Pre-exercise Fueling and Endurance: Effect of a Pre-exercise Almond-raisin Beverage versus a Commercial Sports Beverage on Performance in Trained Male Runners

Wendy N. Bazilian

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PRE-EXERCISE FUELING AND ENDURANCE:
EFFECT OF A PRE-EXERCISE ALMOND-RAISIN BEVERAGE
VERSUS A COMMERCIAL SPORTS BEVERAGE ON PERFORMANCE
IN TRAINED MALE RUNNERS

by

Wendy N. Bazilian

A Dissertation in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Public Health
in Nutrition

June 2003
Each person whose signature appears below certifies that this dissertation, in her/his opinion, is adequate in scope and quality as a dissertation for the degree Doctor of Public Health.

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Pre-Exercise Fueling and Endurance:
Effect of a Pre-Exercise Almond-Raisin Beverage Versus a Commercial Sports Beverage on Performance in Trained Male Runners

by

Wendy N. Bazilian

Doctor of Public Health in Nutrition

Loma Linda University, Loma Linda, California, 2003

Sujatha Rajaram, Chair

Aside from genetics and training, no factor plays as important a role in exercise performance as nutrition. What athletes consume before exercise longer than 60 min influences fuel use, perceived exertion (RPE), heart rate (HR) and time-to-exhaustion (TE). Ingesting carbohydrates (CHO) before exercise can delay exhaustion over placebo, but less is known about combinations of CHO and fat on fuel use and endurance. To date, there are few studies that systematically explore nutritious, whole food products like nuts and dried fruits as pre-exercise fuel sources for endurance exercise.

The purpose of this study was to compare the effects of 2 isocaloric beverages on substrate availability and endurance capacity in trained runners: almond-raisin (AR) beverage (whole food, mixed macronutrient) and a commercial sports (CE) beverage (CHO-only and electrolytes). In a randomized, crossover study, 10 trained, male runners ran to exhaustion on a treadmill at 70% VO_{2_max} twice, separated by 2 weeks. After a
fasting blood sample, subjects drank one of the test beverages, rested for 60 min, and began the trial run. Serial measures were collected including blood samples, respiratory exchange ratios (RER), HR and RPE in 15-30 min intervals. TE was recorded at the end of each run.

Mean TE was similar between the AR and CE trials. However, a significant difference was seen in insulin concentrations during the AR compared with the CE trials throughout exercise, with a significant blunting of insulin observed during the AR trial, but not the CE trial. Free fatty acid concentrations were significantly higher in the AR trial through 60 min, though the between trials effect did not remain significant through exhaustion and recovery. Serum glucose and lactate concentrations, RER, HR, and RPE were similar between the AR and CE trials.

In conclusion, the pre-exercise almond-raisin beverage showed favorable responses in insulin and free-fatty acid concentrations during exercise and a similar mean time-to-exhaustion compared with the carbohydrate-electrolyte beverage. Therefore, the AR beverage may be considered a practical alternative to commonly used commercial sports beverages for endurance exercise.
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A journey of a thousand miles begins with a single step.

(Lao-tzu, 500 BC)

...but no one ever said you had to walk,
so I laced up my running shoes and ran!

(Wendy B., 2003)

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And as for my life’s tune:
As often as possible, skip rather than walk . . . jump rather than stand.

This is just the beginning.

Loma Linda, California
2003
CHAPTER ONE
INTRODUCTION

A. STATEMENT OF THE PROBLEM

Optimal nutrition during exercise is an area of increasing interest today, demonstrated in part by the multi-million dollar sports nutrition industry and increased presence of nutrient-formulated commercial food products in the marketplace. We know that besides physical training and genetics, no other factor plays as important a role in exercise performance as nutrition (1, 2). For endurance events lasting over 60 minutes in duration, pre-, during and post-exercise nutrition are important. In particular, it is well recognized that the consumption of carbohydrate before and during exercise improves performance over placebos, increasing time-to-exhaustion or amount of work accomplished, as well as influencing other exercise parameters like fuel use, respiratory exchanges, ratings of perceived exertion, and heart rates (2-13). Finding meals, snacks and beverages that are beneficial to performance and practical to consume before and during exercise are of particular interest to experts in sports nutrition and medicine, as well as athletes continually seeking the competitive edge.

A.1 Pre-Exercise Nutrition and Pre-Exercise Diet

Pre-Exercise nutrition encompasses two main categories: pre-exercise diet and pre-exercise fueling. The former consists of a comprehensive dietary pattern that an endurance athlete follows in the months, weeks, and days during endurance training and particularly preceding a competitive event like a marathon or triathlon. While this area
warrants further research, for endurance athletes in general, science still supports a high carbohydrate dietary pattern (6-10 grams per kilogram body weight), moderate in fat, and slightly higher protein requirements than the general population (14). Adequate daily calories seem to be the overriding factor in assuring that endurance athletes receive the nutrients and energy needed for effective training and optimal performance.

A.2 Pre-Exercise Fueling

Pre-Exercise fueling refers to the meals, snacks, or beverages that are ingested in the hours or minutes prior to endurance exercise to help optimize performance during a specific event. This is an area of growing interest and previous research has varied regarding the timing, amount, form, nutrient composition, and type of pre-exercise fuels and how they affect exercise performance, as well as more practical aspects like palatability and ease of digestion of the pre-exercise fuel.

Endurance athletes use the pre-exercise meal, snack, or beverage to help them maximize their physical strength and performance so they can work harder and more efficiently. On a biochemical level, therefore, the goal of consuming food prior to exercise translates to supplying the optimal amount and type of carbohydrate at the right time to elevate or maintain blood glucose and its utilization without dramatically increasing insulin or depleting glycogen stores. The critical elements in fuel availability and utilization during exercise thus are glucose, fatty acids, and glycogen stores, as well as insulin and the circulating counter-regulatory hormones.

Insulin plays an important role in fuel availability and tends to increase the burning of carbohydrate and reduce the use of fat when elevated. During prolonged
endurance exercise at moderate intensity, the use of fatty acids becomes essential in providing energy, preserving glycogen stores, and optimizing performance. Once exercise has begun, counter-regulatory hormones like epinephrine act as inhibitors of further insulin increase, thereby favoring the maintenance of blood glucose levels (15). Therefore, finding pre-exercise meals, snacks, or beverages that can bring about these aforementioned effects of maintaining blood glucose without the large increases in insulin, while optimizing fatty acid fuel-use is of particular significance in sports nutrition.

B. PURPOSE OF THE STUDY

The purpose of this study was to determine the effects of a whole food, mixed macronutrient almond-raisin beverage as pre-exercise fuel compared to a commercial, carbohydrate-only beverage that is commonly consumed by athletes. This study was intended to begin to bridge the gap in existing research into the realm of convenient, whole foods that may serve as equal or better sources of pre-exercise fuel compared to commercial sports beverages.

C. RATIONALE AND SIGNIFICANCE

With more and more elite and recreational athletes turning to commercial products such as sports bars and beverages to improve performance, the sports nutrition industry has become big business. Sports bars and beverages are expensive and range in ingredients from pure glucose or combinations of sugars to those that contain all three macronutrients and a vitamin/mineral cocktail mixture. Many commercial products are
highly processed in contrast to whole foods such as nuts and dried fruits. While the traditional pre-exercise snack was a high carbohydrate drink or snack, recent studies (5, 16, 17) are suggesting that combining carbohydrate with fat in pre-exercise snacks may enhance fat oxidation, and thus spare glycogen-use leading to improved endurance.

Although nuts are not considered a staple food, many Americans consume them. The annual per capita consumption of nuts in the United States is 8.5 pounds of which the tree nuts account for 2.7 pounds (18). Among the tree nuts, the most commonly consumed nuts are almonds. The consumption of dried fruits such as raisins, dates, figs, apricots and others is also very common in the United States. The annual per capita consumption of dried fruits is 10.5 pounds, with raisins being the most widely consumed dried fruit (19).

There are several reasons for conducting a study comparing the effects of a nut and dried fruit pre-exercise snack with those of a commercial sports beverage on exercise performance. First, nuts such as almonds are traditionally used either alone or in combination with dried fruits (trail mixes) by sedentary and active people as an energy-boosting snack. However, to our knowledge, the effects of such a mixture on exercise performance have not been tested scientifically. Based on the results of this and future studies, athletes may find they can just as easily turn to nuts and dried fruits than the often more expensive, refined commercial products for purposes of enhancing performance.

Second, nuts such as almonds have a glycemic index that is ~ less than 20. Thus, when combined with dried fruits like raisins, they lower the overall glycemic index or load and may influence fuel use similar to other low GI foods (5, 16, 17) such as lentils,
but without the accompanying gastrointestinal distress. Thus, foods such as nuts and dried fruits can be combined to produce the same effect with regard to glycemic index, fuel use, and exercise performance, but potentially without the gastrointestinal discomfort.

Third, nuts and dried fruits have several naturally existing nutrients that may relate to improved performance, recovery and overall health (20, 21). These include phytochemicals, soluble and insoluble fiber, and antioxidants such as tocopherol that may confer additional health- and performance-related benefits, not specifically addressed in this dissertation. Most commercial sports bars and drinks are not nutritionally optimal and they are designed with the main objective of providing the carbohydrate necessary for fuel during exercise. There are some sports bars or drinks that may contain other macronutrients and added vitamin/mineral cocktail mixes, but they generally do not have the natural phytonutrients that nuts and dried fruits contain.

Finally, nuts such as almonds are rich in monounsaturated fatty acids, the type of fatty acid that is considered neutral with regard to blood lipid levels. In fact, studies have shown that the frequent consumption of nuts reduces several cardiovascular risk factors in both men and women (21-25).

D. OBJECTIVES OF STUDY

The overall objective of this study was to determine the effects of adding nuts (almonds) to a high carbohydrate dried fruit (raisins) pre-exercise beverage on endurance capacity of trained runners. The specific aims are:
1. To determine the effect of an almond-raisin pre-exercise beverage compared to a commercial, carbohydrate-only sports beverage (Gatorade®) on fuel utilization in trained, male runners.

2. To determine the effect of an almond-raisin pre-exercise beverage compared to a commercial, carbohydrate-only sports beverage (Gatorade®) on heart rate, ratings of perceived exertion, respiratory exchanges, and oxygen consumption in trained, male runners.

3. To determine the effect of an almond-raisin pre-exercise beverage compared to a commercial, carbohydrate-only sports beverage (Gatorade®) on endurance capacity in trained, male runners.

E. RESEARCH HYPOTHESES

1. While consuming the almond-raisin pre-exercise beverage, subjects will exhibit a more efficient fuel use as indicated by higher plasma free fatty acids and glucose, lower lactate and insulin levels compared to those consuming the commercial sports beverage.

2. While consuming the almond-raisin pre-exercise beverage, subjects will utilize proportionally more free fatty acids as fuel as indicated by lower respiratory exchange ratios compared to those consuming the commercial sports beverage.

3. While consuming the almond-raisin pre-exercise beverage, subjects will exhibit a greater endurance capacity indicated by longer time-to-exhaustion compared to those consuming the commercial sports beverage.
CHAPTER TWO

REVIEW OF THE LITERATURE

Pre-Exercise Fueling and Endurance Exercise

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ABSTRACT

Nutrition plays a key role in exercise performance. Although evidence has existed for decades showing that the ingestion of carbohydrates before exercise can delay exhaustion over placebo, many questions still remain about the optimal timing, amount, form, type, nutrient composition, and source that can be consumed prior to exercise to enhance fuel use and improve endurance exercise performance. This review focuses on the role of pre-exercise fuels on endurance performance, with current recommendations and future directions in the area of pre-exercise fueling. Pre-exercise fueling refers to the meals, snacks, or beverages that are ingested in the hours or minutes prior to endurance exercise to help maximize stamina and performance during a specific event. It becomes clear from this review that the type, amount, timing, glycemic index, nutrient mixture, and source of the pre-exercise fuel are interrelated and can affect performance during events of long duration. Researchers today are progressively focusing on "real foods" particularly through studies evaluating pre-exercise fuels that vary in glycemic index or macronutrient composition. While results continue to be conflicting, increasing research on whole and refined foods appears to be on the horizon. Because sports nutrition research has a real and pertinent application to the athletes it serves, contemporary investigators tend to recognize the importance of fuel sources having practical attributes like convenience, portability, along with subjective and physiological acceptability. The authors recognize the ongoing necessity of periodic critical reviews of this topic to attempt to elaborate on evolving recommendations and elucidate future directions in the field.
A. INTRODUCTION

Optimal nutrition during exercise is an area of increasing interest today, demonstrated in part by the multi-million dollar sports nutrition industry and increased presence of nutrient-formulated commercial food products in the marketplace. We know that besides physical training and genetics, no other factor plays as important a role in exercise performance as nutrition (1, 2). For endurance events lasting over 60 minutes in duration, pre-, during and post-exercise nutrition are important. In particular, it is well recognized that the consumption of carbohydrates before and during exercise improves performance over placebos, increasing time-to-exhaustion or amount of work accomplished, as well as influencing other exercise parameters like fuel use, respiratory exchanges, perceived exertion, and heart rates (2-9). In this article, the role of pre-exercise meals, snacks and beverages in particular on endurance performance will be reviewed, with current recommendations and the future scope of research in the area of pre-exercise fueling.

B. PRE-EXERCISE NUTRITION

Pre-exercise nutrition can be divided into two main areas: pre-exercise diet and pre-exercise fueling. The pre-exercise diet consists of a comprehensive dietary pattern that an endurance athlete follows in the months, weeks, and days during endurance training and particularly preceding a competitive event like a marathon or triathlon. Traditionally, high carbohydrate diets and “carbohydrate-loading” strategies have been considered as ways for athletes to maximally store glycogen in order to provide readily available energy for an endurance event (10-15). More recently, researchers have also
compared diets higher in fats versus those higher in carbohydrates and their effects on performance parameters including endurance times and the availability of circulating fuels during exercise and found mixed results (16-24).

While this is still a subject of debate and in need of further research, for endurance athletes in general, there is continued support for a high carbohydrate dietary pattern (6-10 g·kg⁻¹ BW), moderate in fat (20%-30% energy), and slightly higher protein requirements (1.2-1.8 g·kg⁻¹ BW) than the general population (15, 25, 26). Also, most public health experts agree that it would not be good practice professionally to recommend a high fat diet amidst our current knowledge about the links between high total and saturated fat consumption and chronic disease risk (21, 24). Carbohydrate-loading recommendations for athletes differ somewhat in terms of specific timing and amounts, but generally reflect an exercise tapering strategy along with an increase in dietary carbohydrate in the week prior to an endurance event on par with 7-10 g·kg⁻¹ BW (12-15). Overall, however, consuming adequate daily calories seems to be the overriding factor in assuring that endurance athletes receive the nutrients and energy needed for training and performance.

Pre-exercise fueling refers to the meals, snacks or beverages that are ingested in the hours or minutes prior to endurance exercise to help maximize stamina and performance during a specific event. This is an area of growing interest and previous research has varied regarding the timing, amount, form, type, nutrient composition, and source of pre-exercise fuels and how they affect exercise performance, as well as more practical aspects like palatability and ease of digestion.
The goal of consuming food prior to exercise is to supply the optimal amount and type of carbohydrate at the right time to increase or maintain blood glucose and its utilization without dramatically elevating insulin or depleting glycogen stores. Ideally, there needs to be adequate glucose and free fatty acids available in the blood for utilization, along with a preservation of glycogen until the very end of exercise. The key players in fuel availability and use during exercise thus are glucose, free fatty acids, and glycogen stores, as well as insulin and the circulating counter-regulatory hormones.

Insulin plays an important role in fuel availability and tends to increase the oxidation of carbohydrate and reduce the use of free fatty acids. During prolonged endurance exercise at moderate intensity, the use of free fatty acids becomes essential in providing energy, preserving glycogen stores and helping optimize performance. Once exercise has begun, counter-regulatory hormones act as inhibitors of further insulin increase, thereby favoring the maintenance of blood glucose levels (27). Therefore, finding pre-exercise meals, snacks, or beverages that can bring about the effects of maintaining blood glucose without the large increases in insulin, while optimizing fatty acid fuel-use, is of particular significance to researchers in the field of sports nutrition.

C. CARBOHYDRATE: THE MASTER PRE-EXERCISE FUEL

Traditionally, researchers have looked primarily at carbohydrate-based meals, snacks, and beverages as preferred sources of pre-exercise fuel. This was due to the knowledge that during exercise, glucose provides a primary fuel source in the blood from digestion of quick absorbing carbohydrates and the ready mobilization of glycogen
stores. Some (4, 7-9), but not all (28, 29) studies have demonstrated improved performance in athletes with a pre-event carbohydrate meal, snack, or beverage.

The amount (percent carbohydrate concentration or grams CHO•kg\(^{-1}\) BW), timing (within 1 hour or longer prior to exercise), form (solid versus liquid), and type of carbohydrate ingested prior to exercise will have different magnitudes of effect on metabolism and performance. The type of carbohydrates can be considered in different categories: biochemical form (glucose versus sucrose or fructose), glycemic index (low, moderate, high), mixtures of macronutrients (combinations of carbohydrates, fats, and protein), and source (whole foods versus isolated nutrients or processed foods).

### C.1 Amount of Pre-Exercise Carbohydrate

The amount of carbohydrates, both in terms of percentage carbohydrate and actual volume ingested, relates to the rate the stomach empties, how it affects fuel use and endurance, and practical considerations of athletes' preferences, gastrointestinal comfort and feelings of fullness. Researchers have looked at different percentages of carbohydrate in beverages to determine the ideal concentration of carbohydrate for optimal gastric emptying, maximal absorption and prolonged endurance. Based on earlier studies that compared different solutions of carbohydrate in beverages (e.g. 6%, 8%, and 10%), we now know that 6% carbohydrate solutions benefit performance and hasten absorption over others (6, 30, 31).

Both Murray et al. (30) and Ryan et al. (31) demonstrated that carbohydrate solutions less than 8% increased gastric emptying, and 8% or higher led to significantly delayed emptying. Ryan et al. (31) also showed increased carbohydrate and water
absorption with water and 6% carbohydrate compared with 8% or 9% carbohydrate solution. Further, Murray et al. (30) found that overall acceptance of the beverage was reduced below 6% carbohydrate solution, leaving 6% as a practical choice both for gastric emptying and athlete preference. Therefore, several commercial beverages are formulated using this proportion (15, 32, 33).

The effects of varying amounts of carbohydrate on endurance have been mixed. In one study, performance was significantly improved when 12 cyclists consumed a 6% carbohydrate beverage prior to cycling and during rest periods compared to water placebo, 8%, or 10% carbohydrate solutions, although physiologic and sensory responses were not different (6). However, McConnell et al. (34) and Millard-Stafford et al. (4) demonstrated that an 8% carbohydrate-electrolyte drink consumed immediately before and during or one hour prior to exercise, respectively, improved performance significantly compared to equal volumes of sweetened placebo or water control, suggesting that a higher percentage such as 8% still confers benefits compared with water alone.

Tsintzas et al. (35) found significantly improved mean performance time when runners consumed a 5% carbohydrate beverage immediately prior to exercise and every 5 kilometers thereafter compared with water. The combination of these and other findings has led to a general recommendation that carbohydrate beverages contain 5-7% carbohydrate for optimal gastric emptying, absorption and performance enhancement (36, 33).

In addition to the formulation of beverages at optimal percent concentration of carbohydrate, scientists have tested different grams of carbohydrate consumed prior to
exercise. In one study, cyclists consumed three different amounts of carbohydrate 4 hours before a performance test (37). Carbohydrate intake ranged from 0.6 to 4.5 g·kg⁻¹ BW (45-312 g) or water placebo. Exercise performance was significantly improved by 15% and carbohydrate oxidation was enhanced when subjects consumed 312 grams compared to placebo.

When large amounts of carbohydrate are consumed 30-60 minutes before exercise, the results are conflicting. Sherman et al. (38) found improvements in exercise over placebo when two different amounts of carbohydrate (1.1 or 2.2 g·kg⁻¹ BW) were consumed one hour before exercise. Both carbohydrate amounts produced similar benefits (12.5%) to performance over placebo. Serum glucose and insulin were significantly higher in both carbohydrate trials than placebo throughout most of the exercise trial.

A classic study by Foster et al. (28) showed significantly decreased performance and significant drop in serum glucose and free fatty acids early in exercise when 75 grams of carbohydrate were consumed 30 minutes before exercise versus water, whereas a more recent re-examination by Chryssanthopoulos et al. (29) found that 75 grams of carbohydrate consumed 30 minutes before exercise resulted in no significant difference in performance times compared with water control. The specific details of optimal amounts of carbohydrate are thus inconclusive and the issue of timing is critical to this consideration.
C.2 Timing of Pre-Exercise Fuel

The amount and timing of carbohydrate consumption are closely interconnected, relating both to the rate the stomach empties and practical considerations of the athlete’s gastrointestinal comfort and feelings of fullness.

C.2.1 Carbohydrate Fueling 3-6 Hours Before Exercise

The timing of the pre-exercise meals, snacks or beverages has varied substantially in research. Larger meals ranging between 100-350 grams carbohydrate have been tested within 3-4 hours of endurance exercise and several (7, 37, 39-43), but not all (44-46) studies have shown improvements in performance.

The proposed mechanisms for improving endurance have included a “topping off” effect of the muscle and liver glycogen stores prior to exercise (47), as well as allowing sufficient time for blood glucose and insulin levels to return to normal before exercise begins (48). Others have suggested that the effects of a high carbohydrate meal several hours prior to exercise may produce similar effects to consuming carbohydrate during exercise (46, 48), and in several cases carbohydrate ingestion 3-4 hours prior and during exercise showed further improvements in performance (40, 41, 43).

In one study that compared identical pre-exercise meals (40 kilojoules per kilogram body weight) at 3 and 6 hours prior to exercise, time-to-exhaustion among female cyclists was significantly longer in the 3 hour pre-ingestion trial than after 6 hours (42), suggesting that 3-4 hours may be a preferable based on this and previous studies (7, 37, 40-43). Among the studies that did not show improvement, effects on performance were neutral and not decreased with comparison treatments or placebo. In sum,
improvements in performance have typically been seen when carbohydrates range between 200-300 grams consumed 3-4 hours before endurance exercise.

C. 2.2 Carbohydrate Fueling Within 60 Minutes Before Exercise

Smaller feedings have been provided within an hour of exercise and yielded conflicting findings in relation to performance outcomes. Early studies have shown that when large amounts of carbohydrates (e.g. 50-75 g) are consumed within an hour, athletes experience a spike in glucose and insulin before exercise, consequently leading to a hypoglycemic response early in exercise, accelerated glucose use and depletion of glycogen stores, fatigue and a resultant decrease in endurance (28, 49). However, more recent research has increasingly challenged this theory, suggesting that the hyperglycemic and hyperinsulinemic responses are transient, and results on performance and substrate utilization continue to be mixed (23).

With smaller pre-exercise feedings (0.7 to 1.5 g CHO•kg\(^{-1}\) BW) within approximately 1 hour, researchers have seen improved (2, 7, 8, 27, 38, 50-53), decreased (28, 49), or neutral (29, 52, 54-57) effects on performance capacity with comparison treatments or placebo trials. One study specifically addressing the timing of carbohydrate found that blood sugar levels remained normalized when 1 g of CHO•kg\(^{-1}\) BW was consumed 30 minutes prior to exercise compared with 60 or 90 minutes, but no improvements were seen in performance times (54). Similarly, Thomas et al. (57) found no differences in performance times between four test meals providing 1 g CHO•kg\(^{-1}\) BW 1 hour prior to exercise, but did show significant inverse relationships between the
glycemic index (GI) of the pre-exercise test foods and plasma levels of glucose and free fatty acid during the later stages of exercise

In a study by Kirwan et al. (52), cyclists who consumed 75 grams of high fiber, whole rolled oats carbohydrate cereal significantly improved performance time over water placebo. However no difference was observed between a second test meal of 75 grams of carbohydrate coming from a low fiber, oat flour cereal compared to the placebo. Two studies by Febbraio and colleagues (55, 56) found no improvements in exercise performance when a high or low GI meal providing 1 g CHO·kg⁻¹ BW was ingested 30 or 45 minutes prior to exercise. Carbohydrate utilization, however, was increased with the high GI meal, but not the low GI meal (55).

