophan: neutral amino acid (i.e. alanine, tyrosine, phenylalanine, leucine, and valine) is the main determinate of brain serotonin concentration</td>
<td>• The ratio of tryptophan: neutral amino acid (i.e. alanine, tyrosine, phenylalanine, leucine, and valine) is the main determinate of brain serotonin concentration</td>
</tr>
<tr>
<td>Yuwiler 1977 (151)</td>
<td>Central Fatigue</td>
<td></td>
<td>• Uptake of plasma tryptophan by the brain is concentration dependent</td>
<td>• Uptake of plasma tryptophan by the brain is concentration dependent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Some albumin bound-tryptophan is stripped from albumin during passage for uptake into the brain, but free-tryptophan is more easily transported</td>
<td>• Some albumin bound-tryptophan is stripped from albumin during passage for uptake into the brain, but free-tryptophan is more easily transported</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Amino acid competition for carrier sites across the blood brain barrier is the most important factor regulating tryptophan uptake into the brain</td>
<td>• Amino acid competition for carrier sites across the blood brain barrier is the most important factor regulating tryptophan uptake into the brain</td>
</tr>
<tr>
<td>Study</td>
<td>Mechanism</td>
<td>Exercise</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Pardridge 1977 (107) | Central Fatigue |                               | • Neutral amino acids compete for a single transport site across the blood-brain barrier with tryptophan  
• The tryptophan: neutral amino acid ratio regulates the brain uptake of these amino acids                                                                                                                                 |
| Asmussen 1979 (4) | Central Fatigue |                               | • Two forms of fatigue exist; central and peripheral fatigue  
• Central fatigue is an expression of lowered arousal                                                                                                                                                         |
| Blomstrand 1988 (10) | Central Fatigue | 1986 Stockholm Marathon or 90 min Army Training | • Plasma concentration of BCAA ↓ during both types of exercise  
• Plasma concentration of free tryptophan was ↑ 2.4-fold  
• ↑ in free tryptophan: BCAA ratio should lead to an ↑ in the rate of tryptophan transport across the blood brain-barrier and an ↑ in synthesis of serotonin  
• Serotonin has been linked to lethargy in humans  
• ↑ concentrations of serotonin in specific areas of the brain may cause central fatigue during prolonged exercise |                                                                                                                                                                                                                                                                                             |
| Blomstrand 1989 (15) | Central Fatigue | Treadmill running to fatigue in rats | • Running to fatigue ↑ ratio of plasma free tryptophan: plasma neutral amino acids and subsequently brain tryptophan (↑ 36%)  
• Brain serotonin was ↑ at fatigue and may play a role in central fatigue |                                                                                                                                                                                                                                                                                             |
### Table 2.5: Protein/Amino Acid Ingestion – Physiological Effect and Proposed Mechanisms (Continued)

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Mechanism</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Sahlin 1990 (121) | TCAI | Cycled to fatigue @ 75% \( \text{VO}_2\text{peak} \) | | • TCAI ↑ initially during exercise and then continually ↓ until fatigue  
• Aerobic energy production may be impaired by depleted TCAI levels |
| Gibala 2002 (59) | TCAI | 90 min leg kicking @ 70% of max one-legged capacity. | Repeatedly measured TCAI | • TCAI ↑ rapidly at the beginning of exercise before slowly ↓ to near resting levels  
• ↓ TCAI did not compromise aerobic energy provision |
| Ivy 2003 (69) | TCAI | 180 min varied intensity cycling (45-75% \( \text{VO}_2\text{peak} \)); TTE @ 85% \( \text{VO}_2\text{peak} \) | • PLA  
• CHO (47g/hr)  
• CHO+PRO (47+12g/hr) | • PRO ergogenic effect proposed as being due to ↑ supply of TCAI |
| Cermak 2009 (25) | TCAI | 90 min cycling @ 69% \( \text{VO}_2\text{peak} \) | • CHO (60g/hr)  
• CHO+PRO (60+20g/hr) | • TCAI pool expansion was similar between trials  
• Muscle glycogen utilization was equal between trials |
| Dechelotte 1991 (37) | Intestinal Absorption | Rest | Humans Jejunum Perfused w/:  
• PLA  
• different concentrations of Glutamine | • Saturation level of glutamine intestinal transport was similar to those previously reported in glucose and alanine |
| Wapnir 1997 (146) | Intestinal Absorption | Rest | Rat Jejunum Perfused w/:  
• PLA  
• different concentrations of Arginine | • 1 to 20 mmol/L of Arginine ↑ intestinal absorption of glucose, sodium, and water |
<table>
<thead>
<tr>
<th>Study</th>
<th>Mechanism</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coëffier 2005 (29)</td>
<td>Intestinal Absorption</td>
<td>Rest</td>
<td>Humans Jejunum Perfused w/ Glutamine, Glutamine &amp; Glucose, Alanine &amp; Glucose, Glucose, PLA</td>
<td>Glutamine and alanine ↑ sodium and fluid absorption in the human jejunum, Neutral amino acids are co-transported with sodium in the intestine that is independent of glucose transporters</td>
</tr>
<tr>
<td>Rowlands 2011 (118)</td>
<td>Intestinal Absorption</td>
<td>150 min @ 50% $W_{max}$</td>
<td>CHO+Glutamine (48+4g/hr), CHO+Arginine (48+0.44g/hr), CHO (48g/hr), PLA</td>
<td>↔ exogenous CHO oxidation; slight ↑ in GI discomfort, ↑ exogenous CHO oxidation (12%); ↓ $O_2$ consumption (2.6%); ↓ the lactate concentration (0.20mmol/L); slight ↑ in GI discomfort</td>
</tr>
<tr>
<td>Rowlands 2012 (120)</td>
<td>Intestinal Absorption</td>
<td>150 min @ 50% $W_{max}$</td>
<td>CHO+PRO (48+12g/hr), CHO (48g/hr), PLA</td>
<td>↔ exogenous CHO oxidation, gut comfort, or perceived exertion; ↑ endogenous CHO oxidation (possibly due to gluconeogenesis)</td>
</tr>
<tr>
<td>Colombani 1999 (30)</td>
<td>Amino Acid Oxidation</td>
<td>Marathon Run</td>
<td>CHO (81g), CHO+PRO (81g+32g)</td>
<td>PRO was absorbed and at least partially oxidized</td>
</tr>
<tr>
<td>Korach-André 2002 (82)</td>
<td>Amino Acid Oxidation</td>
<td>180 min cycling @ 53% $VO_{2peak}$</td>
<td>73.7g alanine Before and During Exercise</td>
<td>50.6g of exogenous alanine was oxidized (68.7% of load), Exogenous alanine accounted for 10% of energy yield along with 5% from endogenous PRO</td>
</tr>
<tr>
<td>Koopman 2004 (81)</td>
<td>Amino Acid Oxidation</td>
<td>150 min cycling; 60 min running; 150 min cycling @ 50% $VO_{2max}$</td>
<td>CHO (50g/hr), CHO+PRO (50+18g/hr)</td>
<td>2-fold ↑ in PRO oxidation</td>
</tr>
</tbody>
</table>
Table 2.5: Protein/Amino Acid Ingestion – Physiological Effect and Proposed Mechanisms (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Mechanism</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valentine 2008 (140)</td>
<td>Amino Acid Oxidation</td>
<td>TTE @ 75% VO_{2peak}</td>
<td>PLA, CHO (78g/hr), CHO+CHO (97g/hr), CHO+PRO (78+19g/hr)</td>
<td>TTE: The isocaloric treatments (CHO+CHO and CHO+PRO) were significantly better than PLA while CHO was not different. At least some of the additive effect of PRO co-ingestion on performance is caused by ↑ calories.</td>
</tr>
</tbody>
</table>

PLA = Placebo; CHO = Carbohydrate; PRO = Protein; BCAA = Branched-Chain Amino Acids; TT = Time Trial; TTE = Time to Exhaustion; TCAI = Tricarboxylic Acid Cycle Intermediates; GI = Gastrointestinal
Figure 2.1: Muscle Amino Acid Metabolism

Alanine Metabolism and Supplementation

Alanine is an amino acid that is readily oxidized/decarboxylated during exercise (149). Moreover, alanine is the principle amino acid extracted by the liver for gluconeogenesis (45). The glucose-alanine cycle (Figure 2.2) is a concept that outlines the production of alanine in the muscle and its subsequent conversion to glucose in the liver (46). As liver glycogen levels decline gluconeogenesis contributes more to hepatic glucose production. Consequently, liver uptake of alanine increases as a function of exercise duration; gluconeogenic pre-cursor uptake increases 2 to 10-fold following four hours of cycling (1). Thus, alanine significantly contributes to the maintenance of blood glucose levels late in exercise. The important role that alanine has on metabolism has raised interest in the metabolic effect that alanine supplementation has during prolonged exercise. As mentioned previously, when supplemented during three hours of cycling, 50.6 g of the 73.7 g of exogenous alanine were oxidized. The peak exogenous alanine oxidation rate (0.35g/min) was only slightly lower than those previously reported when carbohydrate was ingested at a similar rate (0.43g/min). Exogenous alanine contributed 10% of the total energy cost of exercise while endogenous protein contributed another 5%. Therefore, alanine supplementation at a higher rate (30-60 g/hr) is potentially capable of providing substrate to sustain aerobic energy production late in exercise. Currently, only one study has investigated the effect of alanine supplementation on endurance performance. Supplementation produced an ostensibly favorable metabolic effect, but the exercise test was of insufficient duration to challenge endogenous substrate availability and failed to produce an ergogenic effect (79).
## Table 2.6: Alanine Metabolism and Supplementation