Ventura et al. (2) found that ingestion of 75 grams of carbohydrate 30 minutes prior to exercise significantly improved performance in 11 runners versus placebo control. Kirwan et al. (51) also observed improvements with 75 grams of carbohydrate from a moderate GI, high fiber cereal over water control, though no difference was seen between 75 grams of a low fiber oat flour and water. Chryssanthopoulos et al. (29) similarly found that 75 grams carbohydrate consumed 30 minutes before exercise resulted in no significant difference in performance times when compared only with water control. Foster et al. (28), however, showed significantly decreased time-to-exhaustion with 8 male and 8 female cyclists after ingesting a 75 gram carbohydrate snack 30 minutes prior to exercise versus a water control. These various and conflicting findings in the hour prior to endurance exercise have led researchers to focus on the form, GI, combination of nutrients, and source of pre-exercise fuel as a way to predict the benefits to performance related outcomes.
C.3 The Form of Pre-Exercise Fuel

Little has been done specifically comparing solid to liquid pre-exercise fuels *per se*, but comparisons have been made across studies that have used one or the other or both. It appears that solids and liquids are equally effective when consumed *during* exercise on improving performance and raising blood sugar, but it is less clear when specifically considering pre-exercise fueling (58). Two studies have shown similar beneficial effects on performance between liquid and solid forms when the pre-exercise snacks were matched in nutrients (7, 59), suggesting there may be similar benefits from both forms, but certainly more research in this area is needed.

In a practical sense, there is some thought that a *beverage* may be easier to consume and digest, promote hydration when water is added, and be more appealing to athletes in the immediate time preceding an endurance event. On the other hand, the practice of consuming *solid* pre-exercise foods is also very common among athletes and there are some proposed physiological advantages that could support a role for solid pre-exercise fuel sources in the field of sports nutrition. Physiologically, gastric emptying time is slower with solid food compared with liquids (58, 59). Theoretically, a slower, sustained release of glucose could confer an advantage to how fuel is utilized during exercise. Murdoch et al. (59) suggests that solid carbohydrate foods may be a better fuel source for blood glucose than liquid due to the following: 1. if solid food stays in the GI tract longer, a slower, more continuous supply of glucose may be released into circulation; 2. solid foods can provide more carbohydrate per unit weight; 3. solid foods
may be easier to carry and access during an event and cost less, and; 4. solid foods may be more physiologically and psychologically satisfying.

While a number of studies have looked at either liquid (5, 6, 15, 28, 29, 31, 60, 61) or solid (8, 44, 45, 51, 52, 55, 56, 62, 63) forms of pre-exercise fuel, only a few studies have purposely compared liquids versus solids. To date, only three studies to our knowledge have directly sought to compare liquid and solid forms of pre-exercise fuel (7, 59, 64). A few other studies have included both liquid and solid pre-exercise fuel forms among their many treatments, but not usually as a primary objective for comparison. Typically, a liquid form of a moderate or high GI pre-exercise fuel is included in a study as a comparison treatment to other solid food forms of varying glycemic indexes from low to moderate to high (3, 8).

In a study by Neufer et al. (7), subjects consumed 45 grams of liquid or solid carbohydrate or placebo 5 minutes before cycling at 77% \( \text{VO}_2\text{max} \) for 45 minutes followed by a performance trial. Both liquid and solid treatments improved performance and increased serum glucose over artificially sweetened placebo, but no differences were observed between the different forms. Similarly, Murdoch et al. (59) compared solid bananas to a blended banana slurry and an artificially sweetened placebo to provide 1.1 g CHO•kg\(^{-1}\) BW in a total fluid volume of 16 ounces. Subjects ran and then cycled for 90 minutes each, then rested and consumed the slurries before a performance test to exhaustion at 70% \( \text{VO}_2\text{max} \). Both the solid and slurried bananas yielded significantly longer times-to-exhaustion over placebo, but no differences were seen between the two forms. In addition, glucose was significantly greater in both carbohydrate trials over placebo only at exhaustion and no significant differences were noted in serum free fatty
acid or lactate levels between any of the treatments throughout the trials. The researchers suggested that perhaps the high water content of bananas (75% by weight) may have contributed to the lack of differences between the two forms.

Rauch et al. (64) compared a solid energy bar to a liquid carbohydrate (glucose polymer) ingested by subjects before a 330-minute ultra-endurance cycling trial at 55% VO$_{2\text{max}}$ followed by a high intensity performance trial. The two treatments were isocaloric providing 195 calories immediately before and every hour during the trial, but the solid bar provided approximately 39% energy from carbohydrates, 32% energy from fat, and the remainder from protein. No differences were seen in performance during the 330-minute cycling trial, but performance was significantly impaired in the solid bar treatment during the performance trial. Fat metabolism was significantly enhanced during the prolonged ride at 55% VO$_{2\text{max}}$ in the solid bar trial. Because the forms of the pre-exercise fuel varied in macronutrient content and since the intensity of exercise shifted dramatically during the later stages of exercise, it is challenging to draw direct conclusions from this study regarding the form. It does, however, serve as a framework for consideration of mixed macronutrient pre-exercise fueling reviewed in the section below.

In a study by Thomas et al. (8), no differences were seen in any serum fuel levels between liquid glucose and solid high GI potato, though a low GI solid food (lentils) enhanced performance compared to the low GI potatoes, liquid glucose or liquid placebo. In another study comparing glycemic indexes and different foods of mixed macronutrient compositions, a sucrose liquid (high GI) altered blood fuel levels to a similar magnitude.
as the solid high GI rice and potato treatments compared to lower GI foods and fasting control (3).

Based on previous research on form of pre-exercise fuel, it appears that solids and liquids of a similar macronutrient or GI offer similar effects on fuel use and performance. Interestingly, Doyle et al. (65) in a study looking at resting glycemic responses for equal amounts of carbohydrates only in liquid, solid and gel forms found similar total glycemic responses regardless of form, though the time pattern for glucose and insulin responses varied. As researchers have become more aware of the inconsistent findings with regard to exercise performance within studies that include both liquid and solid treatments, the type of fuel—as determined by GI or macronutrient profile—has become an area of increasing attention in exercise nutrition research.

C.4 Pre-Exercise Fuel Type

The type of pre-exercise fuel can be categorized in several ways: chemical form, GI, combinations of macronutrients, or whole and refined foods.

C.4.1 Chemical Form

Fairly extensive research has been done considering the chemical form of carbohydrate and its effect on endurance performance (2, 5, 6, 28, 29, 34, 53, 60, 61, 66-70). There is agreement among most researchers that glucose has a beneficial response on endurance performance when compared to fructose alone or water placebo (2, 5, 34). Ventura et al. (2) demonstrated that pre-event glucose significantly improved performance over fructose or water placebo consumed 30 minutes before exercise in 11
recreational athletes running at 82% \( \text{VO}_2\text{max} \) until exhaustion. Plasma glucose and insulin were higher in the glucose treatment immediately before and 10 minutes after exercise compared with fructose or water, but no differences were seen in lactate levels or oxygen consumption.

When glucose, sucrose, and fructose are compared, glucose and sucrose have similarly beneficial responses over fructose on exercise performance (5). Fructose has been shown to cause gastrointestinal distress and diarrhea in some athletes when used alone as pre-exercise fuel (5, 47, 71), although in one study by Okano et al. (70), fructose ingestion (60 or 85 g) one hour prior to exercise led to improved performance over artificially sweetened placebo without the gastrointestinal distress.

Murray et al. (5) demonstrated that a 6% glucose and 6% sucrose beverage both independently improved exercise performance over a 6% fructose solution in 12 trained cyclists. Gastrointestinal distress was also noted among subjects when they consumed the fructose beverage. Fructose has been studied as a pre-exercise fuel source since it takes longer to metabolize into useable fuel and smaller increases in glucose have been observed. As previously mentioned, Okano et al. (70) did find improvements with fructose over placebo, but several others (2, 5, 60, 61, 66) have not.

Williams et al. (60) found no differences between performance time or blood levels between 50 grams of glucose, fructose, or water consumed immediately before and during a 30-kilometer performance run with 9 athletes. Although performance times were not statistically different, the researchers did observe that the subjects’ self-selected running speeds were significantly slower during the last 15 kilometers of the water trial than the same period on the glucose trial, and when compared to the first 15 kilometers of
the water trial. However, overall performance increases were not seen in comparing any of the three trials.

In one study that examined the oxidation of different types of fuel consumed one hour before cycling at 60% VO$_{2\text{max}}$, researchers noted that ingested glucose and corn starch were both oxidized at a significantly higher percentage than fructose over a two hour exercise period (69). Both plasma glucose and insulin concentrations increased prior to exercise and then showed a transient drop during the first hour of exercise in both the glucose and corn starch trials compared to fructose. Their findings demonstrated that exogenous fructose was less readily available for oxidation than glucose or corn starch, which lends support to the lack of benefits seen in other studies when fructose is used as a pre-exercise fuel.

C.4.2 Glycemic Index

The GI of a food represents the magnitude of the increase in blood glucose occurring after consuming a given food. (72-74). In previously reviewing the research on carbohydrate form, we noted that studies have been conflicting when the glycemic indexes or macronutrient content of the comparison treatments were altered, lending a basis for investigating further into the types of pre-exercise fuels and how they impact fuel use and performance.

In considering the goals of increasing or maintaining a steady source of available glucose and fatty acids for use during exercise while minimizing increases in insulin and preserving glycogen stores, researchers have begun to investigate whether and
to what extent foods with a low GI as compared to a high GI may influence fuel use, and in turn, affect performance (3, 8, 10, 23, 44, 50-52, 55-57, 63, 71, 75-77).

Pre-exercise fuels that have a high GI, such as glucose or foods like potatoes ingested 30-60 minutes before exercise, consistently show hyperglycemic and hyperinsulinemic responses followed by a transient drop in blood glucose due to the shuttling of glucose to the muscles for energy (8, 23, 50). The circulating insulin inhibits the breakdown of fats, therefore making less free fatty acids available for use in prolonged exercise. This response leads to a more immediate use of glycogen stores and potentially poorer exercise performance as demonstrated in earlier studies where large amounts of glucose, a high GI sugar, were consumed within an hour of exercise (28). However, such has not been the case in the subsequent studies directly addressing the impact of GI, and the majority of researchers have supported findings showing that the spike in glucose and insulin is short-lived, and ultimately does not negatively affect and in some cases may actually improve performance (23).

Low GI foods such as lentils and white spaghetti, and some moderate GI foods, when taken prior to endurance exercise produce fuel concentrations in the blood that would theoretically favor performance improvements compared to high GI foods like potatoes and rice (3, 8, 44, 56, 57, 63). Low GI foods or meals have tended to be associated with higher blood glucose and free fatty acid levels later in exercise, suggesting a sustained source of carbohydrate throughout the trial (57). In one study, a strong inverse relationship was seen between GI and plasma glucose and free fatty acids later in exercise, perhaps due to the slower digestion of low GI fuels (57). Studies addressing substrate utilization have also shown that insulin response to the pre-exercise
carbohydrate tends to be reduced in lower GI compared to moderate or high GI foods (3, 8, 63). Additionally, Kirwan et al. (77) observed a prolonged period of euglycemia and greater total carbohydrate oxidation when subjects consumed a moderate GI meal compared with the high GI meal or water control 45 minutes prior to exercise. In sum, lower GI foods do tend to establish a favorable environment for fuel use by blunting the hyperglycemic and hyperinsulinemic responses after ingestion and by maintaining higher levels of glucose and free fatty acids later in exercise (3, 8, 56, 57, 63).

The influence of low GI foods on endurance time or time trial performance, however, are inconsistent and equivocal, although lower GI foods appear to have positive outcomes on, or at least not adversely affect, endurance exercise performance. Some (8, 50, 51, 77), but not all (44, 52, 55, 56, 63, 75) research on GI shows a tendency toward improved performance.

Thomas et al. (8) were the first to investigate the role of pre-exercise fuels of different glycemic indexes on exercise performance and substrate utilization. They found significantly improved performance in 8 trained male cyclists after consuming low GI lentils as a pre-event snack as compared to the high GI potatoes, glucose and water placebo. Pre-exercise fuels were provided at a level of 1 g CHO•kg⁻¹ BW 1 hour prior to cycling at 65-70% VO₂max to exhaustion. DeMarco et al. (50) similarly showed significantly improved performance with a low GI meal versus a high GI meal that were equal in macronutrients, even though glycemic responses between the meals were similar. Meals were designed to provide 1.5 g CHO•kg⁻¹ BW and consumed 30 minutes prior to a 2 hour cycling trial at 70% VO₂max followed by a performance trial.
Two studies conducted by Kirwan and colleagues (50, 77) also showed improvements in performance with moderate GI foods. In the first study, cyclists were fed 75 grams of carbohydrate in a high fiber, moderate GI whole rolled oats cereal or a low fiber, moderate GI, whole oat flour 45 minutes before cycling at 60% VO_{2max} to exhaustion. A water control trial was also included. Mean performance time after the high fiber, rolled oats trial was significantly longer (16%) than the whole oat flour or control, and times were similar between the oat flour and control trials. In the later study, the same 75 grams of moderate GI, whole rolled oats cereal was tested against a high GI puffed rice cereal and water control on subjects cycling again at 60% VO_{2max} to exhaustion (77). Performance time was significantly enhanced in the moderate GI rolled oats trial over the high GI fuel or control, and again no differences were observed between the high GI and control treatments.

While several studies have shown improvements, others have not. Febbraio and colleagues tested high GI instant potatoes or diet jelly control to low GI lentils (45 minutes before) or low GI muesli (30 minutes before), respectively, in two similar studies with male cyclists and found no differences in performance between the trials (55, 56). Subjects rode at 70% VO_{2max} for 120 minutes followed by a 15 minute performance trial. The study with the lentils mimics the earlier study by Thomas et al. (8) where improvements were observed in subjects’ time-to-exhaustion. Burke and colleagues (23) suggest that the measurement of “performance” designed in different studies to measure time-to-exhaustion at a set intensity versus a performance time trial of a set amount of work (e.g. 30 km) in the shortest amount of time may play a role in the differences observed in performance outcomes.
Sparks et al. (63) used a similar design as mentioned above to test low GI lentils to high GI instant potatoes or a diet beverage control. The test meals provided 1 g CHO·kg⁻¹ BW and were ingested 45 minutes prior to exercise. This time, subjects cycled at 70% for 50 minutes followed by a performance trial. Again, no differences in performance were noted between trials, despite differences in fuel metabolism.

Even between studies by the same researchers, variations in findings sometimes occur when different GI pre-exercise fuels are compared. Kirwan and colleagues who on two other occasions found improvements in performance with moderate GI, high fiber rolled oats (51, 77), failed to find a significant difference in another study when a similar 75 grams of the rolled oats were fed to 6 female cyclists 45 minutes before cycling to exhaustion at 60% VO₂max compared to water control.

Two final studies warrant mentioning since they alter the typical time frame, quantity and nutrient composition of those aforementioned. Burke et al. (75) fed subjects a higher amount (2 g CHO·kg⁻¹ BW) of high GI instant potatoes or low GI pasta 2 hours before exercise. A diet jelly control trial was also included and subjects were also provided a carbohydrate beverage to consume during each trial. Subjects rode at 70% VO₂max for 2 hours, followed by a performance trial. Performance times were not different between any treatment. However, this study was among the first to provide isocaloric pre-exercise feedings, along with a similar macronutrient composition between the two test beverages.

Wee et al. (44) similarly provided subjects with isocaloric, matched macronutrient pre-exercise meals that differed in GI. The meals provided 2 g CHO·kg⁻¹ BW (along with 65 g protein and 0.1 g fat) and were ingested 3 hours prior to running at 70% VO₂max.
until exhaustion. The low GI food consisted of boiled lentils and the high GI meal was comprised of toasted potatoes, tuna, sweet corn, toasted crumpets and honey. As with the previous study, no differences were observed in performance, though the authors did observe a relative shift in substrate utilization from carbohydrate to fat in the low GI compared with the high GI meal.

While the glycemic index provides an important tool for classifying foods and mixtures of foods, it is obvious that much is to be done in this area. Though there is a general sense that the low GI foods and meals may confer some benefit on performance, researchers agree that there is still insufficient evidence to make a clear recommendation regarding glycemic indexes of foods consumed prior to endurance exercise (23, 71, 76).

The studies by Burke et al. (75) and Wee et al. (44) that designed the pre-exercise treatments to vary in glycemic index, but control for calories and overall macronutrient profile, provide an important transition into where pre-exercise fueling research has naturally progressed over the past decade. More recent research is shifting toward evaluating mixtures of macronutrients from various sources and their effects on exercise performance and fuel use.

C.4.3 Combinations of Macronutrients

While the research concerning glycemic index and performance is equivocal, Wee and colleagues (44) made an important observation about the overall energy and nutrient composition of some recurring test foods. They noted that several researchers (8, 56, 63), in using lentils and potatoes as their low and high GI foods, failed to consider the fact that lentils have significantly more calories and protein than potatoes,
on par with 208% and 36%, respectively. Wee et al. (44) and Burke et al. (75) subsequently provided isocaloric, matched-macronutrient, high and low GI foods to subjects and did not observe performance benefits. However, they had drawn attention to the importance in future research of considering and controlling for combinations of macronutrients and calories that exist in real food used for pre-exercise fuel.

Naturally, athletes like the general population, do not eat single foods or nutrients, but combinations of nutrients through foods and combinations of foods that contain carbohydrates, protein, fats, vitamins, minerals, and other phytochemicals. With this understanding added to the knowledge that fatty acids provide an important fuel source during prolonged exercise, researchers have begun to address the additions of protein and fat to the mostly carbohydrate pre-exercise fuel package to assess their overall impact on exercise performance and endurance.

Research on the addition of protein in particular is limited, more specifically addressing branched chain amino acids, which are proposed to have a potential role in delaying central nervous system fatigue (26, 78). However, the data are inconsistent (26, 78). In one study, subjects ingested one of three different pre-exercise beverages one hour before repeated shuttle runs to exhaustion alternating between 55% to 95% VO$_{2\text{max}}$ (46). The beverages tested were: 20% carbohydrate, 20% carbohydrate plus 7 grams of branched chain amino acids, or a flavored water placebo. Both carbohydrate beverages resulted in longer endurance times than placebo control, but no differences were seen between the carbohydrate and the carbohydrate plus BCAA trials. Both carbohydrate treatments had higher blood glucose and insulin, and lower free fatty acid levels than the placebo, but again no differences were noted between the trials. While there is evidence
that BCAAs are used as fuel during endurance exercise, the effects of pre-exercise supplementation are not apparent (26).

In a different kind of study looking at pre-exercise protein, Rowlands and colleagues (79) compared the effects of separate high carbohydrate, high protein, and high fat pre-exercise snacks consumed by 12 subjects, 90 minutes before cycling at 55% VO$_{2\text{max}}$ for 1 hour followed by incremental 10-minute tests and a 50-kilometer time trial. All three test meals were isocaloric, providing approximately 4,700 kilojoules (~1100 kilocalories). The macronutrient distributions by percent energy for protein, carbohydrates and fats were: high carbohydrate (10/85/5); high protein (30/40/30); and high fat (10/5/85).

The researchers found mean performance times to be shorter for the high fat meal, but the difference was not significant. There were no differences in performance times between the three treatments, however both the high fat and high protein treatments did show higher oxidation of fatty acids, while the high carbohydrate meal elevated pre-exercise insulin levels and decreased fat oxidation during exercise compared to the other treatments. The authors commented to the interesting similarity in substrate utilization between the high fat and high protein trials, given that the protein treatment had 3 times more carbohydrate, 3 times more protein and 3 times less fat than the high fat treatment (79). Nonetheless, a specific benefit of protein in pre-exercise fuel on performance or substrate utilization remains unclear.

It has been previously pointed out that protein in naturally occurring foods like lentils used in several GI studies adds to the macronutrient profile and total energy of the food item (8, 56, 63) which may influence the rate at which the food is digested and
affect overall substrate metabolism during exercise. Therefore, the role of protein may factor more into influencing the GI of the food or the rate of gastric emptying and absorption of carbohydrate and fatty acids into the blood.

Schabort et al. (39) provided 7 subjects 100 grams of carbohydrate in the form of cereal and skim milk 3 hours before cycling at 70% VO$_{2\text{max}}$ to exhaustion. While the addition of protein to the treatment was not specifically addressed, protein represented 15% of the total macronutrient profile. Compared to a fasting control trial, time-to-exhaustion on the carbohydrate and protein, mixed macronutrient treatment was significantly longer. Thomas et al. (8) also demonstrated performance benefits when subjects consumed lentils (carbohydrates and protein) compared with potatoes (mainly carbohydrate), glucose, and water.

The contribution of proteins within a food or meal may have some relevance in the area of pre-exercise fueling overall, but more attention has been paid to the addition of fats to the traditional carbohydrate-based fuel source. The goals of adding fat is to make more fatty acids, which can provide up to one-half of the energy needed for endurance exercise at 70% VO$_{2\text{max}}$ (10), available for oxidation.

The addition of fat to the traditionally carbohydrate-only pre-exercise fuel is gaining increasing interest in sports nutrition research, emerging from the knowledge that both carbohydrates and fatty acids are important fuel sources—with fat contributing up to 50% of energy—during moderate intensity exercise of long duration. Research on the effect of fat on exercise performance generally falls into two main categories: total fat percentage in the habitual pre-exercise diet mentioned previously, or the role of fat specifically in pre-exercise fuel. There is still a consensus that a carbohydrate-rich diet
and mostly carbohydrate fuel as a pre- and during exercise meal, snack, or beverage is preferable, but researchers are now looking at adding fat to a level that would still enable a maximum steady release of glucose, blunt the insulin rise, and help preserve glycogen stores. A further goal is aiming to increase free fatty acid availability and consequently, increased free fatty acid oxidation during endurance exercise.

Some researchers have begun to evaluate the specific addition of medium-chain triglycerides solutions to carbohydrates. Medium-chain triglycerides have been proposed to increase fatty acids in the blood more quickly because of their faster rate of emptying from the stomach, rapid breakdown and absorption from the small intestine, and potential alternate source of carbon for exercising muscles (22, 80). However, their effects remain unclear, though appear to not alter fuel use or improve endurance (22, 80, 81). This topic has been more thoroughly reviewed elsewhere (80).

Only a few studies have directly sought to determine the effect of fat in pre-exercise fuel. Several studies comparing a high carbohydrate (60-86% of total energy) compared with a high fat (61-90% of total energy) pre-exercise meals consumed 4 hours prior to exercise showed mixed results (20, 45, 82, 83). Whitley et al. (45) found no difference in cycling performance but significant differences in subjects’ fuel levels when comparing a high carbohydrate (86% energy from carbohydrate, 3% energy from fat) with an isoenergetic high fat (20% energy from carbohydrate, 72% energy from fat) meal consumed 4 hours prior to endurance cycling. Subjects cycled for 90 minutes at 70% \( \text{VO}_{2\text{max}} \) followed by a 10 km time trial. No improvements in mean performance time were seen between the treatments, but significant increases in free fatty acids were observed in the high fat and fasting treatments compared to the high carbohydrate meal,
along with higher blood glucose concentrations in the high fat compared to the high carbohydrate meal. Similarly, Okano and colleagues (82, 83) observed favorable fuel shifts in free fatty acids and serum insulin throughout and early in cycling exercise at ~65% VO$_{2\text{max}}$, respectively, but no differences in performance times between the high fat (61% of total energy) and high carbohydrate (58-79% of total energy) meals consumed 4 hours prior to exercise.