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Felig 1970</strong></td>
<td>6 normal weight and 7 obese adults</td>
<td>Rest</td>
<td>Overnight fast and 4 to 6 weeks of total starvation</td>
<td>• ALA is the principal amino acid precursor extracted by the liver for gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• ALA concentration is rate-limiting for gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• With prolonged starvation and subsequent ↓ in plasma ALA, gluconeogenesis and hepatic glucose production</td>
</tr>
<tr>
<td><strong>Müller 1971</strong></td>
<td>Dogs</td>
<td>Rest</td>
<td>Low dose of ALA, high dose of ALA; hyperglycemic w/ high dose of ALA</td>
<td>• When fasted, ALA caused ↑ in plasma glucagon; very slight ↑ in insulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• When exogenous glucose is available ↔ in glucagon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Glucagon mediates gluconeogenesis—ALA causes an ↑ in gluconeogenesis</td>
</tr>
<tr>
<td><strong>Ahlborg 1974</strong></td>
<td>6 Males</td>
<td>Studied at rest and during 240 min of cycling at ~30% VO_{2peak}</td>
<td>12-14 hour overnight fast</td>
<td>• Leg output of ALA ↑ w/ exercise duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Splanchnic uptake of gluconeogenic precursors ↑ 2 to 10-fold after exercise (45% of hepatic glucose output)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• As liver glycogen stores are ↓ gluconeogenesis contributes more to energy production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Blood glucose levels ↓ due to hepatic glucose output unable to keep up with glucose utilization</td>
</tr>
<tr>
<td><strong>White 1981</strong></td>
<td>18 rats assigned to each group</td>
<td>Rest, easy running (2 hours), and hard running (2 hours)</td>
<td>Glucose, alanine, and leucine tracer injections to measure oxidation rates</td>
<td>• Oxidative decarboxylation of amino acids (ALA and Leucine) ↑ during exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• ALA is a readily oxidizable substrate during exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Plasma, muscle, and liver concentrations of ALA ↑ with hard exercise</td>
</tr>
</tbody>
</table>
Table 2.6: Alanine Metabolism and Supplementation (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Williams 1998 (150) | 6 Males  | 180 min treadmill exercise @ 45% VO<sub>2max</sub> | Tracers used to quantify alanine and glutamine rates of appearance and kinetics. | • ALA rate of appearance increased during exercise, but glutamine did not.  
• Amino-N delivery from muscle to liver increased during exercise.  
• ALA is predominant N carrier during exercise. |
| Korach-André 2002 (82) | 6 Active Males | 180 min Cycling @ 53% VO<sub>2peak</sub> | 73.7g ALA Before and During Exercise | • 50.6g of exogenous ALA was oxidized (68.7% of load).  
• Exogenous ALA accounted for 10% of energy yield along with 5% from endogenous PRO. |
| Klein 2009 (79) | 9 Male and 1 Female Cyclist | 45 min @ 75% VO<sub>2peak</sub>; 15 min performance trial (amount of work completed in 15 min) | • PLA  
• CHO (30g prior; 45g during)  
• ALA (30g prior; 45g during)  
• CHO+ALA (30+30g prior; 45+45g during) | • Work Completed: 229 kJ  
• Work Completed: 222kJ  
• Work Completed: 219kJ  
• Work Completed: 218kJ  
• ↔ Performance by treatment  
• Too short for metabolic effect  
• ALA ↑ plasma concentrations of gluconeogenic amino acids, but didn’t affect BCAA  
• Promoted metabolic profile favorable for performance |

ALA = Alanine; PLA = Placebo; CHO = Carbohydrate; BCAA = Branched-Chain Amino Acids; TT = Time Trial; TTE = Time To Exhaustion
Figure 2.2: Alanine-Glucose Cycle

Interleukin-6

Interleukin-6 (IL-6) is a myokine that plays a significant role in metabolism during prolonged exercise. The prevailing thought is that IL-6 is released from skeletal muscle as an energy sensor (109). Therefore, as exercise duration and/or intensity increases skeletal muscle releases additional IL-6 resulting in increased plasma IL-6 (54). Muscle glycogen content plays a large role in the increase in plasma IL-6. When exercise is initiated with low muscle glycogen the exercise-induced IL-6 response is much more prominent (75, 89, 130). Conversely, carbohydrate supplementation minimized the plasma IL-6 response. Therefore, the presence of carbohydrate in skeletal muscle (endogenous or exogenous) inhibits the release of IL-6 and attenuates the plasma IL-6 concentration (44, 99, 101, 128).

IL-6 impacts metabolism through a number of mechanisms. IL-6 increases AMPK activity by increasing the concentration of cAMP and, secondarily, the AMP-ATP ratio (77). This increase in AMPK activity potentiates free fatty acid oxidation and GLUT-4 translocation, which results in skeletal muscle glucose uptake (23). IL-6 also increases hepatic glucose production, by elevating gluconeogenic gene mRNA (PEPCK, G6P, and PGC-1α) as well as gluconeogenic precursor uptake (6, 52, 56, 147). Interestingly, glutamine supplementation, an individual amino acid, augments plasma IL-6 during prolonged exercise (67). Researchers speculated that the increased plasma glutamine concentration signaled for an increase in plasma IL-6 to facilitate hepatic amino acid uptake (67). Regardless of how glutamine supplementation increases plasma IL-6, it appears capable of producing a favorable metabolic effect during prolonged exercise. Thus, the effect of protein/amino acid supplementation on the exercise-induced plasma IL-6 response will provide general insight into the metabolic environment under these conditions.
### Table 2.7: Interleukin-6

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Stith 1994**| Adult Male Rats           | Rest—tested 30, 60, 90, 120, and 180 min after injection | Injected recombinant human IL-6 (rhIL-6)                                 | • IL-6 ↑ plasma corticosterone by central action on the hypothalamus-pituitary-adrenal cortex  
• IL-6 ↑ plasma glucagon and glucose.  
• Hepatic glycogen was ↓ compared to control  
• IL-6 altered the hormonal and CHO profile either by direct action on peripheral organs and/or the central nervous system |
| **Watkins 1994** | Adult Rats                | Rest                      | Injection of 0.5mg/kg of dexamethasone and a varying concentration of IL-6 (10, 50, or 150 μg/kg) | • Amino acid transport in liver was analyzed  
• IL-6 and glucocorticoids were found to work together to ↑ hepatic amino acid uptake  
• A dose-response effect was seen with IL-6 on hepatic amino acid uptake |
| **Fischer 1996** | Human liver biopsies used to obtain hepatocytes |                          | IL-6 and TNF-α in combination with dexamethasone was added to hepatocyte in culture. | • IL-6 and TNF-α exerted stimulatory effect on amino acid transport  
• IL-6 is capable of ↑ alanine and glutamine uptake by the liver |
| **Castell 1997** | 30 Male Marathon Runners  | 1991 and 1993 Brussels Marathons (avg time: 3:45 and 3:42) |                            | • Post-marathon plasma IL-6 ↑ 45-fold                     |
| **Rohde 1997** | 6 Males                   | 60 min of one-legged eccentric leg extension @ 120% of concentric W<sub>max</sub> | Control  
• BCAA—before, during, and after exercise (22.6g) | • BCAA ↓ release of amino acids from muscle—↓ net muscle degradation  
• Plasma IL-6 ↑ after prolonged eccentric exercise (regardless of treatment/muscle protein breakdown) |
Table 2.7: Interleukin-6 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Bruunsgaard 1997**   | 9 Males        | 30 min cycling @ 65% VO$_{2max}$ | • Concentric  
• Eccentric (braking w/ reversed revolutions) | • Plasma IL-6 ↑ 2 hours after eccentric cycling, but not concentric  
• Plasma IL-6 is possibly related to muscle damage |
| **Nehlsen-Cannarella 1997** | 30 Marathon Runners | 150 min running @ 75-80% of VO$_{2max}$ | • PLA  
• CHO (60g/hr) | • Plasma IL-6 ↑ 753% post-run  
• Plasma IL-6 ↑ 441% post-run  
• CHO ingestion ↓ the cytokine response to heavy exertion |
| **Nieman 1998**        | 10 Triathletes | 150 min of running or cycling @ 75% VO$_{2max}$ | • PLA  
• CHO (60g/hr) | • ↑ plasma glucose and insulin; ↓ cortisol and growth hormone; ↓ plasma IL-6  
• ↓ plasma IL-6 from cycling compared to running  
• CHO thought to reduce physiological stress |
| **Ostrowski 1998**     | 16 Males       | Copenhagen Marathon (median time: 3:17:03) | Allowed CHO and fluid ad libitum. | • Plasma IL-6 ↑ ~63-fold immediately post-exercise  
• IL-6 mRNA ↑ in muscle post-exercise  
• IL-6 is locally produced in skeletal muscle |
| **Ostrowski 1999**     | 10 Males       | Copenhagen Marathon (median time: 3:26) | Allowed CHO and fluid ad libitum. | • Plasma IL-6 ↑ ~128-fold immediately post-exercise  
• Plasma IL-6 was more pronounced immediately post-exercise than any other time point following exercise |
| **Starkie 2000**       | 6 Endurance-Trained Males | 120 min of cycling @ 70% VO$_{2peak}$ | • PLA  
• CHO (35g before; ~60g/hr during) | • Plasma IL-6 ↑ 2-fold post-exercise  
• Plasma IL-6 ↑ 2-fold post-exercise  
• Circulating monocytes are not the source of ↑ plasma IL-6 |
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Steensberg 2000 (132)** | 6 Males  | 5 hours of one-legged knee extensor exercise @ 40% $W_{\text{max}}$ | • Plasma IL-6 ↑ 19-fold from rest  
• Net IL-6 release from muscle was 17-fold ↑ than the elevation in arterial IL-6  
• Net IL-6 release from muscle during one minute was half the total plasma content  
• Very high turnover of IL-6 during exercise  
• IL-6 is suggested to contribute to glucose homeostasis during prolonged exercise  
• Source of ↑ plasma IL-6 during prolonged exercise is active skeletal muscle |
| **Henson 2000 (66)** | 15 Elite Female Rowers | 120 min of rowing (~57% $VO_{\text{2max}}$) | • PLA  
• CHO (55g pre-exercise; 73g/hr during exercise) | • Plasma IL-6 was ↔ by exercise and/or CHO |
| **Steensberg 2001 (130)** | 7 Males  | 4-5 hours (until exhaustion) of two-legged knee extensor exercise @ 40% $W_{\text{max}}$ | • Normal muscle glycogen leg  
• Low Muscle glycogen leg  
• Intramuscular IL-6 mRNA ↑; released IL-6 after 2 hours of exercise  
• Augmented intramuscular IL-6 mRNA ↑; released IL-6 after 1 hour of exercise  
• Plasma IL-6 ↑ 36-fold  
• Glycogen availability alters IL-6 production and release in contracting muscle |
| **Keller 2001 (75)** | 6 Males  | 180 min of two-legged knee extensor exercise @ 50-60% $W_{\text{max}}$ | • Normal muscle glycogen  
• Low (60% of normal) muscle glycogen  
• ↑ plasma IL-6, IL-6 gene transcription and mRNA content from rest  
• ↑ plasma IL-6 earlier and higher; much higher IL-6 gene transcription and mRNA content |
Table 2.7: Interleukin-6 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Starkie 2001** *(128)* | 7 Moderately Trained Males | 2 trials of 60 min of cycling @ lactate threshold and 2 trials of 60 min of running @ lactate threshold | • PLA  
  • CHO (64g) | • Exercise resulted in a 21-fold ↑ in IL-6 mRNA expression regardless of mode or treatment  
  • Mode of exercise did not affect ↑ in plasma IL-6, but CHO blunted plasma IL-6 |
| **Lyngso 2002** *(88)* | 7 Experimental and 8 Control Subjects | Rest                                                                      | 2.5 hours of IL-6 infusion causing plasma concentration ~35ng/L | • IL-6 infusion caused ↑ in net glycerol release from subcutaneous adipose tissue while fatty acid release was unchanged  
  • IL-6 caused vasodilation in the splanchnic region and uptake of fatty acids and the gluconeogenic precursors, glycerol and lactate  
  • Splanchnic glucose and triacylglycerol output was ↔  
  • IL-6 caused ↑ in lipolysis and gluconeogenesis |
| **Steensberg 2002** *(133)* | 6 Males | 180 min of two-legged knee extensor exercise @ 55% W<sub>max</sub> |                                      | • IL-6 mRNA ↑ ~100-fold following exercise  
  • IL-6 ↑ in arterial plasma throughout exercise  
  • Net IL-6 release from the contracting limb was ↑ after 120 min of exercise |
| **Steensberg 2003** *(131)* | 6 Males/group | Rest                                                                      | 3 hours of:  
  • high dose of rhIL-6  
  • low dose of rhIL-6  
  • PLA | • ↔ endogenous glucose production, whole-body glucose disposal, and leg-glucose uptake  
  • Expected ↑ in glucose uptake by skeletal muscle with rhIL-6 was not seen |
Table 2.7: Interleukin-6 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keller 2003</td>
<td>6-7 Males/group</td>
<td>Exercise group—180 min cycling @ 60% VO$_{2\text{max}}$ rhIL-6 &amp; Control—Rest</td>
<td>• Exercise • rhIL-6 • Control</td>
<td>• In muscle, IL-6 mRNA ↑ and peaked at the end of exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• In adipose tissue, IL-6 mRNA ↑ 1.5 hours post-exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• rhIL-6 infusion resulted in ↑ IL-6 mRNA levels in the muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 has a positive auto-regulatory role in the muscle</td>
</tr>
<tr>
<td>Febbraio 2003</td>
<td>7 Males</td>
<td>120 min semi-recumbent cycling @ 65% VO$_{2\text{max}}$</td>
<td>• PLA • CHO (64g/hr)</td>
<td>• Decreased plasma IL-6 response; less net leg IL-6 release</td>
</tr>
<tr>
<td>(44)</td>
<td></td>
<td></td>
<td></td>
<td>• Intramuscular glycogen and IL-6 mRNA were ↔ between treatments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 release from muscle may be regulated by substrate availability while IL-6 mRNA is influenced by glycogen content</td>
</tr>
<tr>
<td>Nieman 2003</td>
<td>16 Marathon Runners</td>
<td>180 min treadmill running @ 70% VO$_{2\text{max}}$</td>
<td>• PLA • CHO (60g/hr)</td>
<td>• 875% ↑ plasma IL-6; 35.2-fold ↑ gene expression of IL-6 mRNA</td>
</tr>
<tr>
<td>(102)</td>
<td></td>
<td></td>
<td></td>
<td>• 507% ↑ plasma IL-6; 15.9-fold ↑ gene expression of IL-6 mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• No difference in muscle glycogen content</td>
</tr>
<tr>
<td>MacDonald 2003</td>
<td>8 Male Cyclists</td>
<td>60 min cycling @ 70% VO$_{2\text{peak}}$</td>
<td>• Glycogen Depleted</td>
<td>• IL-6 release from leg seen after 10 min; @ rest α1-AMPK and α2-AMPK activities were ↑; post-exercise α2-AMPK activity was ↑</td>
</tr>
<tr>
<td>(89)</td>
<td></td>
<td></td>
<td></td>
<td>• No IL-6 release from leg or α1-AMPK and α2-AMPK activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Glycogen Loaded</td>
<td>• Plasma IL-6 ↑ similarly in both trials</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• α2-AMPK activity correlated with IL-6 release</td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Activity</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>-------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Lancaster     | Timing—8 | 20 min cycling @ 65% W<sub>max</sub>, followed by a TT lasting ~43 min | Timing: 75g CHO @ 15 or 75 min pre-
Amount: 45 min pre-
PLA; 25g
CHO; 200g CHO | Exercise-induced ↑ in plasma IL-6 was equal in each group (~4-fold) |
| 2003          | Amount—10 Male Cyclists |                               |                                                                           |                                                                                                                                                                                                         |
| (84)          | 8 Male Cyclists | 120 min cycling @ 75% VO<sub>2max</sub> | • PLA (7g CHO)
• Glutamine (7g)
• PRO+Glutamine (27.4+2.5g PRO bound Glutamine+15g sucrose) | PLA IL-6 response: 11-fold ↑
Plasma IL-6 response: 18-fold ↑
Plasma IL-6 response: 14-fold ↑
Glutamine and PRO+Glutamine maintained the plasma glutamine concentration while the PLA had ↓ plasma glutamine
Glutamine uptake by the muscle possibly allowed for a ↑ IL-6 production
↑ glutamine concentration may cause an ↑ demand for IL-6 release from muscle for IL-6 mediated hepatic amino acid uptake. |
| Hiscock       | 7 Male Cyclists | 150 min cycling @ 65% VO<sub>2max</sub> | • PLA
• LCHO (32g prior, 38.4g/hr)
• HCHO (64g prior; 76.8g/hr) | Both CHO supplements attenuated the ↑ in plasma IL-6
CHO attenuated the exercise-induced stress hormone response |
| 2004          | (83)      | Treadmill running-incremental test and fixed speed test | • Control
• IL-6 deficient | IL-6 deficient mice had ↓ endurance and energy expenditure during exercise
RER was ↑ in older IL-6 deficient mice during exercise |
| Fäldt         | Mice     |                               |                                                                           |                                                                                                                                                                                                         |
| 2004          | (42)      | 120 min cycling               | • HI (70% VO<sub>2peak</sub>)
• LO (40% VO<sub>2peak</sub>)
• LO+IL-6 (40% VO<sub>2peak</sub> + rhIL-6 intended to mimic plasma IL-6 concentration of HI) | Rate of appearance and disposal of glucose was ↑ in HI and LO+IL-6 compared to LO
IL-6 contributes to the exercise-induced ↑ in endogenous glucose production regardless of hormonal changes |
<p>| Febbraio      | 6 Males  |                               |                                                                           |                                                                                                                                                                                                         |
| 2004          | (43)      |                               |                                                                           |                                                                                                                                                                                                         |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelly 2004</td>
<td>Mice</td>
<td>60 min swimming</td>
<td>• Control</td>
<td>• Incubation with IL-6 ↑ phosphorylation of AMPK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• IL-6 knockout</td>
<td>• In IL-6 knockout mice P-AMPK and P-ACC was ↓ in muscle and adipose at rest and in response to exercise.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 can activate AMPK and partially contributes to the ↑ in AMPK activity in response to exercise.</td>
</tr>
<tr>
<td>Fischer 2004</td>
<td>7 Males</td>
<td>180 min of two-legged knee extensor exercise @ 50% W&lt;sub&gt;max&lt;/sub&gt;/leg (same relative workload)</td>
<td>Pre and Post 10 wks of one-legged knee extensor training (60 min @ 75% W&lt;sub&gt;max&lt;/sub&gt; 5x/wk)</td>
<td>• Resting muscle glycogen content ↑ ~74% post-training. However, glycogen content ↓ to same extent during exercise.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 mRNA ↑ 76-fold after exercise pre-training and only 8-fold post-training</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Training had no effect on plasma IL-6</td>
</tr>
<tr>
<td>Nieman 2005</td>
<td>15 Male Cyclists</td>
<td>150 min cycling @ 60% W&lt;sub&gt;max&lt;/sub&gt;</td>
<td>• PLA</td>
<td>• The exercise-induced plasma IL-6 ↑ was attenuated with CHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• CHO (74g/hr)</td>
<td>• Muscle IL-6 mRNA ↑ in both trials with no difference between treatments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Muscle glycogen content ↓ 68% during exercise with both treatments</td>
</tr>
<tr>
<td>Keller 2005</td>
<td>7 Males</td>
<td>180 min of two-legged knee extensor exercise @ 50% W&lt;sub&gt;max&lt;/sub&gt;/leg (same relative workload)</td>
<td>Pre and Post 10 wks of one-legged knee extensor training (60 min @ 75% W&lt;sub&gt;max&lt;/sub&gt; 5x/wk)</td>
<td>• Training ↑ basal IL-6 receptor mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• low glycogen leg</td>
<td>• Training ↑ skeletal muscle sensitivity to IL-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• normal glycogen leg</td>
<td>• Exercise-induced ↑ in IL-6 receptor mRNA is unaffected by training status and glycogen levels</td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Activity</td>
<td>Treatment</td>
<td>Outcome</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------</td>
<td>---------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Carey 2006</td>
<td>7 Males; In vitro</td>
<td>Rest</td>
<td>• Hyperinsulinemic-euglycemic clamp and rhIL-6 infusion</td>
<td>• IL-6 increased glucose disposal without affecting the complete suppression of endogenous glucose production.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• IL-6 treatment of L6 myotube</td>
<td>• IL-6 treatment of myotube increased fatty acid oxidation, basal and insulin-stimulated glucose uptake, and translocation of GLUT4 to the plasma membrane.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 increased AMPK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Effects of IL-6 on glucose and fatty acid metabolism likely mediated by AMPK.</td>
</tr>
<tr>
<td>Glund 2007</td>
<td>22 Male Muscle Biopsies</td>
<td>Incubated w/ or w/out IL-6</td>
<td></td>
<td>• IL-6 increased glucose transport 1.3-fold.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 increased glucose incorporation into glycogen (1.5-fold) and glucose oxidation (1.3-fold).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 increased phosphorylation of STAT3 and AMPK and decreased phosphorylation of S6 ribosomal protein.</td>
</tr>
<tr>
<td>Banzet 2007</td>
<td>Rats</td>
<td>Treadmill run to exhaustion</td>
<td>• Control</td>
<td>• Exercise ↑ plasma IL-6 levels, IL-6 mRNA, and p38 MAPK phosphorylation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Calcineurin inhibition</td>
<td>• Calcineurin inhibition blunted the plasma IL-6 and gene transcription.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• P38 MAPK and calcineurin activity are two signaling events involved in IL-6 gene transcription.</td>
</tr>
</tbody>
</table>
Table 2.7: Interleukin-6 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Banzet 2009</strong>&lt;br&gt;(6)</td>
<td>Rats</td>
<td>Treadmill run to exhaustion (~123 min)</td>
<td>Exercise: rhIL-6 injection:&lt;br&gt;• PLA&lt;br&gt;• 3μg/kg&lt;br&gt;• 10μg/kg</td>
<td>• Exercise—plasma glucose, muscle glycogen, and hepatic glycogen ↓ post-exercise. Hepatic glycogen was 5% of resting value.&lt;br&gt;• Exercise—gluconeogenic gene mRNA levels (PEPCK, G6P, &amp; PGC-1α) were ↑&lt;br&gt;• This suggests a key role of gluconeogenesis in hepatic glucose production which was associated with active IL-6 signaling in the liver&lt;br&gt;• rhIL-6 ↑ IL-6 responsive gene mRNA in a dose-dependent manner&lt;br&gt;• During metabolically demanding exercise muscle derived IL-6 could help ↑ hepatic glucose production by upregulating PEPCK mRNA and thus gluconeogenesis</td>
</tr>
<tr>
<td><strong>Kelly 2009</strong>&lt;br&gt;(77)</td>
<td>Rats</td>
<td>Rat EDL muscle incubated w/ IL-6</td>
<td>• IL-6 activated AMPK in muscle by increasing the concentration of cAMP and, secondarily, the AMP-ATP ratio.&lt;br&gt;• This activation resulted in more sustained increase in PGC-1α and UCP3.&lt;br&gt;• IL-6 increased lipolysis and glycogenolysis in muscle.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7: Interleukin-6 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Activity</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fritsche 2010</td>
<td>Rats/mice</td>
<td>60 min treadmill run</td>
<td>Rat hepatoma cells w/ or w/out IL-6</td>
<td>• IL-6 stimulation of rat hepatoma cells ↑ glucose production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• IL-6 deficient</td>
<td>• Injection of IL-6 slightly ↑ PEPCK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• control</td>
<td>• Normal mice had ↑ plasma IL-6, however plasma glucose, and hepatic G6P and PGC-1α were ↔ from IL-6 deficient mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IL-6 doesn’t appear to be essential for ↑ glucose production during fasting or non-exhaustive exercise</td>
</tr>
<tr>
<td>Pedersen 2011</td>
<td>Mice</td>
<td>60 min swimming</td>
<td>• IL-6 deficient</td>
<td>Post-exercise CXCL-1 ↑ in plasma, muscle, and liver.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• control</td>
<td>• ↑ plasma IL-6 and muscle IL-6 mRNA preceded the ↑ in CXCL-1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• The liver CXCL-1 mRNA was completely blunted in IL-6 deficient mice.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Liver CXCL-1 expression is regulated by muscle derived IL-6.</td>
</tr>
</tbody>
</table>