In a similar study, Pitsiladis et al. (20) also compared a high carbohydrate (70%) versus high fat (90%) meal consumed 4 hours prior to exercise. The researchers included intravenous administration of heparin during the high fat test in their protocol to directly elevate free fatty acid levels in the blood. Subjects cycled at 70% VO$_{2\text{max}}$ until exhaustion on two occasions. Time-to-exhaustion was significantly improved in the high fat pre-exercise trial compared with high carbohydrate treatment, thus suggesting that the increased fat availability from both the pre-exercise fat sources along with heparin infusions did lead to increased endurance. The authors make note that the practice of injecting heparin for exercise endurance is not sound professional practice, but for research purposes helped to demonstrate that increased fat availability in the blood may enhance substrate use and overall endurance. Therefore, the continued challenge is to find the optimal pre-exercise food or beverage sources that can attempt to bring about similar favorable fuel changes during exercise.

Horowitz and Coyle (3) considered the metabolic responses to a variety of carbohydrate sources to which fats were added in half of the exercise trials. Subjects complete 7 different trials where 0.7 g CHO$\cdot$kg$^{-1}$ BW were provided 30 minutes before cycling for 1 hour at intensities between 55% and 70% VO$_{2\text{max}}$. The 7 trials consisted of:
high GI potatoes, sucrose, confectionary bar; moderate GI rice, potatoes plus fat (margarine), and rice plus fat (margarine); and fasting control. Fat comprised 35% energy in the confectionary bar and the pre-exercise meals that contained the added fat. The researchers did not test exercise performance, but observed a significant blunting of glycemic response with the addition of fat to both the rice and potatoes. Also, the addition of fat to the potato led to a decreased insulin surge in the athletes. This suggested that a combination of carbohydrate and fat together bring about the goal of slowing glucose into the blood and blunting the insulin surge, which could at least theoretically favor exercise performance through enhanced fuel utilization.

In the study by Rauch et al. (64) a mixed energy bar (39% carbohydrate, 32% fat, 29% protein) did not improve performance over an isocaloric carbohydrate only beverage ingested prior and during a 330-minute cycle, but impaired performance in a later high intensity time trial. During the endurance ride at 55% VO\(_{2\text{max}}\), fatty acid oxidation was enhanced, indicating increased contributions to the availability and use of the mixed macronutrient fuel source from the bar over the carbohydrate beverage during the moderate intensity, prolonged exercise. However, this favorable effect was lost at higher intensities, when the athletes completed the performance trial.

\textbf{C.4.4 Whole and Refined Foods}

A newer approach to pre-exercise fueling research emerges largely from the studies that have sought to evaluate the effects of GI and performance over the past decade. Using "real foods" in sports nutrition has been gaining interest recently due largely to the interest in glycemic interest and in part to finding practical real
food responses. Researchers have been using real, whole foods, and combinations of whole foods in the form of a meal as pre-exercise treatments in sports nutrition studies with endurance athletes. A whole food generally contains a mixture of macronutrients, in addition to other specific vitamins, minerals and phytochemicals. There is also some research starting to look at refined, solid foods like energy and sports bars, gels, and pastes. Very little has been done, however, in comparing whole foods with refined foods, but it appears that this may be the direction in which some researchers are moving.

Foster et al. (28), who used milk as a treatment compared with 75 grams glucose or water control, is likely the first study that used a real food (beverage) to test the effects on performance and fuel utilization. Compared with the water control, they found no differences in performance time or fuel levels when milk (10 g pro, 12.5 g fat, 15 g CHO) was consumed 30 minutes prior to cycling to exhaustion at 80% $\text{VO}_{2\text{max}}$. Thomas and colleagues (8) were the first to compare isolated foods and nutrients of different glycemic indexes, lentils, potatoes, glucose and water, and found improvements with lentils over the other treatments. Horowitz and Coyle (3) also used isolated foods and combinations of foods with fat (margarine) to test the effects of various GI and mixed macronutrient foods. They were the first to comment that the information from their study “shows how the consumption of ‘real foods’ before exercise influences substrate metabolism during subsequent exercise” (3). Since then, researchers have increasingly used real foods as a pre-exercise treatment when evaluating the effects of various pre-exercise fuels.

The literature is limited on whole foods used as pre-exercise fuel, restricted mostly to isolated foods like lentils and potatoes (3, 8, 44, 56, 63), rice (3), muesli (55), bananas (59), and high fiber oats (51, 52, 77). Other studies use combinations of whole
foods like breakfast cereals, fruit, and milk (7, 50, 84) or combinations of potatoes, tuna, corn, crumpets and honey (44). Most of the whole foods research has been in comparing low GI to high GI foods or combinations of foods, though Murdoch et al. (59) used bananas in solid and slurried variations to consider the effect of pre-exercise fuel form on performance. A challenge with many test meals used in prior studies has been the lack of control of calories or macronutrient profile, as discussed in the previous section. Therefore, the results should be interpreted with caution, though there does appear to be a trend toward improved performance and beneficial fuel availability with low glycemic load over high glycemic load whole foods or meals.

While using whole foods as pre-exercise fuel is a growing and important area of research, many of the whole foods tested to date such as lentils, potatoes or rice are typically not practical or preferred options by athletes as an early morning meal or snack (since most endurance events begin early). Additionally, foods tested in previous studies are not always convenient to use as pre-exercise fuel since they require preparation, and some like lentils may potentially cause gastrointestinal distress, an undesirable side effect during endurance events. There appears to be a growing interest and need for research on convenient and practical whole food sources.

If a whole food has other nutritional attributes like fiber, antioxidants or heart-healthy fats, there is a potential for additional health benefits from consuming them.

While whole foods for sport nutrition is a newer area in modern science, athletes and the general population have been consuming items like dried fruits, fruit, nuts, seeds and whole grains to sustain energy for centuries, at least as far as the early Greek Olympics and biblical times (85).
Currently, a large number of athletes and active individuals are turning to commercially manufactured products such as sports bars and beverages to enhance performance. The majority of products that are used are processed from a variety of ingredients and supplemented with a vitamin and mineral cocktail. Commercial products can be designed to have optimal nutrient composition based on sound sports nutrition principles and are typically convenient to use, but they are often expensive and may lack several nutrients and other plant compounds that are naturally found in whole foods.

The literature on processed foods is recent, and while it is certainly increasing, published studies are still lacking. Most research has been published only in abstract form, primarily looking at glycemic response and availability of fuels in the blood and not yet at performance outcomes. Of the studies being done, some compare the carbohydrate-only pastes and gels available on the market, while others are looking at mixed macronutrient commercial sports or confectionary bars and gels (7, 64, 86, 87). In one study with a sports bar, the benefit of increased fat oxidation was observed during exercise after ingesting the mixed nutrient bar over a carbohydrate-only liquid solution of equal calories, but performance was impaired later during a high intensity time trial (64).

Doyle et al. (65) looked at liquid, solid or gel carbohydrate with 20 males and their glycemic responses during exercise. No differences were seen overall, though the time course of glucose and insulin response varied between the different carbohydrate types. Hotell et al. (86) tested blood glucose levels during a 2 hour run after subjects ingested a refined carbohydrate paste versus a placebo consumed before and during exercise. The paste maintained blood glucose significantly higher than placebo, but performance was not evaluated. Rauch et al. (64) tested a mixed nutrient sports bar
versus an equicaloric carbohydrate snack. The bar showed enhanced fat metabolism in 6 male cyclists over the carbohydrate treatment during submaximal exercise, but impaired performance later during a high intensity time trial. Kolkhorst and colleagues (87) compared a name brand, commercial activity bar (contribution energy: 70% CHO, 20% fat, 10% protein) to water placebo consumed 15 min before exercise to exhaustion at 73% VO₂max with seven trained distance runners and found no differences in CHO or fat utilization or improvements in exercise.

Clearly, this is an area of newer investigation with inconsistencies in findings likely due to wide variations in the research protocols employed and differences in overall nutrient composition of the refined foods being tested. While researchers have begun to utilize whole and refined foods as treatments in testing performance and fuel use outcomes, this is still a new area in sports nutrition research. There are no published studies to our knowledge specifically or intentionally comparing the effects of whole food versus processed or refined pre-exercise fuel sources on endurance exercise, though the field appears to be moving in this direction.

D. SCOPE OF FUTURE RESEARCH IN PRE-EXERCISE FUELING

The field of sports nutrition is constantly evolving as athletes and researchers continually strive to find information to provide maximum benefits for performance. The research to date is challenging to interpret but trends in its progress are becoming clear. The goal remains the same: to optimize performance through the maximal usage of fuel stores and maintenance of a ready supply of glucose in the blood. Finding pre-exercise meals, snacks, and beverages that can bring about these effects have a real and practical
application, as snacking during competitive events can be challenging and deleterious to actual performance outcomes like race times. In describing the progression of research on pre-exercise fueling, one can note many gaps in the literature, but a shift from the traditional carbohydrate-only snack to a mixed nutrient snack that may come from a whole or refined food appears to be on the horizon.

It is clear from this review that the type, amount, timing, GI, nutrient mixture, and the source of the pre-event snack are interrelated and affect performance during events of long duration. A habitual diet that is high in carbohydrate and moderate in fat (25-30%) appears to benefit exercise performance and overall health. Pre-exercise fat along with carbohydrate may increase fatty acid levels and breakdown, decrease the GI of the meal, snack or beverage, blunt the insulin responses, and maintain a steady level of blood glucose. However, a question that remains to be investigated is whether a combination of carbohydrate and fat will translate into increased performance. While research on whole food and refined foods is lacking per se, there is a real opportunity in incorporating what is known in the literature to date with current practices of athletes to generate informative and important research that will come to bear on endurance performance indicators in the future.

E. PRACTICAL RECOMMENDATIONS

While there are still questions to be answered, pre-exercise fueling has been shown to benefit performance and enhance endurance in athletes. In counseling athletes on sports nutrition, however, the first priority is to consider his or her individual needs, preferences, and tolerances. The pre-exercise fuel should be convenient, satisfying, and
well-tolerated in the preferred form whether liquid or solid. Additionally, it is of foremost importance that any pre-exercise fuels not cause excess fullness or gastrointestinal discomfort (cramps, nausea, or vomiting) during exercise.

Individual preferences can vary dramatically. For example, some athletes find they cannot eat solid food in the hour prior to exercise without experiencing gastrointestinal distress and therefore must rely on liquid options, while others can tolerate food several hours prior but none at all in the immediate time preceding the event. Adjustments in pre-exercise fueling regimens should be made accordingly.

Glucose and sucrose have shown to benefit endurance athletes over water, but fructose alone is not practical and should be limited since it tends to cause gastrointestinal discomfort in many athletes. On the other hand, since fructose has a low GI and is absorbed more slowly in the intestine, there may be some benefit to its use when consumed in combination with other sources of carbohydrates.

Research to date tends to support lower GI foods and a lower absolute intake of carbohydrate on par with 0.7-1.0 g CHO·kg⁻¹ BW in the hour prior to exercise to avoid large increases in insulin and drops in glucose, leading to a premature reliance on carbohydrates and glycogen stores for fuel. While the effect on performance is still controversial, lower GI and mixed macronutrient foods that include moderate amounts of fat do appear to promote a favorable fuel ratio in the blood during exercise which may lead to increased use of fatty acids as fuel.

Whether whole food sources will lead to equal, worse, or better performance outcomes and fuel availability and use during endurance exercise over commercially manufactured sports products is still unknown, though common sense would suggest
whole foods would be a safe, practical and potentially beneficial pre-exercise fuel.

Researchers now have been presented the challenge and opportunity of further evaluating their benefits through the modern scientific process.
REFERENCES


CHAPTER THREE

METHODS

A. STUDY DESIGN

The Nutrition and Exercise study was a randomized, balanced crossover experimental design. Participants were randomly assigned to one of the two treatment beverages: a whole food, mixed macronutrient almond-raisin beverage or a commercial sports carbohydrate-only beverage as a pre-event snack prior to running to exhaustion on a treadmill. After a two-week period, the exercise test was repeated with the subjects reversing the treatment. This design allows for each participant to serve as his own control.

All aspects of the study were conducted at Loma Linda University (Appendix 3.1).

B. SUBJECTS

Participants in this study were healthy, male endurance runners and triathletes between 20-35 years of age (28.0±1.2 years). The participants were willing to maintain the same training schedule throughout the study period and adhere to the diet and exercise protocol outlined in this study. The male runners all regularly ran thirty or more miles per week.
B.1 Recruiting and Screening

B.1.1 Recruiting

Participants were recruited by advertising through regular media channels (local newspapers, email, and University newsletter) and via posters and flyers posted on the campuses of Loma Linda University; University of California, Riverside; and California State University, San Bernardino. Loma Linda University has a state-of-the-art fitness facility that attracts a number of endurance athletes from the local area who come and train regularly. This facility provided an additional site from which potential participants were recruited. The Loma Linda Lopers, the largest running club in Southern California, also served as a source of potential participants. Recruitment flyers were distributed at weekly workouts for the Lopers by a member of the club. A designated phone line and study-specific email address were set up to receive requests for more information and as a vehicle for ongoing communication with participants.

B.1.2 Screening

A four-stage screening process based on the exclusion criteria detailed below was carried out leading to final selection of study participants. First, interested athletes were called and interviewed over the telephone to assess initial criteria including interest, gender, availability, and who were apparently healthy according to pertinent exercise, diet, medical and schedule-oriented screening questions (Appendix 3.2). Based on the phone screening, those potential subjects who appeared eligible were moved into the next stage.
Second, potential participants who completed the phone interview and met the criteria were invited to a group meeting to discuss the study in more detail. After hearing about the study, interested potential participants were asked to complete a comprehensive general medical history questionnaire (Appendix 3.3), sign an informed consent (Appendix 3.4) and the California Experimental Subject's Bill of Rights (Appendix 3.5), and have a face-to-face interview (Appendix 3.6) with a member of the research team, in addition to completing a 24-hour dietary recall. The responses to the interview questions, 24-hour dietary recall, and informed consent were subsequently reviewed by the primary investigators and the medical history questionnaire was reviewed by a physician.

Potential participants who still met the criteria for selection were scheduled to complete a fasting blood chemical panel to test blood lipids, glucose and electrolyte levels at the Center for Health Promotion at Loma Linda University. Individuals who had values within the normal ranges were then invited to the final screening stage to complete a progressive exercise test to exhaustion (VO$_{2\text{max}}$) on a treadmill to determine their maximal oxygen consumption and electrocardiogram (ECG) test (Appendix 3.7). The results of the VO$_{2\text{max}}$ treadmill test were reviewed by the primary investigators and the ECG printout was evaluated by a physician for any heart rhythm anomalies.

In addition to assessing the fitness status of the participants, the VO$_{2\text{max}}$ screening test also served to familiarize eligible runners with the experimental procedures. Also, for the men that met the criteria of a VO$_{2\text{max}}$ over 50 ml/kg/min., the test results were used to calculate the workload speed and incline corresponding to 70% VO$_{2\text{max}}$ and higher at which all the exercise tests during the study were conducted.
B.1.3 Selection

Based on these screening procedures, participants were invited to participate in the study if they were healthy, had a normal blood chemistry profile, a VO$_{2\text{max}}$ of $\geq 50$ ml/kg/minute, and had not donated blood in the past two months. In addition, participants were selected to enter the study if they continued to meet all of the study criteria and were willing and able to adhere to the training, diet and exercise protocol for the duration of the data collection period.

B.1.4 Incentives

Athletes who participated in the study received a complimentary blood chemical analysis and VO$_{2\text{max}}$ test along with professional interpretation of results at the conclusion of the study. Participants also received meals for the day prior to testing and after completion of each test run. The subjects also received a 45-minute private sports nutrition counseling session following the data analysis phase. Finally, a monetary incentive of $100.00 was given to participants who successfully completed the study.

B.2 Exclusion Criteria

Recruited individuals were initially excluded if they were: female, outside the age range of 20-35 years, not healthy, managing an existing injury, taking multiple supplements, allergic to any ingredient in the test beverages, going to be competing or traveling during the data collection period, and not running at least 30 miles per week on
a regular basis. Subjects with any medical condition or sub-normal nutritional status identified by the blood chemical profile were also excluded from the study as well as those who donated blood within the last two months. Finally, anyone unwilling to maintain his training status and adhere to the diet and exercise protocol for the duration of the study was excluded.

B.3 Sample Size

Based on conservative sample size calculations (Power & Precision, Release 1.20, 1997) for a paired samples t-test with \( \mu = 0 \), using endurance time (time-to-exhaustion) as the primary outcome, at an \( \alpha \) of 0.05, 10 participants were necessary to complete the testing to achieve a minimum of 80% power. Power was determined using a paired samples t-test comparing means. Allowing for a dropout rate of 20%, 13 participants were initially eligible and recruited to participate in this study. Based on previous experience at Loma Linda University in conducting even more rigorous human feeding studies (1, 2), a very high degree of retention was anticipated. Two eligible subjects were unable to enter the study due to personal reasons. Therefore, eleven male runners initially entered the study. One athlete dropped out after the first test run due to family reasons and a non-study related sports injury. Ten runners completed both test runs.
C. PROTOCOLS AND PROCEDURES

C.1 Screening Questionnaires

For the screening phases of the study, a number of questionnaires, procedures, and data collection forms were created to facilitate a thorough screening. The telephone and face-to-face interview questionnaires were developed using a similar format and some of the same questions as a basis from the nut feeding studies done over the past several years at Loma Linda University. Additional questions were added to address specific medical, dietary, supplement usage, and exercise related issues that could have affected selection (Appendices 3.2 and 3.6). The medical history questionnaire from the Loma Linda University Medical Center’s Department of Internal Medicine was utilized in the second phase of screening (Appendix 3.3).

Screening protocols were developed for conducting a VO$_{2\text{max}}$ and ECG test on potential participants based on the manual of instructions for the SensorMedics M Vmax29 Series Metabolic Cart (SensorMedics Corp., Yorba Linda, California), Quinton Q1000 ECG Testing System (A-H Robins Company, Saint Davids, Pennsylvania), and Quinton Q55 Treadmills (A-H Robins Company, Saint Davids, Pennsylvania). A data collection sheet was created to notate information relevant to the operation of the VO$_{2\text{max}}$ and ECG screening tests, as well as for necessary calculations and subsequent administration of actual trial runs (Appendix 3.7).
C.2  Test Beverages

Based on a macronutrient composition established for the almond-raisin test beverage of 1.0-gram carbohydrate per kilogram body weight and 20% energy from fat, a food scientist in the Department of Nutrition utilized the department’s metabolic kitchen to design and standardize a recipe for a 72-kilogram person (Appendix 3.8). After employing a variety of techniques and developing several sample beverages that met the macronutrient profile, members of the department and school sampled a variety of different consistencies and blends of the test beverage. From the taste panel, two beverages were selected for a public taste test to compare a roasted almond/soaked raisin blend versus a soaked and blanched almond/soaked raisin blend.  

A double-blind pilot taste test was conducted in the University’s health and fitness center. Twenty-five recreational and competitive athletes participated in the taste test. A summary of the taste test is provided in Appendix 3.9. Based on the overall ratings, the final preferred recipe was the soaked and blanched almond/soaked raisin blend.  

Based on the standardized recipe, adjusted recipes were created for each of the initial 11 subjects who entered the study based on their body weight to provide 1.0 gram carbohydrate per kilogram and 20% energy from fat (Appendix 3.10). Total kilocalories were also calculated based on the individualized recipes, ultimately providing 5.26 kilocalories per kilogram body weight. The individual beverage ingredients were prepared and weighed the night prior to each test run, stored in separate airtight containers, covered with aluminum foil to keep light out, and refrigerated or frozen based on the ingredient. The complete test beverage was blended from the individual
ingredients on the morning of the test run, 15 minutes prior to consumption and stored in a refrigerator.

The commercial, carbohydrate test beverage was lemon-lime flavored Gatorade® Instant Mix powder. Gatorade® provides 14 grams carbohydrate per 15 grams weight powder. Calculations were made for each of the initial 11 subjects to determine the gram weight of powder needed to provide an isocaloric beverage to the almond-raisin beverage (Appendix 3.10). Gatorade® powder for each subject was weighed and stored in sealed and labeled plastic bags and placed in a dark, cool room until needed. On the morning of the test, the Gatorade® beverage was blended with an equal volume of water as calculated for the almond-raisin beverage 15 minutes prior to consumption and stored in the refrigerator. The concentration of Gatorade® was much greater than recommended in the general directions for the product due to controlling for volume of water. The Gatorade® beverage whose calories are derived exclusively from carbohydrates (sucrose, dextrose) provided the athletes with 1.5 grams per kilogram body weight. Additional ingredients in Gatorade® include citric acid, salt, sodium citrate, monopotassium phosphate, natural lemon and lime flavors with other natural flavors, and Yellow 5. The macronutrient composition of both test beverages can be seen in Appendix 3.11.

C.3 Diet for the Day Prior to Testing

Based on previous research, it is known that with adequate consumption of carbohydrate glycogen stores can be replenished to normal concentrations in 24 hours (3, 4). Additionally, scientists recommend between 7 and 10 grams of carbohydrate in training and pre-event diets of endurance athletes (5-9). Based on this rationale, a partial
diet was designed to provide 7 grams of carbohydrate per kilogram body weight for each study participant to consume in the 24 hours prior to their test run. Guidelines were given to each athlete (discussed below) describing that the foods provided to him comprised mainly carbohydrate sources and did not represent their complete macronutrient or caloric needs for the day. The goal was to provide convenient and familiar food items that would contribute a minimum of 7 grams of carbohydrate per kilogram body weight to ensure adequate and equal glycogen stores between tests. The subjects were encouraged to make sure to finish the contents of their food bags, but to also eat additional food items to fulfill their protein, fat, and energy needs for the day. The subjects were asked to notate additional foods and amounts consumed, bring the food record form to their initial test run, and then repeat their exact diet again on the day prior to the second test run.

The diet was created to provide familiar, portable, easy-to-prepare, commercial food items that could be easily fit in the diet of the study subjects. Nutritional content of all food items was analyzed using Nutritionist Five™ software (First Data Bank, San Bruno, California) from which a reference diet was created for a 72-kilogram body weight man. From the reference diet, calculations and adjustments were made for each of the 10 subjects. A table of the sample food contents and amounts is provided in Appendix 3.12. All items were weighed using the gram scale and portioned into resealable bags or disposable containers. Some of the familiar items included a plain bagel, whole wheat bread, pasta and sauce, yogurt, a bean and cheese burrito, name-brand cereal, crackers and cookies. No nuts, dried fruits, or sports beverages, bars or supplements were permitted in the diet during the day prior to testing. In addition,
complementary items (non-carbohydrate) like cream cheese and other condiments were also provided as optional so the athletes could minimize their food preparation and expenditures for the day.

C.4 Guidelines for the Week and Day Prior to Testing

Study subjects were emailed and called one day preceding the week prior to their test date. They were provided dietary and exercise guidelines created for this study to follow for the whole week before their test date (Appendix 3.13). Some of the guidelines included abstention from alcohol or caffeine, avoidance of nuts, dried fruits, or dietary supplements (other than a standard multivitamin and mineral). When agreeing to participate, the athletes had also been asked to abstain from using any antioxidant supplements for the duration of the study. In terms of exercise, the athletes were asked to continue with their normal exercise regimen, but to avoid any long runs in the few days prior to testing. Instead, they were asked to treat their test run as their long distance run for that week.

Several days prior to testing, arrangements were made to deliver to each participant a bag of convenient, familiar, mostly carbohydrate foods to consume one day prior to his test. During the brief contact with the athlete to provide them their food, they were also provided dietary and exercise guidelines for the day prior to testing (Appendix 3.14). These guidelines were created for this study and explained the contents of their food bags, how to supplement their diet in the day prior, and when to consume their last meal. The guidelines also provided a food record form to track additional foods eaten that they would repeat for the second test (Appendix 3.15). In addition, the subjects were
each given individualized written explanations about the contents of their food bags, how to incorporate additional foods and calories into their day, hydration recommendations, and a checklist of the contents of their bags so they could self-monitor their completion of the contents (Appendices 3.16-3.17).

The participants were instructed to eat something near, but not after, 8:30 p.m. on the night before the test run. Each athlete was called in the evening before 8:30 p.m. to remind him about the test run, review the guidelines, answer questions, and to ensure compliance and consistency of eating times between test runs. The runners were instructed that they should drink water, but no food before arriving to the lab the following morning.

C.5 Protocols During Exercise Runs

Guidelines were created for all aspects of the data collection during the test runs to ensure that the research staff was exact in their recording of measurements and consistent between subjects and within subjects (Appendices 3.18-3.20). The core research team had primary responsibilities, but could oversee or assist with other areas as needed. Additional research assistants had specific duties that they learned and performed with accuracy. All of the research staff attended to the needs of each athlete regarding special requests for extra towels or miscellaneous issues. However, only one researcher for the duration of the study was permitted to coach each athlete during the test run and communicate with him regarding his perceived exertion ratings and specific data-sensitive issues. This helped to provide an unbiased, consistent testing environment between and within subjects’ test runs. Detailed, easy-to-use data collection forms were

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created to facilitate consistent recording of multiple data points during each test run, as well as supplemental checklists and guidelines for the overall set-up and use of the exercise testing laboratory during data collection (Appendices 3.21-3.23).

C.6 Protocols for Blood Sampling and Handling

Procedures and protocols were developed to ensure that blood draws, as well as the handling and processing of samples were safe and consistent within and between subjects (Appendices 3.24-3.25).

A designated, single purpose, movable cart was cleaned with antibacterial, hospital grade disinfectant and restocked each afternoon for the following morning test run. All items were stored in a sealed container until set-up upon the sterile surfaces the following morning at 6:00 am, 30 minutes prior to the arrival of the athlete to the fitness-testing laboratory. Universal Procedures were followed by all members of the research staff for all blood draws including changing and disposal of latex gloves between blood draws and proper disposal of any real or potential biohazard materials in appropriate Sharps containers or biohazard bags. All blood-related materials were single-use, standard, FDA-regulated disposable products.

The protocol that was developed is briefly described here and can be seen in more detail in Appendix 3.24. Blood samples were drawn: upon arrival to the fitness lab at rest, 60 minutes after consumption of the pre-exercise beverage (just prior to starting the test run), every 20 minutes throughout the run, immediately upon termination (exhaustion), and 30 minutes after termination of the test run. The fasting blood was drawn using a standard needle-stick (Vacutainer™, Sterile 21Gx1) procedure into a 10-

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milliliter Vacutainer™ tube. Fifty minutes after the subject consumed the test beverage and warmed up for 5 minutes on the treadmill, a catheter (BD Angiocath I.V. Catheter, 20 GA, 1.16 in. 1.1 x 30 mm, 49 ml/min.) was inserted into the anti-cubital vein in the forearm. A blood draw occurred at that time and was transferred manually with a slow drip procedure into an open Vacutainer™ tube. The catheter was secured with medical tape and a loose Velcro elastic bandage was wrapped on the forearm to prevent the catheter or tape from coming loose during the test run.

All subsequent blood draws during the test runs were performed through the catheter as swiftly as possible while the athlete was leaning or sitting on a stationary table within five feet of the treadmill. After a rubber tourniquet was secured to the bicep region of the arm where the catheter was located below, blood was drawn into the syringe and then transferred into the open Vacutainer™ tube through a slow drip procedure. The catheter was then flushed with a standard 0.9% Sodium Chloride Injection, USP, 5 cc I.V. Flush before recapping the catheter. The site was promptly cleaned, re-taped and secured with the elastic bandage so the athlete could quickly resume his run. Exact times were recorded indicating the treadmill dismount and remount times, as well as the exact time and duration of each blood draw. The final blood draw, 30 minutes after termination of the test run, was conducted using a direct stick method similar to the fasting blood draw.

A separate protocol was developed for the processing of the blood immediately after collection (Appendix 3.25). Approximately 10 milliliters of blood were collected at each sampling and left at room temperature to clot for 20 minutes. The sample was then centrifuged (Clay Adams® Brand TRIAC® Centrifuge, Becton, Dickinson and
Company, Franklin Lakes, New Jersey) for 10 minutes in the adjoining room of the fitness laboratory, and the serum was immediately separated into 1 milliliter and 0.5 milliliter aliquots labeled with subject number, test date, and blood collection sequence. Blood samples were then transferred to an intermediate biochemical samples refrigerator. After all samples were aliquoted for the morning, they were transported in dry ice to the Revco Deep Freezer (Scientific Refrigeration, Orange, California) in the Department of Nutrition and stored at -80°C.

D. STUDY PROTOCOL

D.1 One Week and One Day Prior to Testing

Contact was made via phone, email, and in-person according to the protocol aforementioned in section C.4 and elaborated in Appendices 3.13-3.17. In the research kitchen, all provided foods were measured, weighed, and prepared to standardize total mandatory carbohydrate intake to 7 grams per kilogram body weight as described previously. Participants were allowed to eat and drink additional foods and beverages as long as they were permissible according to the written guidelines and also recorded on the food record form provided for the athletes. The subjects were then instructed to repeat the same diet prior to the second test run.
D.2  Test Day

D.2.1  Prior to Exercise

On the day of the exercise test, one participant per day would arrive at the testing lab by 6:30 a.m. following a 10-hour overnight fast and having refrained from any strenuous activity 24-hours prior to each trial. Upon arrival to the performance lab, an initial fasting blood draw was conducted. Resting blood pressure and pulse were recorded and after 15 minutes, the athlete was provided one of the two pre-exercise test beverages providing 1.0 gram (almond-raisin) to 1.5 gram (Gatorade®) carbohydrate per kilogram body weight that were designed to be isocaloric and of equal volume. The athlete was instructed to consume the test beverage within 10 minutes and then to relax for one hour.

During the period of rest, the athlete received instructions about the testing procedures for the test run. This was repeated during both tests for consistency and to remind the subject of the protocol. Fifty minutes after consumption of the beverage, the athlete was fitted with a Polar A3™ Heart Rate Monitor (Polar Electro, Inc., Woodbury, New York) chest strap and the removable headgear that allows for collection of respiratory gases with the SensorMedics M Vmax29 Series metabolic cart. The subject then warmed up at a self-selected (maximum 3.5 mph) walk for 5 minutes.

After the warm-up period, the athlete dismounted the treadmill and the 20-gauge catheter was inserted into the anti-cubital vein in his forearm for blood sampling. A blood draw was completed at the time of the catheter insertion.
D.2.2 Test Run

At the end of one hour, each athlete began his run to exhaustion at 70% VO_{2\text{max}} on a motorized treadmill. Three stopwatches, the treadmill clock, and the metabolic cart timer were all started simultaneously in case of any interruption or malfunction of one or more timers during the test. All timers were covered or placed out of the view of the athlete. All staff researchers utilized a single centralized clock behind the athlete when recording measurements and times in order to assure consistency and accuracy of times and data recorded. Ambient room temperature was set to a level comfortable to the athlete, recorded, and repeated for his second test run. Music (radio, compact disc, or MP3) was permitted for each athlete, recorded, and repeated (same station, compact discs, or MP3 programming) on the second test run.

During the test, participants were given 4-5 ounces (120 mL) of water every 20 minutes during the exercise test to prevent dehydration (7, 10-12). A series of measurements were taken and recorded on a pre-determined schedule for heart rate, rating of perceived exertion, respiratory gases, and blood draws, discussed in more detail below. If a study participant reached 90 minutes on the treadmill, the speed and incline were incrementally increased by 5% of his VO_{2\text{max}} every 10 minutes until exhaustion. When the subject’s RPE reached 17 on the Borg scale or he communicated being near the end of his performance ability, a series of coaching cheers would commence to encourage the athlete to keep going as long as he could (Appendix 3.20). The exercise test was terminated when the participants showed or communicated complete fatigue, defined as the time at which they could no longer maintain the pre-determined workload or if they complained of muscle cramping, nausea, lightheadedness or inability to continue.
Participants and researchers were blinded to time-to-exhaustion. The exercise test was performed at the same time during both test periods and the two tests were separated by two weeks for each participant.

D.2.3 Post Exercise

Recovery consisted of water consumption ad libitum and a cool down period prior to the final blood draw thirty minutes post-exhaustion. Upon completion of the final measurements, the subject was invited to a post-exercise snack and provided a bag of food to take with him. Follow-up calls were made to each athlete on the day following his test run to ask how he was feeling and answer any questions.

E. DATA COLLECTION

E.1 Body Weight, Height, and Body Mass Index

Body weight, height and body mass index were measured (Health-O-Meter 400 DLT, Continental Scale Corporation, Chicago, Illinois) and calculated during the VO_{2max} treadmill test to exhaustion during the fourth phase of screening, and used to design the individualize test beverage recipes and carbohydrate composition for food provided for consumption during the day prior to testing.

E.2 Heart Rates and Ratings of Perceived Exertion

A resting heart rate measurement was taken after the subject arrived to the fitness lab. Additional heart rates were recorded at the start of the exercise test run, every
15 minutes during the test run, immediately upon exhaustion, and 30 minutes post-exercise. Heart rate measurements were taken manually and with a Polar A3™ Heart Rate Monitor during the test run.

Ratings of perceived exertion (RPE) were collected after 3 minutes and then every 30 minutes throughout the test run using an adapted Borg scale from ranging from 6 to 20 (Appendix 3.26; 13-16). Additional RPE scores were recorded at variable times and more frequently toward the end of the test so the research team could monitor the athlete’s state of fatigue and to gain a subjective estimate of how far he was along in the test.

E.3 Respiratory Gases and Time-to-Exhaustion

Respiratory gas measurements were monitored and collected at the beginning of the test and then every 20 minutes throughout the test run until exhaustion to calculate respiratory exchange ratios to determine substrate utilization, VO₂, and VO₂ per kilogram body weight. Each serial measurement lasted 3.5 minutes. Exact times were notated and subsequent averages were drawn from a 2-minute sample beginning 30 seconds into each gas measurement using 10-second intervals.

The time-to-exhaustion was recorded at the end of each test period for each athlete by two members of the research team.

E.4 Blood Sampling

Blood samples were drawn according to the protocol previously described in this chapter and elaborated in Appendices 3.24-3.25.
F. ANALYSES

F.1 Biochemical Analyses

Serum samples were shipped in duplicate overnight in dry ice to the Center for Exercise Research Substrate Metabolism Laboratory at the University of Michigan, Ann Arbor. Additional aliquots remain in the Loma Linda University Department of Nutrition’s deep freezer at -80°C for potential future analyses.

Serum samples were analyzed for serum insulin, glucose, lactate and free fatty acids. Colorimetric assays were used to assess serum concentrations of both glucose (Trinder-glucose kit, ThermoDMA, Louisville, Colorado) and fatty acids (NEFA-C kit, Wako Chemical USA, Richmond, Virginia) using a spectrophotometer (Biorad, Hercules, California). Serum lactate was determined by measuring the absorbance of NADH after an endpoint enzymatic reaction (17). Serum insulin concentrations were measured by radioimmunoassay (Linco Research Inc, St Louis, Missouri).

F.2 Statistical Analyses

Using the Statistical Package for the Social Sciences Release 10.0.7 (SPSS, Inc., Chicago, Illinois) statistical software, results were analyzed with time-to-exhaustion as the main outcome variable of interest. Results are expressed as means ± SEM. For time-to-exhaustion, the data from the two test periods were compared using a paired samples t-test to compare the difference in means. The assumptions for utilizing a paired samples t-test for this analysis are that the difference variable (created by
subtracting one measured time-to-exhaustion from the other) is normally distributed and that the difference scores are independent of each other (18). Additional analyses were conducted on RER, VO₂, HR, RPE and serum levels of glucose, free fatty acids, insulin, and lactate using analysis of variance (ANOVA) for repeated measures (beverage x time) to evaluate changes over time for time-dependent variables. The assumptions for this test include that the dependent variables are normally distributed for each group, that the variances of the dependent variables are the same for all populations and that the scores on the test variables are independent of each other (18). A paired samples t-test was used to isolate significantly different means when ANOVA indicated significant main effects and to compare within trial changes over time. Statistical significance was set at $P < 0.05$.

Missing data for blood measurements were handled by imputing the within trial means for the corresponding time point, so that a specific case would not be dropped from the analysis, but would also not bias the findings or inflate a treatment effect. Missing blood values occurred due to investigators' decisions to not send exercise-induced hemolyzed samples for analysis, so the analyses of substrate concentrations would yield more accurate results. Of 155 total samples collected during testing, 121 (~80%) were shipped in duplicate and subsequently analyzed.
CHAPTER FOUR

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Pre-Exercise Fueling and Endurance: Effect of an Almond-Raisin Beverage Versus Sports Beverage on Performance in Trained Male Runners

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ABSTRACT

Introduction: What athletes consume before exercise longer than 60 min influences fuel use, perceived exertion (RPE), heart rate (HR) and time-to-exhaustion (TE). Ingesting carbohydrates (CHO) before endurance exercise can delay exhaustion and favor fuel use over placebo, but less is known about combinations of CHO and fat.

Purpose: To compare the effects of 2 isocaloric beverages on substrate availability and endurance capacity: almond-raisin (AR) beverage (whole food, mixed macronutrient) and a commercial sports (CE) beverage (CHO-only and electrolytes).

Methods: In a randomized, crossover study, 10 trained, male runners ran to exhaustion at 70% VO_2max twice, separated by 2 weeks. After a fasting blood sample, subjects drank one of the test beverages, rested for 60 min, and began the trials. Serial measures were collected including blood samples, respiratory exchange ratios (RER), HR and RPE in 15-30 min intervals. TE was recorded at the end of each run.

Results: Mean TE was similar between AR and CE trials. However, a significant difference was seen in insulin concentrations in the AR compared with the CE trials throughout exercise, with a significant blunting of insulin observed during the AR trial. Free fatty acid (FFA) concentrations were significantly higher in the AR group through 60 min, though the between trials effect did not remain significant through 30-min post-
exercise. Serum glucose and lactate concentrations, RER, HR, and RPE were similar between the AR and CE trials.

**Conclusion:** The pre-exercise almond-raisin beverage showed favorable responses in insulin and FFA concentrations during exercise. TE was similar after consuming both the AR and CE beverages. Therefore, the AR beverage may be considered a practical alternative to commonly used commercial sports beverages for endurance exercise.

**Key words:** Pre-exercise feeding, endurance, exercise performance, treadmill running, whole food.
A. INTRODUCTION

For endurance events lasting over 60 min in duration, pre-, during and post-exercise nutrition are important. It is well recognized that ingestion of carbohydrates before and during endurance events over one hour improves performance over non-caloric placebos, increasing time-to-exhaustion or amount of work accomplished, as well as influencing fuel use, respiratory exchanges, perceived exertion, and heart rates (17, 22, 27, 43, 45, 48).

Traditionally, researchers have looked primarily at carbohydrate-based meals, snacks, and beverages as preferred sources of pre-exercise fuel. This is because glucose provides a primary fuel source in the blood from digestion of quick absorbing carbohydrates and the ready mobilization of glycogen stores. Some (24, 27, 42, 45), but not all (2, 9) studies have demonstrated improved performance in athletes with a pre-event carbohydrate meal, snack, or beverage. The amount (percent carbohydrate concentration or g·kg⁻¹ BW), timing (≤1 hour or >1 hour prior to exercise), form (solid versus liquid), and type of carbohydrate (chemical form, glycemic index, mixed macronutrient, whole or processed food source) ingested prior to exercise will have different magnitudes of effect on metabolism and thus performance.

Early studies have shown that when large amounts of carbohydrates (e.g. 50-75 grams) are consumed within an hour, athletes experience a spike in insulin before exercise leading to a hypoglycemic response early in exercise, accelerated glucose use and depletion of glycogen stores, fatigue and a resultant decrease in endurance (9, 14). However, several researchers have increasingly challenged this theory, suggesting that
the hyperglycemic and hyperinsulinemic responses are transient, though results on performance and substrate utilization are still varied (1, 15).

With pre-exercise feedings (0.7 to 1.5 g·kg⁻¹ BW) within approximately 1 hour, researchers have seen improved (6, 10, 20, 21, 27, 39, 42, 48), decreased (9, 14), or neutral (2, 7, 8, 21, 44) effects on performance capacity. This is likely in part due to differences in amount, glycemic index, and energy and macronutrient profiles between treatments, along with differences in how performance was measured.

Naturally, athletes like the general population, do not eat single foods or nutrients, but combinations of nutrients through foods and combinations of foods that contain carbohydrates, protein, fats, vitamins, minerals, and other phytochemicals. With this understanding added to the long-standing knowledge that fatty acids provide important fuel during prolonged exercise, researchers have begun to address the effects of adding protein and fat to the pre-exercise fuel package on exercise performance and endurance. The addition of fat to the carbohydrate-only pre-exercise fuel is gaining increasing interest in sports nutrition research, emerging from the knowledge that both carbohydrates and fatty acids are important fuel sources—with fat contributing up to 50% of fuel—during low to moderate intensity exercise of long duration (5, 46).

Of the few studies specifically addressing fat in the pre-exercise snack, the results on performance are conflicting (31, 35, 50). However, substrate concentrations do tend to reflect a fuel environment in the blood that would theoretically favor enhanced use of fatty acids and glucose, along with glycogen-sparing for prolonged endurance exercise.

Emphasis on testing the effects of “real foods” has been a more recent trend in pre-exercise fueling research, especially prevalent in studies comparing the effects foods
or meals of different glycemic indexes and/or macronutrient profiles (6, 17, 42, 50).
Many studies, though, have used foods like lentils, potatoes or rice that may not be the most practical, convenient, portable, or well-tolerated by athletes before a typical morning competition. Therefore, the role of practical food sources is of interest to researchers and athletes alike (1). Nuts and dried fruits, typically consumed in trail mixes, are familiar, convenient, and healthy, real foods that may offer these qualities and favor performance parameters.

The aim of the present study was to examine the effects of two isocaloric pre-exercise beverages that differed in macronutrient composition and origin: almond-raisin beverage (CHO and fat mixture from whole food sources) and a commercial carbohydrate-electrolyte sports beverage (CHO-only and electrolytes). Both beverages were ingested one hour before an exercise trial to exhaustion. Outcomes of interest included endurance capacity measured in time-to-exhaustion, as well as substrate availability, respiratory exchange ratios, heart rates, and ratings of perceived exertion.

B. METHODS AND MATERIALS

B.1 Subjects

Ten healthy, male endurance runners and triathletes volunteered to participate in this study and completed both trials (Table 1). Of the 11 subjects that initially entered the study, one runner dropped out after the first trial due to a non-study related sports injury and personal reasons. The study received approval from the Loma Linda University Institutional Review Board and written informed consent was obtained
from all subjects. The subjects' mean weight, maximal oxygen consumption (VO$_{2\max}$), and maximum heart rate (HR) were $67.2 \pm 1.5$ kg, $61.9\pm2.1$ mL$\cdot$kg$^{-1}\cdot$min$^{-1}$ and $193\pm1.3$ beats per minute (bpm), respectively. All subjects were experienced runners, training more than 30 miles per week at the time of the study. The subjects were willing to maintain the same training schedule throughout the study period and adhere to the diet and exercise testing protocol. Subjects outside of the age range, unhealthy based on a medical history questionnaire and fasting blood chemical profile, who ran less than 30 miles per week, ingested multiple supplements or prescription medications, or had a VO$_{2\max}$ less than 50 mL$\cdot$kg$^{-1}\cdot$min$^{-1}$ were excluded from the study.

**B.2 Preliminary Measurements**

Subjects completed a series of screening questionnaires, a detailed medical history questionnaire, and fasting blood chemistry panel before being accepted into the study. As part of the four-stage screening process, subjects also completed a VO$_{2\max}$ treadmill running test to exhaustion (SensorMedics M Vmax29 Series Metabolic Cart, SensorMedics Corp., Yorba Linda, CA; Quinton Q1000 ECG Testing System and Quinton Q55 Treadmills, A-H Robins Company, Saint Davids, PA). Subjects' weight, height, and maximum heart rate were measured at that time. Calculations were made to determine treadmill speed and incline at 70% VO$_{2\max}$ and subsequent 5% increments for experimental testing. General dietary information was collected through a 24-hour recall and later analyzed with weight, height and dietary preferences to estimate caloric needs and use in planning foods for the day prior to testing.
B.3 Experimental Design

A randomized, balanced crossover experimental design was used in this study. Subjects were required to complete two exercise trials on a motorized treadmill at 70% VO$_{2\text{max}}$ separated by two weeks. Two different isocaloric treatment beverages providing 1.0 and 1.5 g CHO•kg$^{-1}$ BW were consumed 1 h prior to exercise. Subjects were blinded to their running times and were not informed of the hypotheses being tested.

Subjects were instructed to follow their usual training schedule for the duration of the study, but to refrain from their weekly long distance runs during the week of their trial. Subjects refrained from alcohol, caffeine, supplements, dried fruits, nuts, and commercial sports beverages and bars in the week preceding their exercise trial. Identical foods providing 7 g CHO•kg$^{-1}$ BW were provided for consumption 1 d before each trial to assure adequate glycogen stores and minimize differences between tests. Pre-exercise test beverages were prepared on the morning of the trial and consumed by the subjects at rest in the human performance laboratory (Loma Linda University, Loma Linda, CA).

B.4 Test Beverages

Two isocaloric beverages were used providing different macronutrient compositions. On one occasion, subjects consumed an almond-raisin (AR) beverage specially designed in the research kitchen to provide 1 g CHO•kg$^{-1}$ BW and 20% energy from fat (energy contributions: ~70% CHO, ~20% fat, ~10% protein). The AR beverage was made from whole raw almonds, Thompson seedless raisins, water and ice and provided 370 kilocalories for a 70 kg BW male (5.26 kcal•kg$^{-1}$ BW). On a second occasion, the subjects ingested a 6% carbohydrate-electrolyte (CE), lemon-lime flavored
powder, which was blended with water (Gatorade® Instant Mix powder, Gatorade®, Barrington, IL). Calculations were made for each of the subjects to determine the gram weight of powder needed to provide an isocaloric CE beverage to the AR beverage. The CE beverage whose calories are derived exclusively from carbohydrates (sucrose, dextrose) provided the athletes with 1.5 g CHO•kg⁻¹ BW and no fat. Both test beverages were blended 15 min prior to subjects' consumption on the morning of the trial. In order to control for the form of the pre-exercise fuel and equalize liquid and volume of both treatments, the almonds and raisins treatment was formulated as a beverage. Additionally, since carbohydrate content and volume determine gastric emptying time more than osmolality of different solutions (40), we provided both treatments as beverages of equal volume.

B.5 Procedures

Subjects arrived at the human performance laboratory at 6:30 a.m. after a 10-h overnight fast and having refrained from any strenuous activity for 24 h prior to each trial. After drawing an initial fasting blood sample, HR, and blood pressure, the subjects consumed one of the pre-exercise test beverages and then rested for 1 h. Fifty minutes after consumption of the beverage, the athlete was fitted with a Polar A3™ Heart Rate Monitor (Polar Electro, Inc., Woodbury, NY) and the removable headgear for collection of respiratory gases. After a slow, 5-min warm-up, a catheter (BD Angiocath I.V. Catheter, 20 GA) was inserted into an anti-cubital vein in the forearm and a blood sample was taken.
Sixty minutes following ingestion of the test beverage, subjects began the trial at 70% VO$_{2\text{max}}$. Exercise oxygen consumption (VO$_2$) and respiratory exchange ratios (RER) were collected every 20 min for 3.5 min, immediately followed by a blood draw. HR was notated every 15 min and each subject’s rating of perceived exertion (RPE) using Borg’s 20-point scale was assessed every 30 min throughout the trial (28, 32). Blood samples were collected at fasting, 60 min after ingesting the test beverage (pre-exercise), every 20 min during the trial, immediately upon exhaustion, and 30 min post-exhaustion. During the trial, subjects were required to drink at least 120 mL (4-5 oz) every 20 min at the time of blood sampling to minimize dehydration.