rhIL-6 = Recombinant Human IL-6; PLA = Placebo; CHO = Carbohydrate; PRO = Protein; TT = Time Trial; TTE = Time To Exhaustion
Figure 2.3: Interleukin-6—Effectors and Effect

- Carbohydrate Ingestion
- Skeletal Muscle Contraction (DURATION & intensity)
- Muscle Glycogen Depletion
- Glutamine Ingestion

Legend:
- Increases
- Attenuates

Plasma IL-6

- Gluconeogenic Precursor Uptake & Gene mRNA (PEPCK, G6P, and PGC-1α)
- cAMP and AMP/ATP ratio

Gluconeogenesis

- Hepatic Glucose Production
- AMPK Activity

Lipolysis & FFA Oxidation

GLUT-4 Translocation & Glucose Uptake by Muscle
Summary

Currently, standard practice during prolonged exercise is to ingest fluid at high enough rates to avoid >2% loss of body weight (resulting from fluid loss) while avoiding gastrointestinal distress (125). This rate of fluid replacement is commonly prescribed as 600-1200 ml/hr (31). Also, standard practice during prolonged exercise is to supplement carbohydrate at 30-60 g/hr (114). This practice improves performance by preventing hypoglycemia, sparing liver glycogen, and maintaining high carbohydrate oxidation rates late in exercise (32). Recently, protein and amino acid supplementation have garnered attention for their potential to enhance performance. Whether protein/amino acid was co-ingested with carbohydrate or individually, results have been equivocal with some authors reporting benefits (12, 69, 97, 123) and others finding no effect (18, 27, 41, 90). One glaring deficit in the current literature on the ergogenic effect of protein/amino acid during prolonged exercise is the lack of a definitive mechanism by which protein improves performance. The combination of this deficiency and the equivocal performance findings has fueled debate about the legitimacy of protein/amino acid intake as an acute ergogenic strategy.

Numerous physiological mechanisms have been proposed to explain the amino acid mediated effect on performance. The most promising mechanism based on the current literature is increased energy provision from exogenous amino acids. Specifically, studies investigating protein co-ingestion with carbohydrate that have found positive results were compared to carbohydrate-matched treatments, not calorie-matched treatments (129). When the caloric differences between carbohydrate and carbohydrate-protein treatments were eliminated, performance gains have typically disappeared as well. Thus, it’s likely that the ergogenic effect found in these studies is at least partially derived from the additional energy provision of exogenous amino acids (140). Furthermore, an individual amino acid, alanine, can serve as a significant exogenous fuel source when supplemented during prolonged exercise (82). However, neither protein nor alanine has been supplemented at rates high enough to optimize a metabolic effect during prolonged exercise. Therefore, research utilizing protein and alanine intake rates
equivalent to those recommended for carbohydrate supplementation (30-60g/hr) are required to fully elucidate whether these supplements have an ergogenic value during prolonged exercise.

As exercise duration increases and muscle glycogen stores are utilized, IL-6 serves as an energy sensor and is released from working skeletal muscle (109). Carbohydrate supplementation attenuates this response (99). IL-6 plays a significant role in metabolism during prolonged exercise. Plasma IL-6 increases gluconeogenesis (6, 52, 147), free fatty acid oxidation (23), and skeletal muscle glucose uptake (60). In this way, IL-6 is capable of producing a favorable effect on metabolism during prolonged exercise. Also, glutamine supplementation has been found to augment the plasma IL-6 response to exercise (67). If alanine and/or protein supplementation are also capable of augmenting plasma IL-6 during prolonged exercise it can point to a favorable metabolic effect of these treatments.

The current project was designed to investigate the efficacy of large doses of both protein and alanine intake on prolonged cycling performance. Furthermore, the exercise-induced plasma IL-6 response to these two supplements will be determined.
Chapter Three

Methodology

Subjects

8 endurance-trained cyclists ($VO_{2\text{max}} > 50 \text{ ml/kg/min or } 4.5 \text{ L/min}$), from James Madison University and the surrounding area performed all testing. Prior to any testing, subjects were required to read and sign informed consent forms, which provided details describing the study, the risks and benefits of the study, and confidentiality of the study. All procedures were approved by the James Madison University Institutional Review Board.

Experimental Design

Subjects were asked to complete five total trials, each separated by 5-10 days. Specifically, subjects completed one pre-testing trial, one familiarization trial, and three experimental trials. During the three experimental trials, subjects ingested a whey protein hydrolysate solution, L-alanine solution, or a flavored placebo.

Preliminary Testing

Subjects reported to the Human Performance Laboratory where their height (nearest 0.5 cm) and weight (nearest 0.1 kg) was recorded. Their $VO_{2\text{max}}$ and associated power output ($W_{\text{max}}$) was assessed using a graded exercise test on a Racermate Velotron (Seattle, WA) cycle ergometer. Subjects cycled at a self-selected workload estimated as “a comfortable, but not easy pace for a 1-hour ride.” The workload was be increased by 25 watts every 2 minutes until subjects voluntarily requested to stop due to fatigue or were unable to continue at a cadence $>50$ rpms. Metabolic measurements were assessed throughout each stage of the test using a Moxus Modular Metabolic System (Bastrop, TX). Heart rate was continually assessed using a Suunto heart rate monitor (Vaanta, Finland). $VO_{2\text{max}}$ and $W_{\text{max}}$ were determined from data obtained during the test.
and used to establish intensities for subsequent exercise protocols and for participant inclusion as described above.

**Familiarization Trial**

Subjects performed the protocol that was used during the experimental trials while consuming water only (Figure 3.1).

**Experimental Trials**

Subjects completed three separate experimental trials on a RacerMate Velotron (Seattle, WA) cycle ergometer. Each trial consisted of two exercise phases. The first phase consisted of 120 minutes of steady-state cycling at 55% $W_{\text{max}}$ (determined during preliminary testing). The steady-state portion was immediately followed by a simulated 30-km time trial (TT; ~50 min.), as outlined in Figure 3.1.

All trials were conducted at ambient room temperature. Subjects were asked to void their bladders prior to all trials. An oscillating fan was utilized on “high” speed setting and placed two meters from the handlebars for uniform cooling during trials. Subjects were encouraged to treat the TT portion of each trial as a competitive event and provide a maximal effort. Subjects received no feedback regarding performance during the TT except for distance completed and distance remaining. Researchers provided no verbal encouragement during the TT.

**Treatments**

As outlined above, subjects performed three experimental trials. During these trials, subjects consumed three different solutions. Subjects received 45 g/hr of whey protein hydrolysate (PRO; American Casein Company, Burlington, NJ), 15 g/hr of L-alanine (ALA; Ajinomoto North America, Fort Lee, NJ), or a non-caloric artificially sweetened placebo (PLA), in a randomized double-blind placebo-controlled fashion. Each solution also contained
electrolytes (Sodium and Potassium) and artificial vanilla flavoring (Baker’s Imitation Vanilla; McCormick & Company, Sparks, MD). Immediately prior to each trial, subjects received 250 ml of an experimental beverage. Thereafter, subjects received 250 ml every 15 minutes during the steady state portion of the trial. Another 250 ml was provided at three time points during the 30 km TT (7.5, 15, and 22.5 km). Treatment timing is outlined in Figure 3.1.

Figure 3.1: Experimental Trial Protocol

![Experimental Trial Protocol Diagram]

**Performance and Physiological Data**

*Performance*

Exercise performance was measured using cycling time and mean power output (watts) for the pre-loaded simulated 30-km TT. Three subjects performed repeated pilot trials under placebo conditions (described above) and the coefficient of variation for 30-km TT performance was ~3%.

*Plasma Interleukin-6*

Venous blood samples (3 ml) were collected from an antecubital vein prior to exercise and following 120 min of exercise. Blood was centrifuged and serum samples stored at -80°C until analyzed. Concentrations of serum IL-6 were analyzed with a standard enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN).
Metabolic Measurements

Metabolic measurements assessed using a Moxus Modular Metabolic System (Bastrop, TX) at the following time points: minutes 15, 35, 55, 75, 95, and 115 of the 120-min steady state phase, and at 20-km into the 30-km TT. At each of these points, 5 minutes of expired gas collection was performed and an average of the last three minutes were recorded. Dependent measurements obtained/derived from expired gases included oxygen uptake ($VO_2$), ventilation rate ($VE$), and respiratory exchange ratio (RER).

Blood Glucose and Lactic Acid

Finger-stick blood samples (~0.5 ml) were obtained at the following time points: minutes 20, 40, 60, 80, 100, and 120 of the 120-min steady state phase, and at 20-km into the 30-km TT. Glucose and lactate levels was determined immediately from whole blood using automated instrumentation (YSI 2300 STAT glucose/lactate analyzer).

Heart Rate

Heart rate was recorded at the time-points above (finger-stick time points) using a Suunto heart rate monitor. In addition, average heart rate for the 30-km TT was recorded.

Ratings of Perceived Exertion (RPE)

Subjective ratings of exertion were obtained by having subjects point to a corresponding level of exertion (rated numerically from 6-20) on a Borg RPE scale. RPE was obtained at the time-points indicated above (finger-stick time points).

Gastrointestinal Distress Scale

Subjects were asked to complete a questionnaire (verbally) at minutes 30, 60, 90, and 120 of the 120-min steady state phase of the exercise trials and at 20 and 30-km of the 30-km TT. The
questionnaire contained queries about the presence of GI symptoms at that moment and addressed the following complaints: stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate or defecate. The items were scored on a 10-point scale (1 = not at all, 10 = very, very much). The severities of the GI symptoms were divided into two categories, severe and nonsevere symptoms. Symptoms were only registered as severe symptoms when a score of ≥5 out of 10 was reported. Scores of <5 were reported as nonsevere.