If a subject reached 90 min on the treadmill, the speed and incline were incrementally increased by 5% of his VO$_{2\text{max}}$ every 10 min until exhaustion. The exercise test was terminated when the participants showed or communicated complete fatigue, defined as the time at which they could no longer maintain the predetermined workload or if they complained of muscle cramping, nausea, lightheadedness or inability to continue. Attention was paid by a single investigator during both trials to encourage subjects to a similar extent throughout their trials. Participants and the principal investigator were blinded to time-to-exhaustion. The exercise test was performed at the same time for both trials separated by two weeks.

**B.6 Analytical Techniques**

Oxygen and carbon dioxide exchange was measured through expired air samples using indirect calorimetry (SensorMedics M Vmax29 Series Metabolic Cart,
SensorMedics Corp., Yorba Linda, CA). HR was measured with a Polar A3™ Heart Rate Monitor and a 20-point Borg scale was used to assess RPE.

Ten milliliters of blood were collected at each blood draw and transferred into open Vacutainer™ tubes through a slow drip procedure. Serum was then separated and stored at -80°C for future analyses. Serum samples were analyzed for insulin, glucose, lactate and free fatty acids. Colorimetric assays were used to assess serum concentrations of both glucose (Trinder-glucose kit, ThermoDMA, Louisville, CO) and fatty acids (NEFA-C kit, Wako Chemical USA, Richmond, VA) with a spectrophotometer (Biorad, Hercules, CA). Serum lactate was determined by measuring the absorbance of NADH after an endpoint enzymatic reaction (12). Serum insulin concentrations were measured by radioimmunoassay (Linco Research Inc, St Louis, MO).

**B.7 Statistical Analysis**

The data from the two trials were analyzed using analysis of variance (ANOVA) for repeated measures (beverage x time) to evaluate changes over time for time-dependent variables. A paired samples t-test was used to isolate significantly different means when ANOVA indicated significant main effects and to compare differences in mean time-to-exhaustion and within trial changes. The computer program SPSS 10.0.7 (SPSS, Inc., Chicago, IL) was used for all analyses. Statistical significance was set at \( P<0.05 \). All results are expressed as means ± SEM.

Missing data for blood measurements were handled by imputing the within trial means for the corresponding time point, so that a specific case would not be dropped from the analysis, but would also not bias the findings or inflate a treatment effect.
Missing blood values occurred due exercise-induced hemolysis of some samples (~20%) that were intentionally not sent for analyses.

C. RESULTS

C.1 Serum Insulin, Glucose, FFA, and Lactate Responses

Serum insulin responses over time were significantly different between the AR and CE trials ($P=0.02$) and post-hoc analyses revealed significant differences between trials at pre-exercise, 40 min, 80 min, and at exhaustion. Figure 1 shows a significant spike in insulin in the CE trial between fasting and pre-exercise (fasting: $9.18 \pm 1.98 \mu$U/mL; pre-exercise: $29.80 \pm 7.63 \mu$U/mL; $P=0.033$), whereas no differences were observed in this time frame in the AR trial (fasting: $9.28 \pm 1.40 \mu$U/mL; pre-exercise: $10.96 \pm 1.13 \mu$U/mL; NS). Insulin was significantly lower 60 min after consuming the AR beverage when exercise began than CE (AR: $10.96 \pm 1.13 \mu$U/mL; CE: $29.80 \pm 7.63 \mu$U/mL; $P=0.027$). Additionally, by 40 min into exercise, insulin concentrations had fallen significantly in the CE trial compared to AR, resulting in a more gradual, flat response from the start of exercise ($P<0.01$). A significant blunting of the insulin response was seen throughout exercise, with more subtle shifts in insulin concentrations over time during the AR trial compared with the CE trial. At 80 min and at exhaustion, serum insulin concentrations were significantly lower in the AR compared with the CE trial (80 min—AR: $5.65 \pm 1.18 \mu$U/mL; CE: $14.66 \pm 3.47 \mu$U/mL; $P=0.37$; exhaustion—AR: $4.54 \pm 0.52 \mu$U/mL; CE: $7.72 \pm 1.51 \mu$U/mL, $P=0.027$).
Insulin concentrations fell significantly after exercise began during the CE trial until 40 min when concentrations were significantly lower than basal levels (P=0.04). Insulin levels then rose significantly until 80 min (P<0.05) before dropping again (NS) at the termination of exercise. By 30 min post-exercise, insulin levels were similar to fasting in CE and no differences were noted between the two trials at that time. During the AR trial, insulin levels were similar between fasting to 60 min after ingestion (pre-exercise), increasing significantly between the start of exercise and 20 min (P<0.05), but returning to basal levels by 40 min into exercise (NS). Gradual increases were seen toward the point of exhaustion, and by 30 min post-exercise, insulin concentrations returned to fasting levels in the AR trial (NS).

Serum glucose responses during the AR and CE trials are shown in Figure 2. No significant differences were observed between AR and CE trials over time. There were no differences between fasting levels and any time point during the CE trial. Glucose concentrations remained fairly constant throughout exercise in the CE trial, with a significant rise near the end between 80 min and exhaustion (80 min: 4.9±0.2 mM/L; exhaustion: 5.6 ±0.4 mM/L; P=0.035), and by 30 min post-exercise, concentrations returned to fasting levels. During the AR trial, a marginally significant drop in glucose concentration (P=0.05) was observed between fasting and pre-exercise time. Glucose concentrations rose and leveled out during exercise, remaining significantly elevated over pre-exercise concentrations at 40 min, 60 min, 80 min, and at exhaustion (P<0.05) in the AR trial. Toward the end of exercise in the AR trial, glucose concentrations rose significantly between 80 min and the point of exhaustion (80 min: 4.6±0.2 mM/L;
exhaustion: 5.3±0.3 mM/L; *P*=0.032) and then fell 30 min after exercise to below basal levels (fasting: 4.7±0.2 mM/L; 30 min post: 4.2±0.3 mM/L; *P*=0.01).

Serum FFA concentrations were significantly elevated during the AR trial from pre-exercise through 60 min during exercise (*P*<0.05) compared with the CE trial, but the effect did not remain significant through exhaustion and post-exercise (Figure 3). At 40 min, FFA levels were significantly higher in the AR trial than in the CE trial (AR: 0.232±0.031 mM/L; CE: 0.073±0.005 mM/L; *P*<0.001), and then remained stable until 80 min. Serum FFA levels fell significantly during both trials from fasting to 60 min after ingestion though the drop was attenuated in the AR trial, resulting in a significant difference immediately preceding exercise (AR: 0.117±0.015 mM/L; CE: 0.058±0.004 mM/L; *P*=0.004).

FFA levels dropped significantly during the CE trial from the start of exercise until 20 min and remained low until 40 min, when levels began to rise significantly between 40 and 80 min (*P*<0.01). After 80 min, no significant changes were seen in FFA levels until exhaustion or 30 min post-exercise, though levels were similar in both AR and CE at exhaustion (AR: 0.466±0.073 mM/L; CE: 0.412±0.096 mM/L; NS). In contrast, FFA concentrations began to climb significantly in the AR trial until exhaustion and continued to increase until 30 min post-exercise (*P*<0.05). At exhaustion and 30 min post-exercise in both the AR and CE trials, FFA concentrations were similarly and significantly elevated compared with basal levels.

There were no differences in serum lactate levels at any time point between trials (Figure 4). Mean serum lactate concentrations ranged from 1.24±0.18 and 1.64±0.23 mM/L at fasting to 2.15±0.21 and 2.16±0.31 mM/L at 80 min for AR and CE,
respectively. A significant increase was observed between 80 min and exhaustion followed by a significant decrease and return to fasting levels by 30 min post-exercise during both trials independently ($P<0.05$).

C.2 Endurance

Endurance time-to-exhaustion was similar for both beverages (AR: 106.4±4.9 min; CE: 105.9±5.2 min; NS.) Individual performance times can be seen in Figure 5.

C.3 RER and Oxygen Consumption

The respiratory exchange ratios (RER), collected at 20-min time intervals immediately preceding each blood draw throughout exercise, increased at the beginning of exercise and then gradually and slightly decreased over time, but no differences were seen between the AR and CE trials at any time point. Mean oxygen consumption ($\text{VO}_2$) was similar between both trials at all points during exercise (NS). During both the AR and CE trials independently, however, $\text{VO}_2$ increased by approximately 38% from the beginning of exercise until 80 min (AR—start: 2.55±0.09, end: 3.56±0.11 L/min; CE—start: 2.50±0.07, end: 3.40±0.10 L/min; $P<0.05$). Mean oxygen consumption increased significantly during each trial from the start of exercise through 40 min ($P<0.05$) and also between 60 and 80 min of exercise ($P<0.05$). Physiological responses at start of exercise, 60 min, and at exhaustion are reflected in Table 2.
C.4 Heart Rates and Ratings of Perceived Exertion

The mean heart rates during exercise increased significantly from the start of exercise through the first 15 min (AR—start: 76±4, 15 min: 164±2; CE—start: 82±4, 15 min 166±3; \(P<0.001\)) and then tended to level out until the end of exercise. Mean heart rates at the start of exercise were significantly lower than at exhaustion (exhaustion—AR: 189±4 bpm; CE: 184±4 bpm), but there were no significant differences between the AR and CE trials.

The subjects’ ratings of perceived exertion were similar for both the AR and CE trials with a gradual increase over the course of exercise to exhaustion. Significance was found between time points within each trial starting 30 min (AR) and 60 min (CE) into exercise \(P<0.05\).

D. DISCUSSION

The purpose of this study was to compare metabolic and performance effects between two isocaloric pre-exercise fuel sources that differed in macronutrient profile and origin. The present study demonstrated no differences in performance times between the two pre-exercise beverages. However, significant differences were observed in insulin concentrations during the AR compared with the CE trials throughout exercise, with a significant blunting of insulin observed during the AR trial. Free fatty acid (FFA) concentrations were significantly higher during the AR trial through 60 min, though the between trials effect did not remain significant through 30 min post-exercise. Serum glucose and lactate concentrations, as well as fuel use (RER), oxygen consumption, heart rates, and ratings of perceived exertion were similar in both trials.
Most notably, we observed a significant blunting of insulin from the almond-raisin beverage that contained a mixed macronutrient profile ingested the hour prior to exercise compared to the carbohydrate-electrolyte trial. This is in line with other studies that have compared low glycemic index foods to high glycemic index foods (6-8, 42) or added fat to a high carbohydrate fuel source like potatoes (17). The carbohydrate-electrolyte trial showed a significant spike in insulin prior to exercise, similar to previous research on primarily or exclusively carbohydrate pre-exercise feedings (4, 9, 13, 14, 17, 35). However, unlike several studies, a concomitant hypoglycemic response did not occur in either trial. This may be perhaps due to the timing of the earlier blood draws, as researchers who have noted this response typically observe the peak increase in glucose within the first 30-45 minutes after ingestion (6, 17, 43), followed by a hypoglycemic response or a return to basal levels. Our first post-ingestion blood draw occurred after 60 minutes, potentially time enough for blood glucose to return to and maintain steady concentrations before and during exercise, but without observing the transient rise and fall.

Since insulin shuttles glucose to the muscles for energy and acts as a potent inhibitor of lipolysis, high insulin concentrations can lead to a decreased use of FFA for fuel and an accelerated or premature depletion of glycogen stores that may adversely affect performance. Both the presence of fat and protein in the almond-raisin beverage may have had an effect on decreasing the rate of absorption and the decreased secretion of insulin seen during the AR trial. The addition of fat can lower the glycemic load and delay digestion of a pre-exercise meal over carbohydrates along, resulting in a lower, more moderate effect on raising insulin in response to the ingested fuel as seen when
Horowitz and Coyle added fat in the form of margarine to various foods like potatoes and rice (5). In addition to fat, however, almonds in our mixed macronutrient test beverage also contains protein, which also contributes to the glycemic load, rate of digestion and fuel response.

The protein from the almonds may have an additional role in explaining the blunting effect seen in our study. Previous research on hyper- and normocholesterolemic subjects has shown plant proteins to have a relatively low serum insulin and higher glucagon response compared with animal proteins (19, 37). Plant proteins, like the protein in the almond component of the AR beverage (~10% energy), have a low lysine to arginine ratio (0.24) compared with animal proteins such as casein from milk (2.2) and beef protein (1.3), (47). While arginine in high doses is known to stimulate insulin, arginine obtained through diet in physiological amounts has shown to stimulate glucagon, with a lesser effect on insulin (19, 37). Therefore, plant protein sources high in arginine are associated with a decreased insulin to glucagon ratio, which may have contributed to some extent to the more moderate insulin response seen during the AR trial.

Conflicting evidence exists regarding the addition of fat to the pre-exercise feeding in terms of how the substrate availability and oxidation ultimately affects performance outcomes, even though most studies show similar trends in fuel responses. Our findings are in line with previous research suggesting that serum free fatty acids in particular, as well as fatty acid oxidation do tend to be consistently higher in mixed macronutrient treatments when compared with high carbohydrate or carbohydrate-only comparison treatments (17, 35, 50). Significant elevations were observed in the almond-raisin trial through the first 60 minutes of exercise compared with the CE trial, although
the effect did not remain significant through exhaustion and recovery. Whether there is an acute effect from the almonds ingested in the AR beverage in elevating FFA levels as seen in this study is unclear, but may warrant future consideration since in previous research, we have noted that fasting FFA levels are elevated in individuals fed almonds on a chronic basis over several months (36).

From exhaustion to 30 min post-exercise, FFA concentrations continued to climb significantly, consistent with the theory of "trapping" of FFA in the adipose tissue elucidated in other studies, that may help explain why no performance benefits were seen (5, 18, 33, 34). In studies that measure lipolysis, the data show that the rate of fatty acid appearance at the end of exercise increases dramatically without a simultaneous increase in lipolysis (33). Hypothesized mechanisms for this trapping include increased vasoconstriction toward the exhaustion or exercise at higher intensities lowering albumin availability to carry FFA into circulation and/or a decreased transfer of FFA into the mitochondria for oxidation due to increases in pyruvate, acetyl CoA, and malonyl CoA (33, 34). While we did not measure actual lipolysis in our study, a dramatic increase was observed in FFA concentrations after exercise ended in both trials.

A number of studies have also evaluated the physiologic performance effects of ingesting pre-exercise fuel in the hour prior to exercise. Researchers have observed improved (6, 20, 21, 27, 39, 42, 48, 51), decreased (9, 14), or neutral (2, 7, 8, 21, 44) effects on performance capacity compared to other treatments and/or a placebo trial. In our study, we found similar effects on time-to-exhaustion comparing the two isocaloric beverages providing 1.0-1.5 grams CHO·kg⁻¹ BW of different macronutrient profiles. While the mechanisms have yet to be completely sorted out for performance outcomes,
the inconsistencies of previous findings are likely due to differences between studies with regard to timing (range 5-60 min pre-exercise; 2, 9, 20, 27, 44), amount (range 45-75 g; 1.0-2.2 g CHO•kg⁻¹ BW; 2, 7, 9, 27, 39), glycemic index (6-8, 42), intensity (%VO₂max: range 60-80%; 9, 10, 20, 39, 44), and treatment source (food—e.g. lentils, potatoes, oats; liquid CHO—e.g. glucose, fructose; 2, 6-8, 14, 27, 42, 44, 48).

In studies evaluating the addition of fat to the pre-exercise fuel, effects have also differed, though the timing of the treatments has varied (35, 50, 51) or in some cases, performance was not measured at all (17). In our study, no differences were observed from the exogenous ingestion of high carbohydrate (carbohydrate-electrolyte) or carbohydrate plus moderate fat (almond-raisin) trials. Several earlier studies where subjects were fed either high carbohydrate (70-86% energy) or high fat (61-90% energy) pre-exercise meals showed no differences in performance times, though the high carbohydrate meal elevated pre-exercise insulin levels and decreased fat oxidation during exercise compared to the other treatments (29, 30, 35, 50). Pitsiladis et al. (31) did observe improvements in exercise with a high fat meal (90%) compared to a high carbohydrate meal (70%) with the addition of intravenous heparin administration in the high fat trial to elevate free fatty acids. Though this procedure does not reflect sound practice in free-living athletes, the study did suggest the potential for enhanced fatty acid oxidation leading to improvements in performance.

The amount of fat in the almond-raisin beverage (20% energy) used in our study was also much smaller than several previous studies (61-90%; 29-31, 35, 50), though perhaps reflects a more realistic, practical proportion of the total energy coming from the almonds in the pre-exercise almond-raisin beverage. In one study by Horowitz and Coyle
(17), the treatments that contained fat did comprise a more moderate 35% of total energy. Similar to our findings, they observed a blunted insulin response during exercise with treatments including fat, although performance was not evaluated in that study (17), and we did not observe the accompanying drop in glucose.

We did not observe performance improvements or significant changes in RER after subjects consumed the AR beverage, despite the increases in serum FFA concentrations. This may have to do with the intensity and duration of exercise, even though the set intensity should have still favored fatty acid fuel use. However, it would be interesting in the future to test the athletes at a lower percentage of their VO$_{2\text{max}}$ for a longer duration since 70% VO$_{2\text{max}}$ is on the upper range of the body’s ability to favor FFA use for fuel during exercise (46). Therefore, a lower intensity, longer duration trial may allow us to observe if potential delays in FFA absorption and release might translate to even more enhanced availability and potential performance benefits. Secondly, since the respiratory exchange ratios were not different between the trials, reflecting predominantly CHO oxidation at all times throughout exercise, we note that FFA availability alone may not translate to increased FFA oxidation (drop in RER) or improved performance. We did not specifically measure FFA oxidation in this study.

A potential limitation of this study is our measurement of “performance.” While we used time-to-exhaustion as the performance outcome similar to many other studies (6, 42, 49), some researchers (1, 16) have recently drawn attention to the potential challenges in using this as well as other performance outcomes. In particular, some measures of performance may not be sensitive enough to detect real differences, and there has been criticism in using time-to-exhaustion as compared to a set distance time-trial that may
more accurately mimic actual race conditions. Burke and colleagues (1) suggest that
time-to-exhaustion as a measure of performance may have a low degree of reliability and
therefore may increase the probability of a Type II error—failing to detect a difference
when one actually exists. However, we chose time-to-exhaustion as our performance
outcome by design to control the intensity of exercise (percentage of VO$_{2\text{max}}$) that would
enable us to observe FFA availability and oxidation, since subjects completing a time-
trial may exercise at an intensity higher than 70-75% VO$_{2\text{max}}$, where fat oxidation
becomes limited.

No placebo or fasting trial was included in our study. This is due to the
consistency of previous studies showing improvements in performance and fairly
consistent metabolic differences between various pre-exercise fueling protocols
compared with artificially sweetened placebos, water or fasting controls (3, 27, 38, 39).

A strength of this study was providing isocaloric, though non-significantly
different in CHO amount, beverages (5.26 kcal·kg$^{-1}$ BW). Several previous studies have
compared different pre-exercise fuel sources that are matched in carbohydrate, but differ
substantially in calories and macronutrient composition (8, 41, 42). In addition, the
almond-raisin pre-exercise feeding was provided as a beverage by design to equalize
volume and to create a comparison trial that would be similar to a commercial
carbohydrate-electrolyte beverage. While almonds and raisins may be more commonly
consumed as whole foods in a trail mix, in this preliminary investigation, we were able to
control for interindividual differences in rate of absorption due to the nature of a solid
food and variations in mastication patterns by blending and providing the almond-raisin
treatment in beverage form. Also, since carbohydrate content and volume determine
gastric emptying time more than osmolality of different solutions (40), providing both treatments as beverages of equal volume was further warranted. Future studies evaluating the different food forms of this pre-exercise fuel source, however, would be logical.

Researchers have begun to increasingly utilize whole and refined foods as treatments in testing performance and fuel use outcomes, shifting away from the traditional carbohydrate-only pre-exercise fuel source, but this is still a new area in sports nutrition research. Several researchers mention the importance of considering “real foods” (6, 17, 42), but few to our knowledge have specifically or intentionally compared the effects of whole food versus processed or refined pre-exercise fuel sources on endurance exercise.

While no performance benefits were seen in this study between treatments in comparing almond-raisin and carbohydrate-electrolyte beverages, the potential effects from other nutrients in the whole food source were not assessed. If a whole food is known to have other nutritional attributes like fiber, antioxidants or heart-healthy fats, there is a potential for additional health benefits from consuming them. Finding practical, convenient, and well-tolerated pre-exercise fuel sources will continue to be of interest, but further research in this area is necessary before any recommendations can be made. We do know, however, that while whole foods for sports nutrition is a newer area in modern science, athletes and the general population have been safely consuming items like dried fruits, fruit, nuts, seeds and whole grains to sustain energy for centuries, at least as far as the early Greek Olympics and biblical times (11).
In conclusion, the findings presented here suggest that an almond-raisin, whole food, mixed macronutrient pre-exercise beverage ingested 1 h before exercise is equally beneficial to a well-researched commercial carbohydrate-electrolyte beverage and may be considered an alternative to more commonly used commercial sports beverages for endurance exercise.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of AnhThu Tran, Alfred Quansah, Peter Le and the staff at the Center for Health Promotion at Loma Linda University. In addition, thanks to Jeffrey Horowitz, PhD at the University of Michigan for all the blood assays, and to the Center for Health Research staff for statistical consultation. Special thanks are extended to the athletes who enthusiastically dedicated their time, energy, and athleticism toward making this research possible.

This research was supported by the Center for Health Research and Department of Nutrition at Loma Linda University. The authors have no conflict of interests. The results of the present study do not constitute endorsement of the product by the authors or ACSM.

Additional tables and figures depicting the results can be seen in Appendices 4.1-4.7.
TABLE 1: SUBJECT CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>28±1</td>
<td>23-35</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177±2</td>
<td>168-188</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67±2</td>
<td>62-77</td>
</tr>
<tr>
<td>BMI</td>
<td>21.5±0.3</td>
<td>20-24</td>
</tr>
<tr>
<td>VO₂max (ml·kg⁻¹·min⁻¹)</td>
<td>61.9±2.1</td>
<td>51-72</td>
</tr>
<tr>
<td>Resting HR</td>
<td>61±2</td>
<td>44-74</td>
</tr>
<tr>
<td>Max HR</td>
<td>193±1</td>
<td>183-201</td>
</tr>
</tbody>
</table>

Table 1—Subject characteristics. Values are means ± SEM, N = 10. BMI, Body Mass Index, HR, Heart rate.
| Table 2—Physiological responses at start, 60 min, end of exercise at 70% VO$_{2\text{max}}$ after ingestion of AR and CE. Values are means ± SEM of VO$_2$, RER, and HR measurements during exercise; n=10 subjects. AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. VO$_2$, oxygen consumption, RER, respiratory exchange ratio; HR, heart rate. |
FIGURE 1: SERUM INSULIN CONCENTRATIONS

Figure 1—Serum insulin concentration (μU/mL) at fasting, pre-exercise, 20-min time intervals throughout exercise, at exhaustion and 30-min post exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. AR and CE treatments were significantly different over time \((P<0.05)\). Time points at which AR insulin concentration was significantly different (*) from CE are pre-exercise, 40 min, 80 min and at exhaustion \((P<0.05)\).
Figure 2—Serum glucose concentrations (mM/L and mg/dL) at fasting, pre-exercise, 20-min time intervals throughout exercise, at exhaustion and 30-min post exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. AR and CE treatments were not statistically different at any time point throughout exercise.
**FIGURE 3: SERUM FREE FATTY ACID CONCENTRATIONS**

Figure 3—Serum free fatty acid (FFA) concentration (mM/L) at fasting, pre-exercise, 20-min time intervals throughout exercise, at exhaustion and 30-min post exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. AR and CE treatments were significantly different from pre-exercise through 60 min ($P=0.001$, ANOVA repeated measures), though the between trials effect did not remain significant over time through 30-min post. Plasma FFA levels were statistically different (*) at pre-exercise and 40-min time points (Paired $t$-test, $P<0.05$).
**Figure 4**— Serum lactate concentrations (mM/L) at fasting, pre-exercise, 20-min time intervals throughout exercise, at exhaustion and 30-min post exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. AR and CE treatments were not statistically different at any time point throughout exercise.
Figure 5—Individual performance times-to-exhaustion after AR or CE test beverages. Dotted line represents group means. N=10 for the 2 trials. AR = Almond-Raisin Beverage; CE = Carbohydrate-Electrolyte Beverage.
REFERENCES


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A. SUMMARY AND DISCUSSION

The purpose of this study was to compare metabolic and performance effects of two isocaloric pre-exercise beverages that differed in macronutrient profile and origin. To summarize, the two beverages were: 1.) a specially-formulated almond-raisin beverage providing 1 g CHO•kg\(^{-1}\) BW and 20% energy from fat, and 2.) a commonly consumed commercial carbohydrate-electrolyte beverage providing 1.5 g CHO•kg\(^{-1}\) BW and no fat. This study begins to bridge the gap in sports nutrition research into the realm of convenient, whole foods that may serve as equal or potentially better sources of pre-exercise fuel compared to commercial sports beverages.

The present study demonstrated no differences in performance times between the two pre-exercise beverages. However, significant differences were seen in insulin concentrations during the almond-raisin compared with the carbohydrate-electrolyte trials throughout exercise, with a significant blunting of insulin observed during the almond-raisin trial. Free fatty acid concentrations were significantly higher during the almond-raisin trial through 60 min, though the between trials effect did not remain significant through exhaustion and recovery. Serum glucose and lactate concentrations, as well as fuel use (RER), oxygen consumption, heart rates, and ratings of perceived exertion were similar in both trials.

Most notably, we observed a significant blunting of insulin in the hour prior to exercise from the almond-raisin beverage that contained a mixed macronutrient profile.
compared to carbohydrate-electrolyte beverage. This is in line with our original hypothesis and other studies that have compared low glycemic index foods to high glycemic index foods or added fat to a high carbohydrate fuel source like potatoes. The carbohydrate-electrolyte trial showed a significant spike in insulin prior to exercise similar to previous research on primarily or exclusively carbohydrate pre-exercise feedings, however, unlike several studies, a concomitant hypoglycemic response did not occur in either trial. This may be perhaps due to the timing of the earlier blood draws, as researchers who have noted this response typically observe the peak increase in glucose within the first 30-45 minutes after ingestion (1, 2, 3), followed by a hypoglycemic response or a return to basal levels. Our first post-ingestion blood draw occurred after 60 minutes, potentially time enough for blood glucose to return to and maintain steady concentrations before and during exercise, but without observing the transient rise and fall.

We observed that free fatty acid concentrations were significantly elevated in the almond-raisin trial during the first 60 minutes of exercise compared with the carbohydrate-electrolyte trial, though the effect did not remain significant through exhaustion and recovery. These findings generally support our original research hypothesis that free fatty acid availability would be higher in the almond-raisin trial even though the effect was not significant over time. Additionally, from exhaustion to 30 min post exercise, free fatty acid concentrations continued to climb significantly, consistent with the theory of “fat trapping” of free fatty acid in the adipose tissue elucidated in other studies, that may help explain why no performance benefits can be seen.
A number of researchers have also evaluated the physiologic performance effects of ingesting pre-exercise fuel in the hour prior to exercise or the addition of fat to the pre-exercise fuel and found improved, decreased, or neutral effects on performance capacity in comparison to other treatments or placebos. While the mechanisms have yet to be completely sorted out, the inconsistencies of previous findings are likely due to differences between studies with regard to timing, glycemic index, intensity, nutrient composition and treatment source of the pre-exercise fuel. In our study, we found similar effects on time-to-exhaustion comparing two isocaloric beverages providing 1.0-1.5 grams CHO·kg⁻¹ BW of different macronutrient profiles (carbohydrate-only Gatorade® or carbohydrate plus moderate fat almond-raisin beverage, respectively). We had predicted that the almond-raisin trial would have yielded longer endurance times than carbohydrate-electrolyte trial, but this was not observed despite the favorable metabolic changes found.

In summary, this study demonstrates that a pre-exercise almond-raisin (whole food, mixed macronutrient) beverage had and similar effect on performance time to the carbohydrate-electrolyte (carbohydrate-electrolyte) beverage provided 1 hour before exercise. However, a significant blunting of insulin was seen in almond-raisin trial and insulin responses differed from carbohydrate-electrolyte trial throughout exercise. Enhanced availability of free fatty acids were observed in the almond-raisin trial though the effect did not remain significant over time and did not translate to improved performance. Other measured outcomes were similar between trials.

Consistent to previous research in this area, marked alterations were noted in substrate availability that would suggest enhanced utilization, but exercise performance
still appears remarkably resistant to change. Nonetheless, the almond-raisin beverage showed similar outcomes to the carbohydrate-electrolyte beverage, as well as more favorable substrate availability, lending support for the use of this whole foods pre-exercise fuel source as a practical and beneficial alternative to this common, commercial sports beverage.

B. LIMITATIONS AND STRENGTHS

A potential limitation of this study is our measurement of “performance.” While we used time-to-exhaustion as the performance outcome similar to many other studies (2, 4, 5), some researchers (6, 7) have recently drawn attention to the potential challenges in using this as well as other performance outcomes. In particular, some measures of performance may not be sensitive enough to detect real differences, and there has been criticism in using time-to-exhaustion as compared to a set distance time-trial that may more accurately mimic actual race conditions. Burke and colleagues (7) suggest that time-to-exhaustion as a measure of performance may have a low degree of reliability and therefore may increase the probability of a Type II error—failing to detect a difference when one actually exists. However, we chose time-to-exhaustion as our performance outcome by design to control the intensity of exercise (percentage of VO₂max) that would enable us to observe FFA availability and oxidation, since subjects completing a time-trial may exercise at an intensity higher than 70-75% VO₂max, where fat oxidation becomes limited.

No placebo or fasting trial was included in our study. This is due to the consistency of previous studies showing improvements in performance and fairly
consistent metabolic differences between various pre-exercise fueling protocols compared with artificially sweetened placebos, water or fasting controls (8-13).

A strength of this study was providing isocaloric, though non-significantly different in carbohydrate amount, beverages (5.26 kcal • kg⁻¹ BW). Several previous studies have compared different pre-exercise fuel sources that are matched in carbohydrate, but may differ substantially in calories and other macronutrients like the studies comparing lentils with potatoes (4, 14, 15). Controlling both for calories and carbohydrates provide an additional level of control in evaluating and comparing the results of the two test beverages that differ in source and macronutrient profile.

Additionally, the almond-raisin pre-exercise feeding was provided as a beverage by design to equalize volume and to create a comparison trial that would be similar to a commercial carbohydrate-electrolyte beverage. While almonds and raisins may be more commonly consumed as whole foods in a trail mix, in this preliminary investigation, we were able to control for interindividual differences in rate of absorption due to the nature of a solid food and variations in mastication patterns by blending and providing the almond-raisin treatment in beverage form. Future studies evaluating the different food forms of this pre-exercise fuel source would be logical.

Gastric emptying time was not measured in this study, therefore it is not possible to know the direct contribution of the test meals to fuel concentrations. However, based on previous research, scientists have suggested that actual carbohydrate content and volume of test fuels appear to be more important than differences in osmolality between fuel source in affecting gastric emptying time (16). Additionally, it is conceivable that a longer run time for the athletes would have yielded further differences in fuel
concentrations and performance effects related to digestion time due to potentially delayed or prolonged absorption of the almond-raisin beverage containing carbohydrate, fat, and protein, over time.

Due to the nature of this study and limitations of funding, we were unable to include female or recreational runners in the Nutrition-Exercise study. Therefore, findings cannot be generalized to a population beyond those that fall into the category of healthy, male endurance runners matching our inclusion criteria. However, we had a wide ethnic diversity within a relatively small overall sample size in our study that included Asian, African American, Hispanic and Caucasian ethnicities and that further strengthens the external validity of the study within the subject base selected for and completing the trials.

The pre-trial diets providing 7 grams carbohydrate per kilogram body weight for the athletes to consume in the day prior to exercise was a strength of our study. Controlling for the pre-exercise diet in order to optimize and equalize glycogen stores in subjects before each test run is an important issue, one that is often not or marginally controlled in similar pre-exercise fueling studies.

The study design chose for this investigation offers perhaps the greatest strength to this study in combination with the protocols followed between and during the actual trials. In a randomized, crossover experimental study, subjects serve as their own controls and therefore, eliminate or minimize many of the biological and physiological differences that occur when subjects are matched and placed in groups. In our study, compliance and completion was extremely high due to the study protocols that required specific and regular communication with the subjects.
C. FUTURE DIRECTIONS IN PRE-EXERCISE FUELING RESEARCH

The field of sports nutrition is constantly evolving as athletes and researchers continually strive to find information to provide maximum benefits for performance. The goal remains constant even amidst confusing and sometimes conflicting findings: to optimize performance through the maximal usage of fuel stores and maintenance of a ready supply of glucose and free fatty acids in the blood. Finding pre-exercise meals, snacks and beverages that can bring about these effects have a real and practical application, as snacking during competitive events can be challenging and deleterious to actual performance outcomes like race times.

The research to date is challenging to interpret, but there appears to be a clear shift from the traditional carbohydrate-only to a mixed macronutrient pre-exercise fuel source. Further, comparing mixed macronutrient pre-exercise fuel sources that come from whole versus refined foods appears to be on the horizon. While researchers have begun to utilize whole and refined foods as treatments in testing performance and fuel use outcomes, this is still a new area in the field of sports nutrition. Several researchers have noted the importance of considering "real foods" (1, 4, 5), but few to our knowledge have systematically or intentionally compared the effects of whole food versus processed or refined pre-exercise fuel sources on endurance exercise.

While no performance benefits were seen in this study of one over the other treatment in comparing almond-raisin and carbohydrate-electrolyte beverages, the potential effects from other nutrients in the whole food source were not assessed. If a whole food, like almonds or raisins, has other nutritional attributes like fiber, antioxidants
or heart-healthy fats, there is a potential for additional health benefits from consuming them. As but one example, we are already beginning to understand that phytonutrients and antioxidants in the whole foods may play a role in decreasing exercise-induced oxidant stress (17-21), as well as conferring long-term health benefits (22, 23).

Additional areas for future research extending from our investigation include, but are not limited to:

1. Comparing the effects of the two beverages with subjects completing a longer duration, reduced intensity run, followed by a performance time-trial.

2. Adding a third arm to the study to include whole almonds and raisins in an isocaloric, matched nutrient solid form (trail mix) to compare the effects of solid and liquid forms of the pre-exercise fuel source with each other and to the commercial, carbohydrate-electrolyte beverage.

3. Comparing the antioxidant status of athletes before and after exercise, along with urine metabolites reflecting free radical damage during exercise.

4. Testing the effects of different proportions of fat from the almond source when added to the raisins to evaluate metabolic and performance outcomes and to discover optimal combinations.

5. Extending the study protocol to include female and recreational athletes who are runners or who perform other endurance activities.

Finding practical, convenient, and well-tolerated pre-exercise fuel sources will continue to be of interest, but further research in this area is clearly necessary before any firm recommendations can be made. Whether whole food sources will ultimately lead to
equal, worse, or better performance outcomes and fuel availability and use during endurance exercise over commercially manufactured sports products is still unknown, though common sense would suggest whole foods would be safe, practical and potentially beneficial as pre-exercise fuel. Although whole foods for sports nutrition is a newer area in modern science, we do know that athletes and the general population have been safely consuming items like dried fruits, fruit, nuts, seeds and whole grains to sustain energy for centuries, at least as far as the early Greek Olympics and biblical times (24). Researchers now have been presented the challenge and opportunity of evaluating their benefits through the modern scientific process.
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CHAPTER ONE: INTRODUCTION


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CHAPTER TWO: REVIEW OF THE LITERATURE


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CHAPTER THREE: METHODS


CHAPTER FOUR: PUBLISHABLE PAPER FOR MEDICINE & SCIENCE IN SPORTS & EXERCISE


CHAPTER FIVE: CONCLUSIONS


Appendix 3.1

Facilities and Equipment
FACILITIES AND EQUIPMENT

Laboratories At Loma Linda University

Loma Linda University is a health sciences university. Each one of the facilities described below is part of the University and are located less than one-half mile from the Department of Nutrition.

Human Performance Laboratory

Loma Linda University has a modern human performance laboratory that is approximately 1,500 square feet. The laboratory is currently utilized for teaching purposes, exercise/fitness testing, and is available for use by Loma Linda University faculty researchers. The Nutrition-Exercise Study research staff utilized the laboratory during the morning hours over the course of three months.

The laboratory is equipped with three Quinton Q55 treadmills, Monark cycle ergometers, a 12-lead Quinton Q1000 ECG with connection to the treadmills, a SensorMedics M Vmax29 Series metabolic cart for measuring energy expenditure and fuel utilization at rest and during exercise, and other equipment for measuring exercise parameters including cardiovascular capacity and endurance, strength, flexibility, balance, heart rate (Polar A3™ Heart Rate Monitors), and ratings of perceived exertion (Borg scale).

The fitness laboratory was the site of all fitness testing including VO$_{2\text{max}}$ screening and test runs, as well as the location of blood sample centrifuging and aliquoting, and meeting with athletes for sports nutrition counseling sessions. An adjacent nutrition
kitchen was utilized for final preparation of the test beverages and storage of food for post run meals for the study participants. In the office adjoining the human performance lab, several nurses and an onsite physician were always available should any emergency have arisen during testing.

*Clinical Laboratories, Loma Linda University Medical Center and Emergency Care, Loma Linda University Medical Center*

The investigators in this study did not expect to use any clinical facilities, except those associated with the screening blood draws for the potential subjects. However, should any emergencies have arisen needing immediate care, the Loma Linda University Medical Center is located on the campus of the University, which has comprehensive medical resources available to provide emergency care.

*Research Kitchen*

The U. D. Register kitchen and Laboratory is located in the School of Public Health at Loma Linda University and is next door to the Department of Nutrition. It has been used for several human feeding studies including the nut feeding trials for which the department is well known. The kitchen has two rooms with total floor space of approximately 1,250 square feet. The kitchen has commercial quantity food production equipment for approximately 300 people per meal in half of the room, non-commercial equipment, as well as an eating area for up to 25 individuals.

For the Nutrition-Exercise study, the following equipment was used:

- Washer, single rack, 2 minute cycle
- One electronic balance to weigh food and test beverages for subjects (in grams).
• High speed blenders for preparation of test beverage ingredients
• China, stainless steel, glass and ceramic ware
• Various utensils and spatulas
• One non-commercial range top with 1 non-commercial oven
• One sink with garbage disposal units
• Commercial freezer, single door, with >24 cubic feet volume
• Commercial refrigerator with double doors
• Dry storage for non-perishable food items
• Decking for food preparation
• Tables and chairs

Department of Nutrition, School of Public Health, Loma Linda University

Located in the School of Public Health, the Department of Nutrition provides office space for all designated investigators and support staff. Multiple workrooms furnished with computers, telephones, and locking file cabinets are available. The computers are equipped with Internet access, access to university library holdings and Medline services, and the necessary word processing, spreadsheet, nutrient analysis and statistical software.

The laboratories at the Department of Nutrition also has equipment utilized for this study:

• Large -80°C Revco Deep Freezer (Scientific Refrigeration, Orange, CA), for storage of biological samples
• Clay Adams® Brand TRIAC® Centrifuge (Becton Dickinson Primary Care Diagnostics)
• Storage vials, Vacutainers™, and serum sample boxes.
Appendix 3.2

Telephone Screening Questionnaire
LOMA LINDA UNIVERSITY DEPARTMENT OF NUTRITION
NUTRITION-EXERCISE STUDY

TELEPHONE SCREENING QUESTIONNAIRE

Date ______________

Name ____________________________________________

<table>
<thead>
<tr>
<th>Last</th>
<th>First</th>
<th>Middle Initial</th>
</tr>
</thead>
</table>

Home Address ____________________________________________

Contact Information

Phone (home) ____________________________
(work) ____________________________
(cell) ____________________________
(fax) ____________________________
Email ____________________________

1. What is your age? __________ years 2. What is your gender?

[ ] Male  [ ] Female

3. What is your race?

[ ] Caucasian  [ ] Hispanic
[ ] African American  [ ] Asian
[ ] Other Please specify ____________________________

4. What is your occupation?

[ ] Employed on LLU campus  [ ] Employed outside of LLU campus
[ ] Homemaker  [ ] Retired
[ ] LLU student Please indicate which school & department __________________
[ ] Non-LLU student. Please indicate which University or College __________

5. What is your weight? ____________ pounds or ____________ kg

6. What is your height? ____________ inches or ____________ cms
7. Have you experienced any significant (5 lb. or more shift in weight) weight changes in the last year?
   [ ] No
   [ ] Yes  Number of pounds gained _______ or pounds lost ______.
   Over what time period was the weight lost or gained?______

8. Do you have any health condition or medical problems that you are currently managing?
   [ ] No.
   [ ] Yes. Please specify ________________________________

9. Do you currently take any medications?
   [ ] No
   [ ] Yes. List all over-the-counter and prescription medications taken in the last month.________________________

10. Do you currently take any supplements such as vitamins, minerals or herbs?
    [ ] No
    [ ] Yes. List all supplements or herbs taken in the last month.________________________

11. Do you train with any sports/ergogenic aids or supplements?
    [ ] No.
    [ ] Yes. Please specify names, brands and amounts. ________________________________

12. Are you currently recovering from any illness or injury?
    [ ] No.
    [ ] Yes.

13. When was your most recent medical physical examination? __________________________

14. If you have had recent blood cholesterol or blood sugar levels taken, do you recall any of the following?
    Total Cholesterol ______ mg/dl, LDL ______ mg/dl, HDL ______ mg/dl
    Triglycerides ________ mg/dl, Glucose ________ mg/dl

15. Do you smoke or use tobacco products?
    [ ] No
    [ ] Yes. Please specify amount smoked and type of tobacco used.
16. How often do you drink alcohol (beer, wine, liquor)?
   [ ] Never
   [ ] Occasionally
   [ ] A few times per week
   [ ] Daily

17. What is your dietary pattern?
   [ ] Omnivore
   [ ] Lacto-ovo Vegetarian
   [ ] Vegan
   [ ] Other. Please specify ________________________________

18. How often do you consume peanut butter, other nut butters or eat nuts?
   [ ] Never
   [ ] Occasionally
   [ ] A few times per week
   [ ] Daily

19. Do you have any known food allergies or intolerances?
   [ ] No
   [ ] Yes. Please specify. ________________________________

20. How often do you engage in regular vigorous activities (brisk walking, jogging, bicycling, etc.) to work up a sweat?
   [ ] Never
   [ ] Occasionally
   [ ] A few times per week
   [ ] Daily

21. Do you run in official races or events?
   [ ] No.
   [ ] Yes. Please specify how often. ________________________________
       For how long? ________________________________

22. How long have you been running? ________________________________

23. How many miles do you run each week?
   [ ] 0-10
   [ ] 11-20
   [ ] 21-30
   [ ] >30
24. Are you currently training for a specific event that will fall between Oct.-Jan.?
   [ ] No.
   [ ] Yes. Please specify when, where, event, distance. ______________________________

25. Did you have any sports related or other injuries in the last year?
   [ ] No.
   [ ] Yes. Please specify injury and approximate time. ______________________________

26. What prompted your interest in this study?
   ______________________________

27. Do you have any travel plans in next 4 months (October-January)?
   [ ] No.
   [ ] Yes. When and for how long? ______________________________

28. Would you be able to arrange to spend 3 mornings at the Loma Linda University’s fitness lab?
   [ ] No. List constraints. ______________________________
   [ ] Yes.

29. How did you find out about the study?
   [ ] Newspaper [ ] Trading Post [ ] Flyer
   [ ] E-Mail or Internet [ ] Other Please specify ______________________________

Interviewers initials _______ Date _______

(For Investigators Only)
Evaluation checklist:

Age 20-35? ______ NO ______ YES
Male? ______ NO ______ YES
Run >30 miles/week ______ NO ______ YES
Nut allergy ______ NO ______ YES
Report healthy? ______ NO ______ YES
Available Oct-Jan? ______ NO ______ YES
Call for next screening phase? ______ No ______ Yes ______ Maybe
Appendix 3.3

Medical History Questionnaire
### PERSONAL HISTORY

- **Birthplace**
- **Nationality**
- **Marital Status**
- **Occupation**
- **Residence Past 5 Years**
- **Education Through**
- **Recreation**
- **Exercise**
- **Average Per Day**
- **Alcohol**
- **Tobacco**
- **Tea, Coffee**
- **Medicines Taken Regularly**

### PERSONAL PAST HISTORY

- **Measles**
- **Whooping Cough**
- **Polio**
- **Scarlet Fever**
- **Diphtheria**
- **Meningitis**
- **Infectious Mono**
- **Viral Fever**
- **Tuberculosis**
- **Exposure To TB**
- **Malaria**
- **Hepatitis**
- **Cancer**
- **Other**
- **Injuries**
- **Asthma**
- **Back Trouble**
- **Pneumonia**
- **Pleurisy**
- **Asthma**
- **Emphysema**
- **Rheumatic Fever**
- **High Blood Pressure**
- **Heart Disease**
- **Anemia**
- **Blood Transfusion**
- **Nephritis**
- **Ulcera**
- **Hernia**
- **Bladder Infections**
- **Kidney Disease**
- **Heart Infections**
- **Glaucoma**
- **Nose Bleeds**

### FAMILY HISTORY

- **Anemia**
- **Blood Tendency**
- **Leukemia**
- **Recurrent Infections**
- **Infectious Diseases**
- **Chronic Lung Disease**
- **Tuberculosis**
- **High Blood Pressure**
- **Kidney Disease**
- **Asthma**
- **Severe Allergies**
- **Mental Illness**
- **Convulsions Or Fits**
- **Migraine Headaches**
- **Diabetes**
- **Gout**
- **Obesity**
- **Thyroid Trouble**
- **Peptic Ulcer**
- **Chronic Arthritis**
- **Cancer**

### MEDICAL RECORD IDENTIFICATION

**Name:**
**Birth Date:**
**Medical Record #:**
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Appendix 3.4

Informed Consent
**Loma Linda University**

**School of Public Health**

**Effect of pre-exercise snacks on endurance capacity in trained runners**

Loma Linda, California 92350  
(909) 824-4546  
FAX: (909) 824-4087

**Informed Consent**

**Purpose and Procedures**

You are invited to participate in a research study because you qualify as a healthy subject based on the criteria established by the researchers. The purpose of this research is to study the effect of consuming an almond raisin smoothie vs. Gatorade sports drink prior to starting exercise on performance. Participation in this study will require the following commitments for a 2-month period.

You will be interviewed by one of the researchers to determine your commitment to the study. First you will go through an initial procedure. This will include taking your height and weight, getting a basic blood work up and measuring your physical fitness by testing your oxygen consumption during exercise on a treadmill. On the day before the exercise test, the research staff will provide all meals to you. You will be required to consume only what is provided and no meals from outside can be consumed. For this, you will be required to visit the Research Kitchen (Nichol Hall, Loma Linda University) for breakfast and dinner. Lunch and all snacks will be packed 'to go' and given to you at the time of the breakfast. Both vegetarian and non-vegetarian meals will be provided based on your preference. Typically consumed foods will be used in the preparation of the meals. Participation in the study also requires you to drink only non-alcoholic beverages and water to remain fully hydrated. On the day of the exercise testing you will be required to come to the exercise lab following 12 hours over night fasting. You will be required to consume either the almond raisin smoothie or Gatorade. No other food will be permitted until 30 minutes after the exercise testing is completed.