**Dietary and Exercise Controls**

Subjects were asked to: 1. Maintain consistent dietary habits for 72 hours prior to each trial, 2. Record food intake 24 hours prior to their first experimental trial, 3. Replicate their exact food intake for the 24 hours preceding each subsequent experimental trial, 4. Refrain from heavy and unaccustomed exercise for 48 hours prior to each experimental trial, 5. Maintain consistent exercise habits between trials and record all physical activity performed during the 72 hours preceding each experimental trial, and 6. Abstain from alcohol and caffeine for 24 hours and 12 hours prior to the experimental trials, respectively. Subjects performed all trials in the fed state. Additionally, subjects consumed a liquid meal replacement (Ensure® Shakes; 20-25% of daily caloric intake) in the evening prior to each trial (7-9 hours prior). Two hours prior to all experimental trials, subjects consumed a standardized meal consisting of ~500 kcals.

**Statistical Analyses**

Treatment effects on 30-km TT performance, VO2, VE, HR, RPE, blood glucose, and blood lactate were analyzed with a repeated-measures ANOVA, with treatment as the within subject factor. Pairwise comparisons were performed using paired t-tests with a Bonferroni correction. Friedman Tests were applied to the following variables that were not normally distributed: GI distress symptoms, blood glucose (min 120), VE (20-km TT), VO2 (min 120), and
RPE (min 20). The serum IL-6 response to exercise (pre vs. post- 120-min FI), for each treatment, was analyzed with Wilcoxon Signed Ranks tests. Significance was set at P < 0.05. All data are presented as means ± SE.
Chapter Four

Manuscript
Cycling Time Trial Performance is Not Enhanced by
Either Whey Protein or L-Alanine Intake During Prolonged Exercise

Authors: Adam B. Schroer, Michael J. Saunders, Daniel A. Baur, Christopher J. Womack, and Nicholas D. Luden

Institution: James Madison University, Harrisonburg VA, 22807

Contacts: Adam Schroer, schroeab@jmu.edu
Michael Saunders, saundemj@jmu.edu
Daniel Baur, baurda@jmu.edu
Christopher Womack, womackcx@jmu.edu

Address of Correspondence:
Nicholas D. Luden, Ph.D.
Department of Kinesiology
James Madison University
Harrisonburg VA, 22807
Phone: (540) 568-4069
E-mail: ludennd@jmu.edu
ABSTRACT

Previous studies have reported that adding protein (PRO) to a carbohydrate (CHO) solution can enhance endurance performance. This ergogenic effect may be a function of additional calories from supplemental protein/amino acids, but this thesis has not been directly examined. Additionally, L-alanine (ALA) is readily oxidized when provided during exercise; the impact that this has on metabolism and prolonged endurance performance is unknown. The purpose of this investigation was to assess performance and various cardiovascular and metabolic outcomes during prolonged cycling, to independently gauge the efficacy of whey PRO hydrolysate and L-alanine (ALA) supplementation. Eight trained male and female cyclists (age: 22.3±2.0 years, weight: 70.0±2.8 kg, VO\textsubscript{2max}: 59.4±1.7 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) performed 120 min of constant-load cycling (55% of peak power, 161.9±7.4 W) followed by a 30-km time trial (TT) under placebo (PLA), PRO, and ALA conditions. TT performance was not different between treatments (PLA: 57.6±1.6 min, ALA: 58.8±1.5 min, PRO: 58.8±1.8 min). VE, VO\textsubscript{2}, heart rate, rating of perceived exertion, blood glucose, blood lactate, and gastrointestinal distress were also similar across experimental conditions. Conversely, serum interleukin-6 (IL-6) levels following 120 min of cycling were elevated above rest with PLA (pre: 0.56±0.16, post: 3.14±0.84) and ALA (pre: 1.14±0.46, post: 2.62±0.71) (p<0.05), but not with PRO intake (pre: 0.81±0.25, post: 1.55±0.32). The ingestion of PRO or ALA alone does not appear to enhance performance during prolonged cycling. Thus the ergogenic effects of CHO+PRO co-ingestion reported by others are likely not the result of additional energy from protein \textit{per se}. 
INTRODUCTION

The capacity for carbohydrate (CHO) intake to enhance endurance performance has been widely recognized since early work demonstrated that CHO provision preserves blood glucose levels, carbohydrate oxidation rates, and consequently improves performance (Coyle et al. 1983). The ergogenic potential of protein (PRO) has only recently been addressed. Initial studies indicated that adding small doses of PRO (~9-12 g·hr⁻¹) to standard CHO treatments (30-60 g·hr⁻¹) enhanced performance in time-to-fatigue trials to a large extent (29-36%) (Ivy et al. 2003; Saunders et al. 2004). Subsequent research has been mixed, as PRO co-ingestion with CHO was beneficial in some studies (Ferguson-Stegall et al. 2010; Ghosh et al. 2010; Saunders et al. 2007; Saunders et al. 2009), but not others (Breen et al. 2010; Osterberg et al. 2008; Romano-Ely et al. 2006; Valentine et al. 2008; van Essen and Gibala 2006). Importantly, recent meta-analyses have indicated a probable ergogenic benefit when PRO is provided with CHO (Stearns et al. 2010; Vandenbogaerde and Hopkins 2011).

Although adding PRO to a CHO solution can benefit endurance performance under certain conditions, a major limitation of the current literature is the lack of a mechanistic explanation for how PRO may facilitate this response. The benefits of CHO+PRO are most prominent when tested against CHO-matched formulas (Ivy et al. 2003; Saunders et al. 2007; Saunders et al. 2009; Saunders et al. 2004), prompting speculation that the ergogenic effect of CHO+PRO may simply be a function of supplemental calories. This is a logical thesis but it awaits confirmation, as the effects of ingesting PRO calories in isolation have not been examined. Moreover, the potential interactive properties of CHO and PRO (Rowlands et al. 2012a; Wapnir et al. 1997) invites the possibility that PRO may influence performance only when in the presence of exogenous CHO. This study was therefore conceived to provide insight into a possible mechanism through which PRO alters endurance performance. Specifically, the current project was designed to evaluate whether or not a moderate dose (45 g·hr⁻¹) of whey PRO hydrolysate delivered during prolonged cycling influences time trial (TT) performance.
Using this same experimental framework, a separate purpose of the study was to evaluate the ergogenic effect of ingesting L-alanine (ALA), an amino acid constituent of whey PRO (and many other PRO sources). ALA is the principle amino acid extracted by the liver for gluconeogenesis, and is oxidized/decarboxylated during endurance exercise (Felig et al. 1970; White and Brooks 1981). Exogenous ALA is readily oxidized (51 of 73 g ingested) during prolonged exercise (Korach-André et al. 2002). Further, when provided at ~25 g·hr⁻¹, ALA is oxidized (0.35 g·min⁻¹) at rates similar to CHO (0.43 g·min⁻¹) (Korach-André et al. 2002; Peronnet et al. 1990). Thus it is theoretically possible for ALA to enhance endurance performance through mechanisms related to CHO metabolism (i.e. conserving liver and/or muscle glycogen). Although ALA supplementation does not appear to improve performance lasting ~60 min (Klein et al. 2009), the potential for the metabolic effect of ALA to enhance prolonged exercise performance has not been investigated.

We were also interested in gaining insight into the impact that PRO and/or ALA intake may have on serum interleukin-6 (IL-6) levels. IL-6 is a myokine released from active skeletal muscle that is capable of impacting metabolism (Pedersen et al. 2007). For instance, IL-6 enhances hepatic amino acid uptake and gluconeogenic gene expression (PEPCK), thereby promoting gluconeogenesis (Banzet et al. 2009; Fischer et al. 1996; Watkins et al. 1994). The magnitude of the IL-6 response to exercise is partially mediated by metabolic conditions; muscle glycogen depletion potentiates the plasma IL-6 response to exercise (Keller et al. 2001; MacDonald et al. 2003; Steensberg et al. 2001), whereas CHO supplementation attenuates plasma IL-6 levels (Febbraio et al. 2003; Nehlsen-Cannarella and Fagoaga 1997). Therefore, IL-6 levels were measured to provide general information about how PRO and ALA may influence the metabolic environment during prolonged exercise.
MATERIALS AND METHODS

Subjects

Ten healthy endurance trained cyclists (VO\textsubscript{2max} >50 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) from James Madison University and the Harrisonburg area volunteered to participate in this study. Two subjects withdrew prior to completion because of circumstances unrelated to the study, resulting in complete data from eight subjects (four males and four females). Subjects were provided written and oral information about experimental procedures and potential risks prior to giving informed consent. All procedures were approved by the James Madison University Institutional Review Board prior to any testing. Subject characteristics are displayed in Table 1.

Cardiorespiratory Fitness

Subjects performed an incremental exercise test to exhaustion on a bicycle ergometer (Velotron, Racermate, Inc., Seattle, WA, USA) to determine VO\textsubscript{2max}. Subjects began the test at a self-selected workload estimated as “a comfortable, but not easy pace for a 60-min ride.” Power was then increased by 25 W every two-min until the subject reached volitional exhaustion. Metabolic measurements were assessed throughout each stage of the test using a Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, PA, USA). VO\textsubscript{2max} was determined by the highest 30-sec mean oxygen uptake value. Peak power at VO\textsubscript{2max} (W\textsubscript{max}) was defined by the power corresponding to the final successful stage, and was used to prescribe workload for the 120-min constant-load segment of subsequent trials.

Exercise Trials

Subjects completed four trials (one familiarization trial followed by three experimental trials) on the aforementioned cycle ergometer. Trials consisted of 120-min of constant-load cycling at 55% W\textsubscript{max} (162 ± 21 W) followed by a simulated 30-km TT, each separated by 5-14 days. The familiarization trial was identical to the experimental trials (see below), except that no
blood samples were obtained, and subjects received only water while cycling. Subjects were asked to void their bladder prior to all trials. A pedestal fan was placed ~2 m from the handlebars and utilized on high speed setting for uniform cooling during each trial. Subjects were encouraged to treat the TT portion of each trial as a competitive event and provide a maximal effort. Subjects did not receive performance feedback during the TT other than elapsed- and remaining distance, and no verbal encouragement was provided during the TT.