You will be required to consume 4-6 ounces of water (1/2 cup) every 15 minutes during the exercise and this will be provided. You will be required to run on a treadmill till you reach the point of exhaustion. The total running time will be dependent on your endurance level. Prior to starting exercise a trained nurse will place an in-dwelling catheter in an area inside the elbow. This will prevent the need for multiple needle sticks while exercising. Blood will be drawn twice before beginning the exercise, twice after stopping exercise and every 20 minutes during exercise. The total number of blood draws will depend on each subject and their total time to exhaustion. At each blood draw about

I have read this informed consent  
_____ Initials  _____ Date

(Page 1 of 3)
Effect of pre-exercise snacks on endurance capacity in trained runners

1 tablespoon of blood will be drawn using a tube that will withdraw blood from the area inside the elbow. This is a crossover study and you will be required to repeat all procedures (but consume a different drink) after a month.

Risks
The risks to you are the pain and minor bruising at the site of the catheter needle insertion and possible exhaustion following the exercise testing. There might be some discomfort while running from the inserted catheter. Apart from the blood drawing and exercise testing, none of the other procedures are associated with risk or significant discomfort. The committee at Loma Linda University that reviews human studies (Institutional Review Board) has determined that participating in this study exposes you to minimal risk.

Benefits
The potential benefits to you are to learn more about your physical fitness level and specific nutritional plans to follow prior to exercise to improve performance. The potential benefits to nutritionists are increased knowledge of the relationship of food and nutrients in improving endurance.

Participants' Rights
Participation in this study is voluntary. Your decision whether or not to participate or terminate any time will not affect your present or future medical care.

Confidentiality
Any published document resulting from this study will not disclose your identity without your permission.

Additional Costs
There is no cost to you for participating in this study.

Reimbursement
Since your completion of the entire protocol is important for the success of the whole study, you will be paid the sum of $100 for completion of the study. You will also receive a one half an hour session with a dietitian free of charge. This reimbursement is available at the end of the two months of your participation and completion of all required tests. You will receive only $25 if you withdraw after the completion of the first month.

Research Related Injury
In the event of physical injury resulting from this research due to the exercise testing and blood drawing, appropriate emergency medical treatment will be provided. If you should
Effect of pre-exercise snacks on endurance capacity in trained runners

sustain injury as a result of this research, no other provisions has been made for financial payments or other forms of compensation. Please contact Dr. Sujatha Rajaram at 558-4598 in the event of research related injury.

Impartial Third Party Contact
If you wish to contact a third party not associated with this study regarding any questions or complaint you may have about the study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, phone (909) 558-4647 for information and assistance.

Informed Consent Statement
I have read the consent form and listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities. I may call Sujatha Rajaram, PhD during routine office hours at (909-558-4598) or during non-office hours at (909-335-9336). I have been given a copy of this consent form.

Signatures

Signature of subject __________________________ Date ________________

Signature of witness __________________________

I, the Principal Investigator, attest that the requirements for informed consent from the research project described in this form have been satisfied, that the participant has been provided with a copy of the Experimental Subject's Bill of Rights, that I have discussed the research project with the participant, and explained to him in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the participant to ask questions and that all questions asked were answered.

Signature of Investigator __________________________ Phone Number __________________________ Date ________________
Appendix 3.5

California Experimental Subject’s Bill of Rights
CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

You have been asked to participate as a subject in an experimental clinical procedure. Before you decide whether you want to participate in the experimental procedure, you have a right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be used.
3. Be given a description of any attendant discomforts and risks to be reasonably expected from the experiment.
4. Be given an explanation of any benefits to the subject to be reasonably expected from the experiment, if applicable.
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.
6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if complications should arise.
7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved.
8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time, and that the subject may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of a signed and dated written consent used in relation to the experiment.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any elements of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision.

I have carefully read the information contained above in the “California Experimental Subject’s Bill of Rights” and I understand fully my rights as a potential subject in a medical experiment involving people as subjects.

Date Patient

Time Parent/Legal Guardian

If signed by other than the patient, indicate relationship:

Relationship Witness

151
Appendix 3.6

Face-To-Face Screening Questionnaire
LOMA LINDA UNIVERSITY DEPARTMENT OF NUTRITION
NUTRITION-EXERCISE STUDY

FACE-TO-FACE SCREENING QUESTIONNAIRE

1. Where do you live?

2. What kind of work do you do?

3. What is your travel plans between November 2002 and January 2003?

4. Why do you want to be a part of this study?

**Medical/Health**

1. Have you ever been on a weight reduction or gaining diet in the last 5 years?
   [ ] No.
   [ ] Yes. Weight loss _____ Weight gain _____
   Please explain when and for how long. ________________________________
   What kind of diet? _______________________________________________

2. Do you have any health condition or medical problems that you are currently managing?
   [ ] No.
   [ ] Yes. Please specify ____________________________________________

3. Do you currently take any medications?
   [ ] No
   [ ] Yes. List all over the counter and prescription medications taken in the last month.
   _______________________________________________________________

4. When was the last time you had a cold or felt sick?

5. Do you have an aversion or discomfort with needles – injection, blood draws?
   [ ] No
   [ ] Yes
6. If you have had recent blood cholesterol or blood sugar levels taken, can you recall any of the following values? Approximately when were these done? ________

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<th>mg/dl</th>
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<td>LDL</td>
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</tr>
<tr>
<td>HDL</td>
<td>________</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>________</td>
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<td>Glucose</td>
<td>________</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>________</td>
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**Exercise**

1. How long have you been running?
2. How many miles do you run each week?
3. How many times do you run each week?
4. How long do you run during your workouts?
5. How long have you been following this routine?
6. How often do you compete in official events/races?
7. Have you ever had VO$_{2\text{max}}$ testing at a fitness lab?
   [ ] No
   [ ] Yes. Approximately when? __________________________
   Do you recall your VO$_{2\text{max}}$ last time you were tested?  

8. Under what circumstances do you change or discontinue your exercise routine?

**Alcohol**

1. Do you drink alcohol?
   [ ] No
   [ ] Yes

2. How often do you drink alcohol?

3. What type and how much do you typically drink?

4. Under what circumstances do you change your alcohol consumption pattern?
Tea/Coffee

1. Do you drink coffee/tea?
   Coffee? _____ No  _____ Yes
   Tea? _____ No  _____ Yes
   Caffeinated? _____ No  _____ Yes

2. How often do you drink coffee or tea?

3. What type and how much is typical per day?

Soda

1. Do you drink regular or diet soda?
   [ ] No
   [ ] Yes

2. How often?

3. What type and how much typical per day?

4. Is it difficult to abstain from caffeinated coffee, tea or soda for a week or more?
   Coffee _____ No  _____ Yes
   Tea _____ No  _____ Yes
   Soda _____ No  _____ Yes

Supplements

1. Do you use any dietary, vitamin, mineral, or herbal supplements?
   [ ] No
   [ ] Yes

2. Which ones?

3. In what amounts and how often?
4. Do you use other sports/ergogenic supplements or aids?
[ ] No
[ ] Yes

5. Which ones?
________________________________________________________________________
________________________________________________________________________

6. In what amounts and how often?
________________________________________________________________________
________________________________________________________________________

7. Do you use any caffeine-containing sports supplements?
[ ] No
[ ] Yes

8. Which ones?
________________________________________________________________________
________________________________________________________________________

9. In what amounts and how often?
________________________________________________________________________
________________________________________________________________________

**Diet**

1. What is your dietary pattern?
[ ] Omnivore
[ ] Lacto-ovo Vegetarian
[ ] Vegan
[ ] Other. Please specify ____________________________

2. Are there any meats that you do not eat?

3. Do you have any food intolerances, food allergies or dislikes?
[ ] No
[ ] Yes. To what items and when was your last allergic reaction?
________________________________________
(nuts? dried fruits?)

4. Have you ever consumed sports beverages or sports bars?
During athletic training?  _____No  _____Yes
During athletic event?  _____No  _____Yes
At a non-sports related time?  _____No  _____Yes
5. What brands do you consume?

6. Do you have any known intolerance or allergy to sports beverages or bars?
   [ ] No
   [ ] Yes

Which one/s.

7. Do you follow a special diet before an endurance event?
   [ ] No
   [ ] Yes

If so, what kind?

For how long prior to the event do you follow this diet?

8. Do you have any particular eating routine before, during, or after long runs or events?
   [ ] No
   [ ] Yes

   Before    During    After

   Please describe.

9. When preparing for an event, what are some of your favorite or most common pre-event foods (1 day before; few hours before)?

Research
1. Have you participated in previous research at LLU or elsewhere?
   [ ] No
   [ ] Yes

2. Were you able to successfully complete the study?
   [ ] No. Please explain why.
   [ ] Yes

3. Would you be able to arrive to the LLU fitness lab early in the morning on 3 separate occasions?
   [ ] No. Please explain why.
   [ ] Yes
4. Does the distance to LLU or you work schedule pose a real or potential problem for you to be a part of this study?  
[ ] No.  
[ ] Yes. Please explain why. ________________________________

5. Is there anything else in addition to what we have covered that you would like us to know?

_____________________________________

(FOR INVESTIGATORS ONLY)

Scoring

1. Dietary Compliance

   Reliability  _____ poor  _____ fair  _____ good  _____ excellent  
   Honesty    _____ poor  _____ fair  _____ good  _____ excellent

2. Distance/work  _____ problem  _____ no problem

3. Motivation/commitment  
   _____ problem  _____ no problem

FINAL ASSESSMENT
   _____ problem  _____ no problem

Ranking  

   1  2  3  4  5
   (least) (highest)

Subject Name ___________________________  Interviewers Initials _______  Date _______
Appendix 3.7

$V_{O_2}^{max}$ Subject Form
LLU Nutrition-Exercise Study Subject VO_{2max} Form

NAME: DATE:
SUBJECT NUMBER: SSN:

DOB:

People present:

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<td>MINIMUM 5 MINUTE COOL DOWN (press stop exercise on ECG, let treadmill run at 1.2 mph)</td>
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<td>85% VO_{2max}</td>
<td>speed, and incline</td>
</tr>
<tr>
<td>90% VO_{2max}</td>
<td>speed, and incline</td>
</tr>
<tr>
<td>95% VO_{2max}</td>
<td>speed, and incline</td>
</tr>
</tbody>
</table>

Comments:

160
Appendix 3.8

Almond-Raisin Test Beverage Recipe
Almond-Raisin Test Beverage for 72 kg BW Individual

Raisin paste (RP):
Combine raisins and water (2:1) into a blender.
Set blender on “puree” for 20 seconds
Set blender on “liquefy” for 1 min

Soaked almond:
Weigh 200g of raw almonds
Add 2 cups of water
Soak for 2 hours
Drain almonds
Add almonds into boiling water for 1 min
Drain

For 1 serving based on 15g almond:
19.3g of soaked almonds into blender
Add 7.5g water
Blend until almonds become paste
Product: Soaked Almond Paste (SAP)

Beverage:
26.8g SAP* + 108g RP* + 75g water*
Set blender at “liquefy” for 1 min
Add 100g crushed ice*
Drink immediately

If crushed ice is not available, ice cubes (same weight) can be added into the blender during the last 15 seconds of blending.

* Indicates weighed items for 72 kg individual; require adjustments based on weight of particular individual to receive test beverage.

Preparation and Storage Notes:
Prepare SAP, RP, water, and ice separately, and no more than 1 night in advance. Store ingredients in separate, air-tight containers and cover with aluminum foil and plastic wrap. Label with subject number and test date. Place items in refrigerator (ice in freezer.)

Prepare in blender just prior to consumption on morning of exercise test run.
Appendix 3.9

Almond-Raisin Beverages Taste-Test
Almond-Raisin Beverage Taste Test
August 12, 2002

Two almond-raisin beverages were prepared according to recipes developed specifically for the Nutrition and Exercise study in Loma Linda University Department of Nutrition's metabolic kitchen in the School of Public Health. The almond-raisin beverages were both designed to provide 20% fat, with the other 80% to be comprised of mostly carbohydrate and some protein. Both beverages consisted only of raisins, almonds, and water. The two almond-raisin beverages only differed in the way the almonds were prepared to add to the beverage. One recipe used toasted almonds prepared by baking dry, raw almonds at 300°F as a single layer on cookie sheets in the Loma Linda University research kitchen ovens. The second recipe used soaked almonds prepared by soaking dry, raw almonds in an established volume of water for 2 hours, draining them, boiling for 1 minute and draining again. Water was adjusted in the recipes so water volume overall was equal in both beverages.

After preparation of the recipes, the two beverages were labeled A (soaked almond-raisin beverages) and B (toasted almond-raisin beverages). Both beverages were divided into 3 clear plastic cups each and transported to the Loma Linda University health and fitness center for a taste test with randomly selected recreational athletes entering and leaving the facility. Three stations were set up at the front desk for individuals to sample and judge the almond-raisin beverages. A brief assessment tool was used for tasters to rank from 1 (poor) to 5 (excellent) in taste, appearance, texture, viscosity and give an
overall score for beverages A and B. Finally, tasters were asked to compare the two beverages and give an overall preference for A or B.

Twenty-five individuals participated in the taste testing at the health and fitness center. Beverage A (soaked almonds) scored higher in taste, appearance, texture and overall score. Beverage B (toasted almonds) scored higher in viscosity. Thirteen testers preferred beverage A to beverage B; 11 testers preferred beverage B to beverage A; and one tester preferred neither.

A table reflecting the averages of the scores can be found below. As a result of this taste test, beverage A was selected as the test beverage to be used in the Nutrition and Exercise study.

<table>
<thead>
<tr>
<th>Averages</th>
<th>Beverage A (1-5)</th>
<th>Beverage B (1-5)</th>
<th>Neither (1-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>3.44</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>3.14</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>3.34</td>
<td>3.22</td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td>3.18</td>
<td>3.36</td>
<td></td>
</tr>
<tr>
<td>Overall score</td>
<td>3.38</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>Overall preference (number)</td>
<td>13</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>
Appendix 3.10

Individual Subject Calculations for Test Beverages
### Individual Subject Calculations for Test Beverages

#### ALMOND-RAISIN TEST BEVERAGE

<table>
<thead>
<tr>
<th>Subject</th>
<th>BW</th>
<th>RAISIN</th>
<th>ALMOND</th>
<th>SAP</th>
<th>RP</th>
<th>H2O</th>
<th>Ice</th>
<th>α</th>
<th>CHO</th>
<th>CHC</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD</td>
<td>72</td>
<td>72.00</td>
<td>15.00</td>
<td>26.80</td>
<td>108.00</td>
<td>75.00</td>
<td>100.00</td>
<td>1.000</td>
<td>72</td>
<td>1.0</td>
<td>378.6</td>
</tr>
<tr>
<td>16</td>
<td>66</td>
<td>65.95</td>
<td>13.74</td>
<td>24.55</td>
<td>98.93</td>
<td>68.60</td>
<td>91.60</td>
<td>0.916</td>
<td>66</td>
<td>1.0</td>
<td>346.8</td>
</tr>
<tr>
<td>14</td>
<td>91</td>
<td>90.72</td>
<td>18.90</td>
<td>33.77</td>
<td>136.08</td>
<td>94.50</td>
<td>126.00</td>
<td>1.260</td>
<td>91</td>
<td>1.0</td>
<td>477.0</td>
</tr>
<tr>
<td>18</td>
<td>68</td>
<td>67.97</td>
<td>14.16</td>
<td>25.30</td>
<td>101.95</td>
<td>70.83</td>
<td>94.40</td>
<td>0.944</td>
<td>68</td>
<td>1.0</td>
<td>357.4</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>65.95</td>
<td>13.74</td>
<td>24.55</td>
<td>98.93</td>
<td>68.70</td>
<td>91.60</td>
<td>0.916</td>
<td>66</td>
<td>1.0</td>
<td>346.8</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>65.02</td>
<td>13.55</td>
<td>24.20</td>
<td>97.52</td>
<td>67.73</td>
<td>90.30</td>
<td>0.903</td>
<td>66</td>
<td>1.0</td>
<td>341.9</td>
</tr>
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<td>10</td>
<td>63</td>
<td>63.00</td>
<td>13.13</td>
<td>23.45</td>
<td>94.50</td>
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<td>87.50</td>
<td>0.875</td>
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<tr>
<td>7</td>
<td>62</td>
<td>61.99</td>
<td>12.92</td>
<td>23.08</td>
<td>92.99</td>
<td>64.58</td>
<td>86.10</td>
<td>0.861</td>
<td>62</td>
<td>1.0</td>
<td>326.0</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>68.98</td>
<td>14.37</td>
<td>25.67</td>
<td>103.46</td>
<td>71.85</td>
<td>95.80</td>
<td>0.958</td>
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<td>362.7</td>
</tr>
<tr>
<td>17</td>
<td>64</td>
<td>64.01</td>
<td>13.34</td>
<td>23.83</td>
<td>96.01</td>
<td>66.68</td>
<td>88.90</td>
<td>0.889</td>
<td>64</td>
<td>1.0</td>
<td>336.6</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>77.04</td>
<td>16.05</td>
<td>28.68</td>
<td>115.56</td>
<td>80.25</td>
<td>107.00</td>
<td>1.070</td>
<td>77</td>
<td>1.0</td>
<td>405.1</td>
</tr>
<tr>
<td>19</td>
<td>72</td>
<td>72.00</td>
<td>15.00</td>
<td>26.80</td>
<td>108.00</td>
<td>75.00</td>
<td>100.00</td>
<td>1.000</td>
<td>72</td>
<td>1.0</td>
<td>378.6</td>
</tr>
</tbody>
</table>

#### GATORADE TEST BEVERAGE

<table>
<thead>
<tr>
<th>Subject</th>
<th>BW</th>
<th>AR Kcal</th>
<th>Gram powder</th>
<th>Grams CHO/kg</th>
<th>Grams CHO/kg</th>
<th>CE Kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD</td>
<td>72</td>
<td>378.6</td>
<td>113.58</td>
<td>106.01</td>
<td>1.5</td>
<td>378.6</td>
</tr>
<tr>
<td>16</td>
<td>66</td>
<td>346.8</td>
<td>104.04</td>
<td>97.10</td>
<td>1.5</td>
<td>346.8</td>
</tr>
<tr>
<td>14</td>
<td>91</td>
<td>477.0</td>
<td>143.10</td>
<td>133.56</td>
<td>1.5</td>
<td>477.0</td>
</tr>
<tr>
<td>18</td>
<td>68</td>
<td>357.4</td>
<td>107.22</td>
<td>100.07</td>
<td>1.5</td>
<td>357.4</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>346.8</td>
<td>104.04</td>
<td>97.10</td>
<td>1.5</td>
<td>346.8</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>341.9</td>
<td>102.60</td>
<td>95.70</td>
<td>1.5</td>
<td>341.9</td>
</tr>
<tr>
<td>10</td>
<td>63</td>
<td>331.3</td>
<td>99.39</td>
<td>92.76</td>
<td>1.5</td>
<td>331.3</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>326.0</td>
<td>97.80</td>
<td>91.00</td>
<td>1.5</td>
<td>326.0</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>362.7</td>
<td>108.81</td>
<td>101.56</td>
<td>1.5</td>
<td>362.7</td>
</tr>
<tr>
<td>17</td>
<td>64</td>
<td>336.6</td>
<td>100.98</td>
<td>94.25</td>
<td>1.5</td>
<td>336.6</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>405.1</td>
<td>121.53</td>
<td>113.43</td>
<td>1.5</td>
<td>405.1</td>
</tr>
<tr>
<td>19</td>
<td>72</td>
<td>378.6</td>
<td>113.58</td>
<td>106.01</td>
<td>1.5</td>
<td>378.6</td>
</tr>
</tbody>
</table>

**Almond-Raisin beverage calories:**
1. \( \alpha (72 \text{ g}) = Y \text{ g raisins} \)
2. \( 15 \text{ (BW)} = X \text{ g almonds} \)
3. Calories per gram: Raisins=4; Almonds=6.04
4. Calories=\( 4 \ Y + (6.04) \times X \)

**Legend:**
- Standard: Initial calculations based on standard 72 kg BW
- BW: Body weight (kilograms)
- Raisins: Whole raisins (grams)
- Almonds: Whole unsalted raisins (grams)
- SAP: Soaked almond paste (grams)
- RP: Raisin paste (grams)
- H2O: Water (grams)
- Ice: Crushed Ice (grams)
- \( \alpha \): Conversion factor for calculating subjects' recipes
- CE: Carbohydrate-Electrolyte (Gatorade beverage)
- AR: Almond-Raisin beverage
Appendix 3.11

Nutrient Profiles of Almond-Raisin and Carbohydrate-Electrolyte Test Beverages
Test Beverages: Nutrient Profiles

<table>
<thead>
<tr>
<th>Test Beverages</th>
<th>Nutrient Profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Almond-Raisin</strong> *</td>
<td><strong>Gatorade®</strong> ++</td>
</tr>
<tr>
<td>- CHO: 70 g</td>
<td>- CHO: 103 g</td>
</tr>
<tr>
<td>- Fat: 8 g</td>
<td>- Fat: 0 g</td>
</tr>
<tr>
<td>- Pro: 6 g</td>
<td>- Pro: 0 g</td>
</tr>
<tr>
<td>- Fiber: 5 g</td>
<td>- Fiber: 0 g</td>
</tr>
<tr>
<td>- Calories: 370</td>
<td>- Calories: 370</td>
</tr>
<tr>
<td>* 1 g CHO·kg⁻¹ BW</td>
<td>* 1.5 g CHO·kg⁻¹ BW</td>
</tr>
<tr>
<td>* 20% energy from fat</td>
<td>* 0% energy from fat</td>
</tr>
</tbody>
</table>

| MUFA | 4.9 g |
| PUFA | 1.9 g |
| SFA | 0.7 g |
| Sodium | 17 mg |
| Potassium | 764 mg |
| Calcium | 77 mg |
| Magnesium | 71 mg |
| Vitamin E | 10 mg |
| Iron | 2.4 mg |
| Lys:Arg | 0.24 |

Sodium 736 mg
Potassium 764 mg

Not a significant source of calories from fat, SFA, cholesterol, dietary fiber, vitamin A, vitamin C, calcium, iron.