Treatments

We implemented a randomly-counterbalanced, double-blind, placebo-controlled study that was designed to compare the effects of three separate treatment conditions on performance, cardiovascular and metabolic physiology, and serum IL-6. The treatments were: 1) PRO: 45 g·L⁻¹ of whey protein hydrolysate (American Casein Company, Burlington, NJ, USA), 2) ALA: 15 g·L⁻¹ of L-alanine (Ajinomoto North America, Fort Lee, NJ, USA), and 3) PLA: non-caloric artificially sweetened placebo (Splenda, Fort Washington, PA, USA). Each solution also contained 470 mg·L⁻¹ sodium chloride (Morton Salt, Chicago, IL, USA), 200 mg·L⁻¹ potassium chloride (NOW Foods, Bloomingdale, IL, USA), and 4.1 g·L⁻¹ artificial vanilla flavoring (Baker’s Imitation Vanilla; McCormick & Company, Sparks, MD, USA). Immediately prior to each trial, subjects received 250 ml of treatment beverage. Thereafter, subjects received a 250 ml feeding every 15 minutes during the constant-load portion of the trial, and at three points during the TT (7.5, 15, and 22.5 km). Although optimal rates of exogenous PRO provision are unknown, previous work has examined PRO co-ingestion at rates ranging from ~5-20 g·hr⁻¹, rates that are particularly low when compared to those recommended for CHO (30-60 g·hr⁻¹). This invites the possibility that higher doses of PRO are required to optimize the ergogenic effect of PRO. Therefore, a PRO ingestion rate of 45 g·hr⁻¹ was selected as it falls within the range of recommended carbohydrate delivery rates (30-60 g·hr⁻¹) during prolonged exercise (Coyle 2004). Previous research investigating amino acid oxidation rates utilized an ALA delivery rate of ~25
g·hr⁻¹ (Klein et al. 2009). However, ALA was delivered at 15 g·hr⁻¹ in the present study, because higher doses were not well-tolerated during pilot testing.

### 30-km TT Performance

Finishing time and mean power output (watts) during the 30-km TT were used as performance criteria. We have previously assessed the reproducibility of cycling time/power measurements using identical equipment in our laboratory. Using a similar performance trial (20-km of cycling over a simulated hilly course), the coefficient of variation (CV) between repeated trials (under placebo conditions, following a familiarization trial) was 1.4% for time, and 2.6% for power output (Goh et al. 2012). Further, we obtained repeatability data for this protocol from three pilot subjects under placebo conditions and the CV for 30-km TT performance was ~3% for time.

### Metabolic Measurements

Oxygen uptake (VO₂), expired ventilation (VE) and respiratory exchange ratio (RER) were assessed using a Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, PA, USA) at the following time points: 15-20 min and 115-120 min of constant-load cycling, and at 20-km of the TT. These time points were selected to correspond with early exercise, late exercise, and the TT. Aggregates of the final three minutes of each phase were recorded.

### Heart Rate and Rating of Perceived Exertion

HR (Suunto, Vaanta, Finland) and rating of perceived exertion (RPE; 6-20 Borg Scale) were recorded at 20- and 120 min of constant-load cycling, and at 20-km of the TT.
Blood Glucose and Lactate

Finger-stick blood samples (~0.5 ml) were obtained at the following time points: 20- and 120 min of constant-load cycling, and at 20-km of the TT. Glucose and lactate levels were determined immediately from whole blood using automated instrumentation (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, OH, USA).

Gastrointestinal Distress Scale

Subjects verbally indicated their perceived level of gastrointestinal (GI) distress at 30- and 120 min of constant-load cycling and at 20- and 30-km of the TT. Utilizing a 10-point scale (1 = not at all, 10 = very, very much), subjects rated the following symptoms: stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate or defecate (Jentjens et al. 2002).

Serum Interleukin-6

Venous blood samples (~3 ml) were collected from an antecubital vein prior to exercise and following 120 min of constant-load exercise (~3 min post-exercise). All samples were obtained under standardized postural conditions (reclined in a chair). Blood was allowed to clot for 30 min prior to being centrifuged at 3 000 rpm for 10 min. Serum samples were stored at -80°C until analyzed. Quantikine high sensitivity enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) were used to measure IL-6 in serum, according to manufacturer’s instructions.

Dietary and Exercise Controls

Subjects were instructed to: 1) Practice consistent dietary habits for 72 hours prior to each trial, 2) Record food intake 24 hours prior to their first experimental trial and replicate their exact food intake for the 24 hours preceding each subsequent experimental trial, 3) Refrain from heavy
and/or unaccustomed exercise for 48 hours prior to each experimental trial, 4) Maintain consistent exercise habits between trials and record all physical activity performed during the 72 hours preceding each experimental trial, and 5) Abstain from alcohol and caffeine for 24 hours and 12 hours prior to the experimental trials, respectively. Subjects performed all trials in the fed state. Specifically, subjects consumed 20-25% of their estimated daily caloric expenditure (Harris-Benedict equation) in the form of a liquid meal replacement (Ensure® Shakes, Abbott Laboratories, Abbott Park, IL, USA) in the evening prior to each trial (immediately prior to sleep). Two hours prior to all exercise trials, subjects consumed a standardized meal consisting of ~500 kcals (cereal with milk, orange juice, and strawberry yogurt).

Statistical Analyses

Data that displayed normality (RER, HR, and lactate) were analyzed with 2 x 3 repeated-measures ANOVAs, with time (early and late constant-load exercise) and treatment (PLA, ALA, and PRO) as within-subject factors. The effects of treatment on 30-km TT performance and RER, HR, and lactate during the TT were analyzed with repeated-measures ANOVAs (treatment as within-subject factor). Wilcoxon Signed Ranks tests were applied to examine the effects of constant-load exercise (treatments were aggregated within each time point) and Friedman Tests were performed to determine the effects of treatment (within each time point) on the following variables that were not normally distributed: GI distress symptoms, blood glucose, VE, VO2, and RPE. The serum IL-6 response to exercise (pre vs. post- 120-min constant-load), for each treatment, was analyzed with Wilcoxon Signed Ranks tests. Significance was set at P < 0.05. All data are presented as means ± SE.
RESULTS

30-km Time Trial Performance

Time trial performance was not influenced by treatment (Figure 1). Performance times were: PLA, 57.6 ± 1.6 min; ALA, 58.8 ± 1.5 min; PRO, 58.8 ± 1.7 min. Likewise, average power output during the TT was similar across treatments: PLA, 175 ± 14 W; ALA, 166 ± 11 W; and PRO, 167 ± 13 W.

VO$_2$, VE, RER, HR, and RPE

VE, RER, HR, and RPE during exercise are displayed in Table 2. No treatment differences were observed for any variable during the TT. Likewise, there was no treatment effect for RER or HR during constant-load cycling, although there was a main time effect. Specifically, RER was diminished and HR elevated at min 120 compared to min 20 of constant-load cycling (p<0.05). Similarly, VE, VO$_2$, and RPE were not influenced by treatment during constant-load cycling, but all three variables were elevated at min 120 compared to min 20 of constant-load cycling (p<0.05).

Blood Glucose and Lactate

Blood glucose and lactate data are displayed in Table 3. No treatment differences were observed for either variable during the TT. Likewise, there was no time or treatment effect for blood lactate during constant-load cycling. Similarly, blood glucose was not influenced by treatment or time during constant-load cycling.

GI Distress Symptoms

GI distress symptoms were not different between treatments at any time point. With the exception of the symptom urge to urinate, all subjective GI ratings were non-serious (≤ 4) during constant-load cycling, indicating no GI distress at these time points. Four subjects experienced
serious nausea symptoms at the end of the 30-km TT (0, 1, 3 instances in PLA, ALA, and PRO, respectively), and two of these subjects also reported serious symptoms at 20-km (2 instances in PRO). Individual ratings of nausea during the TT are presented in Table 4. Average ratings of nausea symptoms at 30-km were: PLA, 1.25 ± 0.25; ALA, 2.00 ± 0.68; PRO, 3.13 ± 0.83. Finally, with the exception of an increase in the ‘urge to urinate’ (p<0.05), GI distress symptoms were similar between min 20 and min 120 of constant-load cycling.

**Serum Interluekin-6**

Post-exercise IL-6 levels were elevated above rest with PLA (rest: 0.56 ± 0.16 pg·ml⁻¹, post-exercise: 3.14 ± 0.84 pg·ml⁻¹) and ALA (rest: 1.14 ± 0.46, post-exercise: 2.62 ± 0.71) (P < 0.05), but not PRO (rest: 0.81 ± 0.25, post-exercise: 1.55 ± 0.32). Resting and post-exercise concentrations are displayed in Figure 2.
DISCUSSION

We assessed 30-km cycling TT performance and various cardiovascular and metabolic outcomes during prolonged cycling to gauge the efficacy of PRO and ALA supplementation. Most notably, 30-km TT performance was not affected by either PRO or ALA intake. This was affirmed by the global physiological response, as cardiovascular and metabolic parameters were also similar across experimental conditions. The only notable disparity among treatments was that IL-6 levels increased with both PLA and ALA, but not PRO. This is the first evidence that PRO feedings can attenuate the IL-6 response to prolonged exercise, albeit not in a manner that altered substrate utilization, blood glucose levels, or performance.

Previous studies have found the functional worth of CHO+PRO supplementation during endurance exercise to be most obvious when compared to CHO-matched formulas (Ivy et al. 2003; Saunders et al. 2007; Saunders et al. 2009; Saunders et al. 2004), suggesting that the gains in performance are simply a function of additional energy (calories). We tested this inference by evaluating the isolated effects of PRO on performance (i.e. in the absence of CHO). In an attempt to maximize the potential ergogenic effect, a markedly higher dose of PRO (45 g·hr\(^{-1}\)) was provided relative to previous investigations (6-20 g·hr\(^{-1}\); Breen et al. 2010; Ivy et al. 2003; Martinez-Lagunas et al. 2010; Osterberg et al. 2008; Romano-Ely et al. 2006; Saunders et al. 2009; Saunders et al. 2004; Valentine et al. 2008; van Essen and Gibala 2006). One inherent risk of higher dosing is impaired gastric emptying (Hunt and Stubbs 1975) and consequential gastrointestinal (GI) distress (Rehrer et al. 1989), which could potentially mask an otherwise beneficial effect on performance (Rowlands et al. 2012b). While GI symptoms were not statistically different between treatments in the current study, three of the eight subjects experienced severe nausea (≥ 5 on 10 point scale) immediately following the 30-km TT under PRO conditions. However, among the five subjects that did not experience GI distress with PRO intake, only one completed the TT faster in the PLA condition. Thus, it is likely that the current data accurately reflect the inability of PRO to enhance endurance performance.
These findings indicate that previously documented improvements in performance cannot be explained by additional PRO calories *per se*, but rather may be related to ‘non-caloric’ mechanisms of PRO. For example, PRO consumption may increase intestinal absorption rates of sodium, water, and glucose. Neutral amino acids are co-transported with sodium across the intestines via glucose independent transporters (Coëffier et al. 2005). This additional sodium absorption across the gut may facilitate better transport of fluid and CHO via solvent drag (Rowlands et al. 2012a; Van Loon et al. 1996; Wapnir et al. 1988; Wapnir et al. 1997). Therefore, the potential exists for PRO co-ingestion to support the effects of CHO-based beverages on performance. More specific to the current investigation, this dynamic is heavily influenced by fluid osmolarity such that high amino acid concentrations cease to aid sodium and fluid absorption (Wapnir and Lifshitz 1985; Wapnir et al. 1988; Wapnir et al. 1997). Considering the relatively high PRO dose used in the current study, it is likely that fluid and sodium absorption were not altered, which is consistent with the lack of performance and physiological differences between treatments.

In the current study serum IL-6 levels were elevated following 120 minutes of constant-load cycling with PLA and ALA treatments, but were unchanged with PRO. This is the first evidence that IL-6 levels are attenuated by PRO intake during prolonged exercise. Muscle glycogen depletion has been shown to potentiate the release of IL-6 from the muscle during exercise (Keller et al. 2001; MacDonald et al. 2003; Steensberg et al. 2001). Conversely, CHO ingestion diminishes the release of IL-6 from the muscle during prolonged exercise, likely due to the preservation of endogenous CHO stores (Febbraio et al. 2003; Nehlsen-Cannarella and Fagoaga 1997; Nieman et al. 1998; Starkie et al. 2001). In view of these results, it is tempting to speculate that PRO increased muscle energy provision and attenuated endogenous CHO depletion. However, endogenous CHO preservation typically results in higher levels of CHO oxidation (and RER) late in exercise, which can benefit TT performance (Bergström and Hultman 1967; Coyle et al. 1986; Coyle et al. 1983; Hargreaves et al. 1984). Because PRO did not
influence either of these variables (RER or performance), it is unlikely that endogenous CHO utilization was attenuated.

Another possible explanation for the attenuation of IL-6 is related to muscle damage. The IL-6 induction with exercise appears to be partially evoked by muscle damage due to its role as an inflammatory cytokine (Bruunsgaard et al. 1997), although it appears to play a secondary role to energetics (Croisier et al. 1999; Pedersen 2012; Toft et al. 2002). The relatively subtle rise in serum IL-6 levels with the PLA and ALA treatments (2.58 and 1.49 pg·ml⁻¹, respectively) was of a magnitude that could be credited to muscle damage, rather than a response to limited energy availability (Toft et al. 2002). Further, PRO co-ingestion has commonly attenuated creatine kinase and myoglobin levels, indirect markers of muscle damage, following prolonged exercise (Thomson et al. 2011; Valentine et al. 2008). Therefore, it is possible that an attenuation of muscle damage generated by PRO intake inhibited the IL-6 response to exercise.

Similar to PRO, ALA supplementation failed to improve TT performance. This extends previous work demonstrating that performance in a relatively short duration time trial (~60 min) was unaffected by ALA provision (Klein et al. 2009). Exogenous ALA is oxidized nearly as efficiently as CHO during exercise when ingested at 25 g·hr⁻¹ (Korach-André et al. 2002; Peronnet et al. 1990), making ALA an ideal model to determine whether or not nitrogenous sources are capable of affecting performance through the provision of supplemental calories. However, it appears that any ALA oxidation that occurred in the current study was insufficient to impact prolonged exercise performance. It remains possible that higher doses of ALA are required to exhibit a metabolic effect. However, larger ALA doses elicited severe GI discomfort during pilot testing, as well as a clear ergolytic effect. Therefore, ALA rates were reduced to 15 g·hr⁻¹ to alleviate these symptoms. Nonetheless, the lack of a performance effect likely was not the result of inadequate energy provision per se, as CHO supplementation has been shown to improve performance with ingestion rates of 15 g·hr⁻¹ (Smith et al. 2010). Altogether, this
provides further evidence that nitrogenous protein sources do not impart an ergogenic effect through caloric means.

Neither PRO nor ALA affected VO$_2$, RER, HR, RPE, blood glucose, or blood lactate at any time point. The lack of a physiological response to PRO intake aligns with the preponderance of relevant work examining CHO+PRO ingestion (Ivy et al. 2003; Saunders et al. 2007; Saunders et al. 2004; Valentine et al. 2008). However, there is recent evidence that the presence of PRO (with CHO) can favorably impact HR and/or RPE during exercise (Hall et al. 2013; Martinez-Lagunas et al. 2010; McCleave et al. 2011). As stated previously, low concentrations of amino acids are capable of augmenting intestinal absorption of sodium and fluid (Van Loon et al. 1996; Wapnir et al. 1988; Wapnir et al. 1997). This implies that PRO and/or ALA inclusion may better preserve blood volume and stroke volume during prolonged exercise, which would subsequently maintain HR late in exercise (Ekelund 1967). Perceived exertion during endurance activity is commonly linked to HR (Borg and Linderholm 1967) and can potentially explain lower RPE with CHO+PRO in these past studies (Hall et al. 2013; Martinez-Lagunas et al. 2010). Our data suggests that PRO/ALA alone do not influence these variables. However, as previously noted, the PRO intake rate in this study may have been too high to generate increased fluid absorption rates (Wapnir et al. 1988; Wapnir et al. 1997), negating this potential mechanism. No studies are available to either confirm or refute the conclusion that ALA consumption does not impact these cardiovascular and metabolic variables. If ALA had provided a critical amount of exogenous energy in this study, akin to CHO intake, RER and blood glucose would have likely been elevated compared to PLA (Coyle et al. 1986). However, this was not observed, agreeing with the lack of performance improvement from the ALA treatment.

The present study demonstrates that prolonged TT cycling performance and various cardiovascular and metabolic parameters are not impacted by either 45 g·hr$^{-1}$ of whey PRO hydrolysate or 15 g·hr$^{-1}$ of ALA. Although ALA can be efficiently oxidized during exercise, ALA supplementation did not impact performance in the current investigation. Higher doses of ALA
may be required to elicit meaningful metabolic effects but do not appear to be well-tolerated by the GI system. Also, the similar performance times between PRO and PLA suggest that the ergogenic effects of CHO+PRO co-ingestion reported by others are not the result of additional PRO calories *per se*. Rather, it seems that the mechanism through which the inclusion of PRO enhances performance requires the interaction of the two macronutrients (i.e. exogenous CHO oxidation) and/or lower rates of PRO intake.
Table 4.1: Subject Characteristics

<table>
<thead>
<tr>
<th>Subjects, n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>VO$_{2\text{max}}$, ml·kg$^{-1}$·min$^{-1}$</th>
<th>W$_{\text{max}}$, Watts</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>22.3 ± 2.0</td>
<td>173.1 ± 2.6</td>
<td>70.0 ± 2.8</td>
<td>59.4 ± 1.7</td>
<td>294 ± 14</td>
</tr>
</tbody>
</table>

Note: Values are expressed as means ± SE. W$_{\text{max}}$, peak power at VO$_{2\text{max}}$
<table>
<thead>
<tr>
<th></th>
<th>20 min</th>
<th>120 min</th>
<th>20-km</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO$_2$ (L·min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>2.55 ± 0.10</td>
<td>2.71 ± 0.11</td>
<td>2.83 ± 0.18</td>
</tr>
<tr>
<td>ALA</td>
<td>2.49 ± 0.11</td>
<td>2.68 ± 0.10</td>
<td>2.80 ± 0.18</td>
</tr>
<tr>
<td>PRO</td>
<td>2.48 ± 0.09</td>
<td>2.74 ± 0.10</td>
<td>2.78 ± 0.17</td>
</tr>
<tr>
<td><strong>VE (L·min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>59.6 ± 2.0</td>
<td>63.2 ± 2.2</td>
<td>72.0 ± 6.6</td>
</tr>
<tr>
<td>ALA</td>
<td>59.4 ± 2.4</td>
<td>63.2 ± 2.4</td>
<td>70.0 ± 5.9</td>
</tr>
<tr>
<td>PRO</td>
<td>58.8 ± 2.6</td>
<td>64.4 ± 2.9</td>
<td>72.4 ± 7.4</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>0.89 ± 0.01</td>
<td>0.83 ± 0.01</td>
<td>0.82 ± 0.01</td>
</tr>
<tr>
<td>ALA</td>
<td>0.90 ± 0.02</td>
<td>0.84 ± 0.01</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>PRO</td>
<td>0.90 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td><strong>HR (b·min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>137 ± 5</td>
<td>144 ± 5</td>
<td>153 ± 6</td>
</tr>
<tr>
<td>ALA</td>
<td>133 ± 3</td>
<td>141 ± 2</td>
<td>150 ± 4</td>
</tr>
<tr>
<td>PRO</td>
<td>140 ± 5</td>
<td>146 ± 5</td>
<td>152 ± 5</td>
</tr>
<tr>
<td><strong>RPE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>12.4 ± 0.3</td>
<td>13.4 ± 0.5</td>
<td>15.8 ± 0.6</td>
</tr>
<tr>
<td>ALA</td>
<td>12.1 ± 0.2</td>
<td>13.3 ± 0.5</td>
<td>16.0 ± 0.6</td>
</tr>
<tr>
<td>PRO</td>
<td>11.9 ± 0.2</td>
<td>13.1 ± 0.5</td>
<td>15.8 ± 0.3</td>
</tr>
</tbody>
</table>

**Note:** Values are expressed as means ± SE. VO$_2$, oxygen consumption; VE, pulmonary ventilation; RER, respiratory exchange ratio; HR, heart rate; RPE, rating of perceived exertion; PLA, placebo; ALA, L-alanine; PRO, whey protein hydrolysate.

All variables (VO$_2$, VE, RER, HR, and RPE) were different (p<0.05) at min 120 compared to min 20 of constant-load cycling.
Table 4.3: Blood glucose and lactate at 20 and 120 min of constant-load cycling (55% $W_{\text{max}}$), and at 20-km during the time trial

<table>
<thead>
<tr>
<th></th>
<th>20 min</th>
<th>120 min</th>
<th>20-km</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BG (mg·dL$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>73.7 ± 2.4</td>
<td>75.7 ± 2.3</td>
<td>72.9 ± 2.8</td>
</tr>
<tr>
<td>ALA</td>
<td>74.4 ± 3.2</td>
<td>72.6 ± 1.4</td>
<td>69.5 ± 2.0</td>
</tr>
<tr>
<td>PRO</td>
<td>68.2 ± 2.0</td>
<td>71.6 ± 0.6</td>
<td>72.3 ± 1.9</td>
</tr>
<tr>
<td><strong>BLa (mmol·L$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>ALA</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>PRO</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

Note: Values are expressed as means ± SE. BG, blood glucose; BLa, blood lactate; PLA, placebo; ALA, L-alanine; PRO, whey protein hydrolysate.
Table 4.4: Individual ratings of nausea at 20 and 30-km of time trial

<table>
<thead>
<tr>
<th>Subject</th>
<th>20-km PLA</th>
<th>20-km ALA</th>
<th>20-km PRO</th>
<th>30-km PLA</th>
<th>30-km ALA</th>
<th>30-km PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: PLA, placebo; ALA, L-alanine; PRO, whey protein hydrolysate.
Figure 4.1: 30-km time trial performance with PLA, ALA, and PRO treatments. Values are expressed as means ± SE.
Figure 4.2: Concentrations of IL-6 in arterial serum before and following 120 minutes of constant-load cycling with PLA, ALA, and PRO treatments. Values are expressed at means ± SE. *P<0.05 compared to Rest.
Subjects Wanted for Cycling Study

The Human Performance Laboratory at JMU will be conducting a study examining the effects of ingesting different beverages on cycling performance.

Who Are We Looking For?

- 18-45 years old
- Experienced cyclists (performing cycling exercise on a regular basis)

What Will You Be Asked to Do?

- Complete preliminary fitness testing/screening
- Participate in four exercise protocols, each of which will consist of 3 hrs of cycling on a computerized bicycle ergometer. Beverages will be provided during each session
- Receive laboratory assessments (including blood sampling) during each session
- Each of the four exercise protocols above will be separated by 5-10 days

What are the benefits of participation?

- Free evaluation of aerobic capacity (VO₂peak) and physiological data from a race simulation
- $75 for study completion

For more information, please contact Dr. Nick Luden at ludennd@jmu.edu and (540) 568-4069, or Adam Schroer at schroeab@dukes.jmu.edu and (812) 239-4356.
Purpose

You are being asked to volunteer for a research project conducted by Dr. Nick Luden, Dr. Mike Saunders, Adam Schroer, and Dan Baur from James Madison University titled “Effect of Both Protein and Alanine Consumption on Performance and the Plasma Interleukin-6 Response during Prolonged Cycling”.

The primary goal of this study is to determine the effect of sports beverages with differing amounts of nutrients (carbohydrate, fat, and protein) on cycling performance and the physiological response to prolonged cycling.

Experimental Procedures

You will be asked to report to James Madison University’s Human Performance Laboratory (Godwin 209) on five occasions. Specifically, you will be asked to participate in one preliminary trial, one familiarization trial, and three experimental exercise trials, each separated by 5 to 10 days. The preliminary trial will require approximately 45 minutes, whereas the familiarization trial and each experimental exercise trial will require approximately 3 hrs each, for a total time commitment of 12 hrs 45 min. Detailed information for each of these trials is provided below:

Preliminary Trial – Visit 1 – 45 minutes

Prior to any data collection, you will be asked to complete health history and screening questionnaires to ensure that you meet the study criteria and that you do not have any risk factors that would prevent you from performing heavy exercise. In the process of completing these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and body weight and you will then be asked to perform a cardiovascular fitness test on a bicycle ergometer to determine your peak oxygen consumption (VO₂peak). At the beginning of the test, you will be asked to ride the stationary bicycle ergometer at an initial workload that is ‘fairly easy’. The workload will be increased every two minutes during the test. You will be encouraged to continue to cycle until you request to stop due to fatigue or are unable to continue at a cadence of >50 revolutions per minute.

Familiarization Trial and Experimental Exercise Trials – Visits 2, 3, 4, and 5 – 3 hrs each

You will be asked to complete each trial on a stationary bicycle ergometer. Each trial will consist of two distinct exercise phases. Specifically, each trial will include an initial steady-state segment of 120 min at 55% W_max (‘moderate intensity’). The steady-state ride will be immediately followed by a simulated 30-km time trial (~60 minutes). Total exercise time will be approximately 3 hrs. During each of these five trials, you will receive water or a protein sports beverage at various time-points. Each of the trials will
be separated by a minimum of 5-10 days. A different beverage will be provided during each trial. You will consume the beverages according to the following schedule:

- 250 ml of the assigned beverage will be provided in a bottle immediately prior to exercise.
- 250 ml will be provided every 15 min during the steady state portion of the trial.
- 250 ml will also be provided at 7.5, 15, and 22.5 km of the time trial.

You will be instructed to consume the beverages within 5 minutes during exercise. The beverages will have slightly different ingredients during each trial (see Study Treatments below).

You will be asked to void your bladder prior to each trial. You will also be encouraged to treat all time trials as a competitive event, and provide a maximal effort. You will receive no feedback regarding performance during the time-trials, except for the distance completed and distance remaining in the trial. The researchers will not provide any verbal encouragement.

You will be asked to complete the following procedures during each protocol:

**Exercise Performance**
Performance will be measured by your finishing times (and average power output) in the 30-km time trial. Again, these are to be approached as competitions.

**Metabolic Measurements**
Metabolic measurements such as oxygen uptake, ventilation, etc. will be measured with a metabolic cart at the following time points: minutes 15, 35, 55, 75, 95, and 115 of the 120-min steady state phase, and at 20-km into the 30-km time trial. At each of these points, 5 minutes of expired gas will be collected. To do this, you will be asked to breathe through a mouthpiece/breathing apparatus that collected your expired breath. This apparatus will NOT be worn during exercise other than at the indicated time points.

**Blood Glucose and Lactic Acid**
A total of 8 finger-stick blood samples (~0.5 ml) will be obtained at the following time points: minutes 20, 40, 60, 80, 100, and 120 of the 120-min steady state phase, and at 20-km into the 30-km time trial. Each of these samples will be obtained by puncturing your fingertip with a small lancet. A very small amount of blood (~2 drops) will be collected at each time point.

**Heart Rate**
You will be asked to wear a heart rate monitor around your chest. Heart rate will be monitored throughout each exercise session.

**Ratings of Perceived Exertion (RPE)**
You will be asked to provide subjective ratings of your exertion level. You will do this by pointing to a corresponding level of exertion (rated numerically from 6-20) on a Borg RPE scale.

**Gastrointestinal Distress Scale**
You will be asked to complete a questionnaire (verbally) at several points throughout the exercise session. The questionnaire contains questions regarding the presence of GI problems at that moment and addresses the following complaints: stomach problems, GI
cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate or defecate.

**Plasma IL-6**

Blood samples (~3 ml) will be obtained via venipuncture from the antecubital vein prior to exercise and after 120 min of exercise. Blood samples will be collected while you are in the seated position.

**Dietary and Exercise Controls**

You will be asked to complete a diet record for the 24-hr preceding each treatment trial. You will also be asked to bring your initial diet record with you to the laboratory on the morning of your first experimental trial (diet record from previous day). You will be provided with a copy of this dietary log, which is to be used as a template when replicating your dietary habits for the 24-hrs leading up to each of the following trials. You are also asked to refrain from heavy exercise for 48-hours prior to each trial. You will be asked to keep a record all physical activity performed during the 72-hr preceding each treatment trial and to maintain consistent exercise habits between each of these trials. You are to consume your final ‘self-selected’ meal no less than 12 hours prior to the start of the exercise trials (i.e. dinner on the evening prior to testing). Approximately 7-9 hrs prior to each trial (the night before), you will consume a liquid meal replacement (Ensure® Shakes) at an amount corresponding to 20-25% of daily caloric intake. 2 hrs prior to all experimental trials, you will consume a standardized breakfast consisting of ~500 kcals (provided by the researchers). Finally, you will be asked to abstain from alcohol for 24 hrs preceding each trial and caffeine for 12 hrs preceding each trial.

**Risks**

You are expected to be honest about disclosing all known risk factors to the researchers. According to the American College of Sports Medicine, the risks associated with maximal exercise/testing for healthy individuals are very minimal. To be included in this study, you will need to meet the criteria for “low risk”. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, at least one investigator present at all testing sessions will be CPR certified.

The exercise protocol may result in minor-moderate levels of muscle soreness and fatigue for 1-2 days following each exercise session. However, the level of muscle soreness is expected to be lower than levels normally experienced when you perform other ‘normal’ activities that are not part of your regular exercise routine (i.e. if a cyclist played a game of basketball with friends for 2 hours).

The consumption of relatively large amounts of sports drinks can increase the risk of digestive issues; include symptoms such as nausea, stomach cramping, bloated feeling, vomiting, dizziness, and diarrhea. These symptoms may cause mild discomfort for a short-term period, but are not life-threatening. Digestive symptoms will be monitored throughout testing, and tests will be terminated if your symptoms become severe enough to require you to cease exercise. The risks of venipuncture and finger stick blood sampling include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or
skin irritation. This risk is considered to be minimal, and all safety precautions for handing blood samples will be followed according to OSHA protocols. The investigators have been trained in phlebotomy and completed JMU blood-borne pathogen training.

**Benefits**

The benefits associated with this project include a free VO$_{2\text{peak}}$ assessment, and a $75 payment for study completion. In the case of withdrawal, payments will be pro-rated such that you will receive $25 for the completion of each experimental trial (steady-state + TT). Participation in this novel research project will also contribute to our understanding of nutritional influences on recovery from endurance exercise.

**Confidentiality**

All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with individual data. The researchers retain the right to use and publish non-identifiable data. All data will be kept secured in a locked cabinet. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed. Final aggregate results will be made available to you upon request.

**Participation & Withdrawal**

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

**Questions about the Study**

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Adam Schroer  
Kinesiology  
James Madison University  
schroeab@dukes.jmu.edu

Dr. Nick Luden  
Kinesiology  
James Madison University  
ludennd@jmu.edu

**Questions about Your Rights as a Research Subject**

Dr. David Cockley  
Chair, Institutional Review Board  
James Madison University  
(540) 568-2834  
cocklede@jmu.edu
Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

Name of Participant (Printed)

Name of Participant (Signed)                        Date

Name of Researcher (Signed)                        Date
Subject Prescreening Information

Age: _______

Height: _______  Weight: _______

Average Exercise Habits over the Past 2 Months:

Avg. # days of exercise per week: _______________

Avg. # of days of aerobic exercise per week: _______________

Avg. # of days of cycling per week: _______________

Do you have a muscle or joint injury/condition that precludes the completion of exercise protocol?

Do you have any food allergies?
AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire
Assess your health status by marking all true statements

History
You have had:

- [ ] a heart attack
- [ ] heart surgery
- [ ] cardiac catheterization
- [ ] coronary angioplasty (PTCA)
- [ ] pacemaker/implantable cardiac
defibrillator/rhythm disturbance
- [ ] heart valve disease
- [ ] heart failure
- [ ] heart transplantation
- [ ] congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Symptoms

- [ ] You experience chest discomfort with exertion
- [ ] You experience unreasonable breathlessness
- [ ] You experience dizziness, fainting, or blackouts
- [ ] You take heart medications

Other Health Issues

- [ ] You have diabetes
- [ ] You have asthma or other lung disease
- [ ] You have burning or cramping sensation in your lower legs when walking short distances
- [ ] You have musculoskeletal problems that limit your physical activity
- [ ] You have concerns about the safety of exercise
- [ ] You take prescription medication(s)

If you marked that you have diabetes but did not mark any other statements, physician’s approval will be necessary before engaging in the study.

Cardiovascular risk factors

- [ ] You are a man older than 45 years
- [ ] You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal
- [ ] You smoke, or quit smoking within the previous 6 months
- [ ] Your blood pressure is > 140/90 mmHg
- [ ] You do not know your blood pressure
- [ ] You take blood pressure medication
- [ ] Your blood cholesterol level is > 200 mg/dl
- [ ] You do not know your cholesterol level
- [ ] You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
- [ ] You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)
- [ ] You are > 20 pounds overweight

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.

- [ ] None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.
REFERENCES


66. Henson D a, Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Shannon M, Bolton MR, Davis JM, Gaffney CT, Kelln WJ, Austin MD, Hjertman JM, Schilling BK.