(15 g powder = 14 g CHO and 50 kilocalories)
+ Gatorade® Lemon-Lime Instant Powder

* Based on a 70 kg BW male

Calculations done manually and with Nutritionist Five™ and Food Processor software programs.
Appendix 3.12

Final Menu Calculations Sample of Diets for Subjects
### Final Menu Calculations Sample of Diets for Subjects

#### WORKSHEET FOR DAY PRIOR FOODS PROVIDED

<table>
<thead>
<tr>
<th>Subject</th>
<th>Body Weight in kg</th>
<th>7 grams CHO/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>016</td>
<td>66</td>
<td>462</td>
</tr>
<tr>
<td>014</td>
<td>91</td>
<td>637</td>
</tr>
<tr>
<td>018</td>
<td>68</td>
<td>476</td>
</tr>
<tr>
<td>006</td>
<td>66</td>
<td>462</td>
</tr>
<tr>
<td>009</td>
<td>65</td>
<td>455</td>
</tr>
<tr>
<td>010</td>
<td>63</td>
<td>441</td>
</tr>
<tr>
<td>007</td>
<td>62</td>
<td>434</td>
</tr>
<tr>
<td>005</td>
<td>69</td>
<td>483</td>
</tr>
<tr>
<td>017</td>
<td>64</td>
<td>448</td>
</tr>
<tr>
<td>002</td>
<td>77</td>
<td>540</td>
</tr>
<tr>
<td>019</td>
<td>72</td>
<td>508</td>
</tr>
</tbody>
</table>

#### Food

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>Weight (g)</th>
<th>CHO</th>
<th>Pro</th>
<th>Fat</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature Valley Oats &amp; Honey Granola Bars</td>
<td>2</td>
<td>29</td>
<td>4</td>
<td>6</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Nabisco Nilla wafers</td>
<td>6</td>
<td>22.5</td>
<td>15.75</td>
<td>0.75</td>
<td>4.5</td>
<td>105</td>
</tr>
<tr>
<td>Nabisco Oreos</td>
<td>2</td>
<td>22.7</td>
<td>16</td>
<td>1.3</td>
<td>4.7</td>
<td>107</td>
</tr>
<tr>
<td>Pepperidge Farm Goldfish</td>
<td>55pc/30g</td>
<td>30/1.1 oz</td>
<td>19</td>
<td>3</td>
<td>6</td>
<td>150</td>
</tr>
<tr>
<td>Banana (med. 4 oz)</td>
<td>1</td>
<td>114/4 oz</td>
<td>27.6</td>
<td>1.2</td>
<td>0.5</td>
<td>109</td>
</tr>
<tr>
<td>Banana (med. 4 oz.)</td>
<td>1</td>
<td>114/4 oz</td>
<td>27.6</td>
<td>1.2</td>
<td>0.5</td>
<td>109</td>
</tr>
<tr>
<td>Yoplait yogurt (French vanilla) 6 oz.</td>
<td>1</td>
<td>33</td>
<td>5</td>
<td>1.5</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Sara Lee Bagel (plain)</td>
<td>1</td>
<td>52</td>
<td>9</td>
<td>1</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>TreeTop Apple Juice</td>
<td>1</td>
<td>5.5 oz</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Bohemian Hearth Whole Wheat Bread</td>
<td>1</td>
<td>35</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>Bohemian Hearth Whole Wheat Bread</td>
<td>1</td>
<td>35</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>La Romanella Fusilli pasta</td>
<td>2c</td>
<td>321</td>
<td>109</td>
<td>18.7</td>
<td>2.7</td>
<td>560</td>
</tr>
<tr>
<td>Prego Pasta Sauce</td>
<td>3/4 cup</td>
<td>31.5</td>
<td>3</td>
<td>6.75</td>
<td>195</td>
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<tr>
<td>Total cereal</td>
<td>2c</td>
<td>80</td>
<td>67</td>
<td>8</td>
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<td>293</td>
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<tr>
<td>1 tbsp jam</td>
<td>1 tbsp</td>
<td>20</td>
<td>13.8</td>
<td>0.1</td>
<td>0.01</td>
<td>56</td>
</tr>
<tr>
<td>Don Miguel Bean and Cheese Burrito</td>
<td>1</td>
<td>142</td>
<td>47</td>
<td>11</td>
<td>8</td>
<td>290</td>
</tr>
<tr>
<td><strong>TOTAL CHO and CALORIES</strong></td>
<td></td>
<td></td>
<td>542.25</td>
<td>72.25</td>
<td>46.86</td>
<td>2834</td>
</tr>
</tbody>
</table>
Appendix 3.13

Guidelines for Week Prior to Exercise Test
Loma Linda University Nutrition-Exercise Study

Guidelines for the Week Prior to Your Fitness Testing Date.

Name: [Redacted]

Test Dates: Tuesday, December 3 and Tuesday, December 17

Start following these guidelines on the week prior to Tuesday, December 3
   Tuesday, December 17

Begin on Tuesday, November 26 for December 3 test.
Begin on Tuesday, December 10 for December 17 test.

**YOU WILL BE PROVIDED ADDITIONAL, SPECIFIC RECOMMENDATIONS FOR THE DAY BEFORE YOUR FITNESS TEST.**

**DIET**

**SEPARATE RECOMMENDATIONS WILL BE PROVIDED FOR THE DAY BEFORE THE FITNESS TEST.**

Follow your normal or usual diet for the week prior (6 days) to the fitness test, with the following limitations:

AVOID:

✓ Alcohol (wine, beer, liquor)
✓ Caffeinated foods (black and green tea, regular coffee, regular cola, Mountain Dew, coffee ice cream, Excedrin pills/tablets) NOTE: if you are a habitual coffee drinker and get withdrawal headaches, you may consume up to 1 cup (8 oz. Coffee) daily.
✓ Dried fruits: raisins, dates, dried figs, dried apricots, dried papaya, dried mango, dried apples, dried pineapple, etc.
✓ Nuts: almonds, pecans, peanuts, cashews, walnuts, etc. NOTE: if you consume PB&J sandwiches, you may continue to do so at 1 per day.
✓ Sports bars and beverages (Powerbars, Gatorade, Gu2O, Gu, MetRx bars, etc.) - General meal replacement bars OK as a snack or meal replacement (except on day before test).
CONTINUE:
✓ If you use protein powders to help you meet your caloric or nutritional needs, you may continue to do so **IF they DO NOT include** other supplements or antioxidants, except for the DAY before the test.
✓ Aim for at least 8 cups of water daily.

SUPPLEMENTS

**Please avoid antioxidant supplements (Vitamin C, E, Selenium, Antioxidant combinations) for the DURATION of the study (from today until AFTER the second fitness test).**

AVOID:
✓ Herbal supplements
✓ Single vitamin or mineral supplements
✓ Antioxidant supplements.
✓ Sports supplements: muscle building powders, ergogenic aids,

CONTINUE:
✓ General multi-vitamin/mineral supplement (only if you are already taking one).
✓ Any prescription medications required for your health.

EXERCISE

AVOID:
✓ A “long run” for the week within 5-6 days of the fitness test.
✓ Major changes in your exercise routine for the DURATION of the study.
✓ Strenuous exercise on the day PRIOR to the fitness test.

CONTINUE:
✓ Your normal exercise routine, except plan for your weekly “long run” to occur on the fitness test day.

CALL WENDY AT (___) ___-___ WITH ANY QUESTIONS
OR EMAIL THE RESEARCH TEAM AT lluexercisestudy@hotmail.com.
Appendix 3.14

Guidelines for Day Prior to Exercise Test
Loma Linda University Nutrition-Exercise Study

Guidelines for the Day Prior to Your Fitness Testing Date.

Name: [redacted]
Test Date: Tuesday, December 17, 2002
Start following these guidelines on: Monday, December 16

GENERAL GUIDELINES:
Below you will find guidelines for the day prior to the test. Please follow these guidelines closely. It is important for you to eat as much as you like in terms of calories and quantity, so you may add freely to the food you will be provided, with some limitations. Consume a healthy diet with moderation in carbohydrates, fats, and protein. Consume all of the food provided to you by the research team.

The majority of your carbohydrate needs will be supplied for you based on your body weight. All items provided are lacto-ovo vegetarian. Supplement these items provided to you with protein and fat sources that you like to eat (animal: meat, poultry, fish; or vegetarian sources are fine). In addition, aim for at least 8 cups of water. Refrain from vigorous exercise on the day prior to the fitness test.

Be sure to record the additional foods you consume on the attached page. Don’t eat or drink anything past 8:30 pm and try to get a good night’s rest.

CALL WENDY AT (###) ###-### WITH ANY QUESTIONS OR EMAIL THE RESEARCH TEAM AT lluexercisestudy@hotmail.com.

Thank you!

The Nutrition-Exercise Research Team
Sujatha Rajaram, Joan Sabate, Bryan Haddock
Wendy Bazilian, AnhThu Tran
GUIDELINES FOR THE DAY PRIOR TO FITNESS TEST

DIET

✓ You will be provided with a variety of foods for the day before the fitness test.

✓ Please consume ALL of the foods provided to you. You can consume them anytime, in any order for meals and snacks throughout the day.

✓ Please do not consume anything except water after 8 pm and come to the fitness lab the next morning in a “fasted” state.

✓ You may consume any additional food items to fit your hunger and personal caloric/food preferences, but PLEASE AVOID THE FOLLOWING:

AVOID:
✓ Alcohol (wine, beer, liquor)

✓ Caffeinated foods (black and green tea, regular coffee, regular cola, Mountain Dew, coffee ice cream, Excedrin pills/tablets)
NOTE: if you are a habitual coffee drinker and get withdrawal headaches, you may consume up to 1 cup (8 oz. Coffee).

✓ ALL dried fruits: raisins, dates, dried figs, dried apricots, dried papaya, dried mango, dried apples, dried pineapple, etc.

✓ ALL nuts: almonds, pecans, peanuts, cashews, walnuts, etc.
NOTE: Please avoid peanut butter.

✓ ALL sports bars and beverages (powerbars, Gatorade, Gu2O, Gu, MetRx bars, etc.)
CONTINUE:
✓ If you use protein powders to help you meet your caloric or nutritional needs, you may continue only IF the ingredients are exclusively protein (whey, egg, etc.) and do not include other supplements, vitamins, minerals, antioxidants, etc.
✓ Aim for at least 8 cups of water during the day.

**Please record all additional foods or beverages you consume during the day. You will be asked to repeat this day’s foods/beverage pattern before the 2nd fitness test.

SUPPLEMENTS

**Please avoid antioxidant supplements (Vitamin C, E, Selenium, Antioxidant combinations) for the DURATION of the study (from today until AFTER the second fitness test).

AVOID:
✓ Herbal supplements
✓ Single vitamin or mineral supplements
✓ Antioxidant supplements.
✓ Sports supplements: muscle building powders, ergogenic aids,

CONTINUE:
✓ General multi-vitamin/mineral supplement (only if you are already taking one).
✓ Any prescription medications required for your health.

EXERCISE

AVOID:
✓ Vigorous exercise. A light and short morning run is fine, if you must.

**RECORD YOUR FOOD INTAKE ON THE NEXT PAGE AND BRING WITH YOU ON THE DAY OF YOUR FITNESS TEST.
Appendix 3.15

Sample of Subject Food Record
Nutrition Food Record for (name):

Please write down everything you eat and drink **IN ADDITION TO** the foods you have been provided by the research team. You do not need to record the foods we provided.

Bring this form with you on the day of your fitness test. You will be asked to repeat this food intake pattern before your second fitness test.

Date: ____________________  Day of the week: ____________________

<table>
<thead>
<tr>
<th>Meal/Time (Breakfast, lunch, dinner, snack)</th>
<th>Name of Food or Drink</th>
<th>Description/How prepared</th>
<th>Amount Consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

If you did any exercise (light intensity only, please), record it here:

Exercise type: ____________________

How long/far: ____________________
Appendix 3.16

Food Bag Guidelines for Day Prior to Test Run
Dear [ ],

Enclosed are your carbohydrates for the day prior to your fitness run.

These foods will provide you the minimum amount of carbohydrates for you based on your body weight so we can assure that your glycogen stores (stored sugars in the liver) are filled before your test run tomorrow.

A few items of note:

1. Please consume ALL of the contents in the large brown bag.
2. In the small bag marked “optional” we have included a few items: cream cheese, extra jam. You can choose or not choose to eat these. They are provided as an extra if you like to eat your bagel with cream cheese, for example. They will offer no additional advantage to us for the study and are strictly “optional”.
3. The foods contained here SHOULD NOT be the only items you eat the day before your fitness test. They provide the CARBOHYDRATES, with only modest amounts of protein and fat. PLEASE CONSUME OTHER ITEMS TO ACCOMPANY THE ITEMS PROVIDED TO YOU. You can make a deli sandwich with the bread or put butter on your toast. You can add vegetables or meat to your spaghetti sauce, salsa to your burrito, regular or low fat dairy milk, rice milk, soy milk or other items to your cereal, etc. These are just a few examples.
4. You CAN eat additional carbohydrates. We are providing you the minimum amount to assure that your glycogen stores are adequate for the research/fitness run on your test date. If you want additional bread, fruit or require additional calories, they can come from whatever foods (carbohydrates, protein or fat) that you like (with the exception of those items on the guidelines for the day prior to testing like alcohol, caffeine, nuts, dried fruits, sports beverages/bars).
5. Record any additional foods you eat on the form included with your guidelines for the day prior to testing. You will be asked to repeat this diet on the day prior to your second test date.

6. You can eat the foods in the bag in any order you like, spread throughout the day. You be the gauge of when and how much to eat at each meal and snack. Just be sure to consume everything in the bag.

Again, we’d like to remind you to please consume ALL of the items in the bag. If we have overlooked a personal dislike (e.g. you actually despise bananas...), please call Wendy at (___) ___-___, so she can help you make an appropriate substitution.

Since this bag provides mainly your carbohydrate sources (with some protein and fat), in an effort to provide you responsible and healthy advice, we are including the general dietary guidelines for good nutrition for your information.

Carbs: Consume 45-65% of total calories
Fat: 20-35% of total calories
Protein: 15-20% of total calories
Fruit: 2-4 daily servings
Vegetables: 3-5 daily servings
Water: At least 8 cups/daily.

Most importantly, eat an amount that is normal and comfortable for you throughout the day. Do not try to alter your normal diet drastically or stick to any of the general dietary guidelines if they are not a part of your normal, comfortable, and current eating routine.

Make your last meal mainly carbohydrates, and do not eat anything after 8:30 pm. You may continue to consume water and you can brush your teeth before coming to the fitness lab. See you on the test date by 6:30 am (please be prompt).

Please call Wendy at (___) ___-___ if you have ANY questions about your diet!

Enjoy!

Sincerely,

The LLU Nutrition-Exercise Research Team
Appendix 3.17

Food Bag Checklist for Day Prior to Test Run
Food Intake for Day Prior to Test

(Name)  

Food items:  

Please be sure to eat all of the following items provided on the day prior to the test.  

   _____ Bagel  
   _____ Total cereal  
   _____ 2 slices whole wheat bread  
   _____ 2 bananas  
   _____ Yogurt (needs refrigeration)  
   _____ Apple juice  
   _____ Burrito (needs refrigeration)  
   _____ Goldfish  
   _____ Nilla wafers  
   _____ Oreos  
   _____ Jam  
   _____ Granola bars  
   _____ Pasta (needs refrigeration)  
   _____ Pasta sauce (needs refrigeration)  

   _____ Write down your additional foods/times/amounts on food record sheet provided. (Bring to fitness test).  
   _____ Eat last meal or snack near 8:30 pm and focus on mostly carbohydrates at that meal.
Appendix 3.18

Exercise Test Protocol for Heart Rates, Ratings of Perceived Exertion, and Water
LLU Nutrition-Exercise Study – Heart Rate, Borg, Water Recording

Heart Rate
✓ Make sure polar heart rate monitor is attached snugly and reading heart rates. Moisten sensors before fitting to subject.
✓ Record readings in the table below every 15 minutes throughout the test.
✓ Optional additional readings are OK in addition to every 15 minutes (required). You can use the "comments" column to note additional heart rate and times.
✓ After fitness test is over: detach heart rate monitor and clean sensor with alcohol swabs; hang elastic bands to dry.

Borg Scale
✓ Refresh athlete’s knowledge of Borg RPE scale.
✓ Also record periodically during the run, by asking the athlete what his RPE is.

Water
✓ Get gallon of water near by with measuring cup and Dixie 5 oz drinking cups.
✓ Measure and provide 4 oz. (1/2 cup) water every 20 minutes to the athlete min. More as desired.
✓ Be conscious of their breathing and gait for timing of passing water to them.

GENERAL NOTES:
Blood work and Facemask (Gas) Measurements
✓ Will occur every 20 minutes throughout the test. These need to occur on time.
✓ Provide assistance as indicated and record other measurements at times that do not interfere with these measurements.
✓ If the facemask is on or blood work being done, collect information immediately after they are completed (mask off) and athlete has resumed comfortable pace/breathing.

The Athlete:
✓ Everything must be done to pay attention to the athlete and make the testing experience as comfortable as possible.
✓ Watch for athlete pace and breathing – assess the right time to take measurements, ask RPE and provide water.
✓ Coach the athlete along to do his best (see coaching instructions).
✓ DO NOT PROVIDE exact times or specific information to athlete.

*** IT IS ESSENTIAL TO PAY ATTENTION TOWARD THE END OF THE TEST (WHEN SUBJECT BECOMES FATIGUED) TO NOTATE EXACT TIME OF STOPPING and to IMMEDIATELY reduce treadmill speed to a recovery walk pace of 3.0 mph.
Appendix 3.19

Quinton Q1000 Treadmill Protocol for Test Runs
LLU Nutrition-Exercise Study Fall 2002
TREADMILL PROTOCOL FOR TEST RUNS

1. Turn treadmill “ON”; turn off the “REC POWER,” and set “protocol” to MANUAL.
2. Warm subject up at max 3.5 mph, 0% incline.
3. Start test by pressing **START EXER** when signaled and **immediately** bring treadmill speed and incline up to designated speed (70% VO$_{2\text{max}}$ for the subject) indicated on testing form.
4. At blood draw times (every 20 minutes), immediately and quickly bring the SPEED ONLY down to 4.0 mph for a fast walk while physician draws blood. (If the subject is still jogging at that pace and not walking swiftly, bring the speed down to 3.8 mph) Notate times in minutes and seconds.
5. When physician completes the blood draw and has re-secured the catheter, bring the speed immediately back up to the pace it was at prior to blood draw.

AT 90 minutes:
✓ Bring speed and incline up to 75% VO$_{2\text{max}}$ for the subject as indicated on testing form.

AT 100 minutes:
✓ Bring speed and incline up to 80% VO$_{2\text{max}}$ for the subject as indicated on testing form.

AT 110 minutes:
✓ Bring speed and incline up to 85% VO$_{2\text{max}}$ for the subject as indicated on testing form.

AT 120 minutes:
✓ Bring speed and incline up to 90% VO$_{2\text{max}}$ for the subject as indicated on testing form.

AT 130 minutes:
✓ Bring speed and incline up to 95% VO$_{2\text{max}}$ for the subject as indicated on testing form.

AT EXHAUSTION:
✓ Pay attention to signs of fatigue throughout the test.
✓ When the subject puts his hands on the treadmill bar or signals to stop, **IMMEDIATELY press STOP EXER** and treadmill will go automatically to 1.2 mph and 0 incline.
✓ Write down exact time that the test ended.
✓ Assist physician in collecting final blood sample.
✓ Assist subject in cool down (minimum 5 minutes), adjust speed for subject.
Appendix 3.20

Coaching Guidelines
Providing encouragement to the athletes is important throughout the test runs. It is equally important to be consistent in how the research team coaches the athletes – for every athlete and for the same athlete from one run to the next.

Guidelines:
✓ General encouragement is preferred; no specific times or distances.
✓ Increase the encouragement as the subject begins to fatigue.
✓ Use the following phrases:

Excellent job.
Come on, NAME!
Push it. Push yourself.
You’re doing great.
You’re cranking.
Great job.
Work it out.
Let’s go.
Work those arms, legs.
Use your breath, arms, legs.
You can do it.
Come on, keep on going!
You make it look easy.
Good pace, keep it up.
Way to go!
Whoa-hoo!
Appendix 3.21
Data Collection Form 1
Nutrition-Exercise Study – Athlete Fitness Test Form (TEST 1)

NAME: [redacted]       DATE: 12/16/02
SUBJECT NUMBER: 022
TEST BEVERAGE: S       ID: [redacted]
DOB: [redacted]

70% VO2max: 7.5 speed 5.0% incline
75% VO2max: 7.5 speed 6.0% incline
80% VO2max: 7.5 speed 7.5% incline
85% VO2max: 7.5 speed 9.0% incline
90% VO2max: 7.5 speed 10.5% incline
95% VO2max: 7.5 speed 13.0% incline

***EXACT TIME OF EXHAUSTION!!!

MUSIC? ___
H2O every 15 min. mandatory + ad libitum

TEMP: ___

People present:

<table>
<thead>
<tr>
<th>MEASUREMENT/ ACTIVITY</th>
<th>VALUE/COMPLETE (Check)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEIGHT (lbs)</td>
<td>145</td>
</tr>
<tr>
<td>HEIGHT (in)</td>
<td>67</td>
</tr>
<tr>
<td>INITIAL BLOOD DRAW</td>
<td></td>
</tr>
<tr>
<td>RESTING BP/TIME (6:40-45 – before food)</td>
<td></td>
</tr>
<tr>
<td>RESTING PULSE (6:40-45 – before food)</td>
<td></td>
</tr>
<tr>
<td>TEST DRINK</td>
<td>(S)</td>
</tr>
<tr>
<td>CONSUMPTION TIME (6:45)</td>
<td></td>
</tr>
<tr>
<td>REST ONE HOUR UNTIL ___ TIME</td>
<td></td>
</tr>
<tr>
<td>CHECK ADD’L FOODS RECORD FOR DAY PRIOR/MAKE COPY</td>
<td></td>
</tr>
<tr>
<td>POLAR HEARTRATE MONITOR</td>
<td></td>
</tr>
<tr>
<td>BATHROOM BREAK</td>
<td></td>
</tr>
<tr>
<td>CATHETER INSERTION TIME (~7:40)</td>
<td></td>
</tr>
<tr>
<td>BLOOD DRAW (1 hour post snack)</td>
<td></td>
</tr>
<tr>
<td>WARM-UP (5 MIN) AT (MAX. 3.5 MPH, 0 INCLINE)</td>
<td>SPEED: _ COMPLETE:</td>
</tr>
</tbody>
</table>

Comments:
<table>
<thead>
<tr>
<th>MINUTE</th>
<th>MEASUREMENT/ACTIVITY</th>
<th>VALUE/COMPLETE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE clip</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*3</td>
<td>Borg Scale</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*17-20 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
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</tr>
<tr>
<td>20</td>
<td>Blood draw</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>*30</td>
<td>Borg Scale</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*37-40 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Blood draw</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*57</td>
<td>Borg Scale</td>
<td></td>
</tr>
<tr>
<td>57-60 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
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<tr>
<td>60</td>
<td>Blood draw</td>
<td></td>
</tr>
<tr>
<td>60 (1 hr.)(before dismount)</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>75 (1 hr., 15 min.)</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*77-80 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
<td></td>
</tr>
<tr>
<td>80 (1 hr., 20 min.)</td>
<td>Blood draw</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>*90 *** Increase treadmill</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>90 (1 hr., 30 min)</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Borg Scale</td>
<td></td>
</tr>
<tr>
<td>*95-100 GAS MEASURE</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
<td></td>
</tr>
<tr>
<td>100 (1 hr., 40 min.)</td>
<td>Blood Draw</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>PULSE</td>
<td></td>
</tr>
<tr>
<td>*115-120</td>
<td>Face mask/sensor ON/NOSE CLIP</td>
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</tr>
<tr>
<td>120 (2 hours)</td>
<td>Blood Draw</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>4 oz. Water</td>
<td></td>
</tr>
<tr>
<td>*120</td>
<td>Borg Scale</td>
<td></td>
</tr>
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</table>

Borg near the end: 115-120  Time: 130
<table>
<thead>
<tr>
<th>MEASUREMENT/ACTIVITY</th>
<th>VALUE/COMPLETE (Check)</th>
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</thead>
<tbody>
<tr>
<td>STOP TIME (exact)</td>
<td></td>
</tr>
<tr>
<td>BLOOD DRAW IMMEDIATELY</td>
<td></td>
</tr>
<tr>
<td>PULSE IMMEDIATELY at exhaustion</td>
<td></td>
</tr>
<tr>
<td>START 30 MINUTE TIMER FOR LAST BLOOD DRAW</td>
<td></td>
</tr>
<tr>
<td>MINIMUM 5 MINUTE COOL DOWN</td>
<td></td>
</tr>
<tr>
<td>WATER ad libitum</td>
<td></td>
</tr>
<tr>
<td>FINAL BLOOD DRAW 30 MIN. POST (not include cool down)</td>
<td></td>
</tr>
<tr>
<td>BLOOD PRESSURE 30 MIN. POST</td>
<td></td>
</tr>
<tr>
<td>PULSE 30 MIN. POST</td>
<td></td>
</tr>
<tr>
<td>SNACK/LUNCH/JUICE</td>
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</table>

**Comments:**
Appendix 3.22
Data Collection Form 2
Nutrition-Exercise Study – Athlete Fitness Test Form 2 (TEST 1)

HEART RATE, BLOOD DRAW, WATER

NAME: [Redacted]
SUBJECT NUMBER: 022
TEST BEVERAGE: S
DATE: 12/17/02
ID: [Redacted]
DOB: [Redacted]

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>70% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>5.0% incline</td>
</tr>
<tr>
<td>75% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>6.0% incline</td>
</tr>
<tr>
<td>80% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>7.5% incline</td>
</tr>
<tr>
<td>85% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>9.0% incline</td>
</tr>
<tr>
<td>90% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>10.5% incline</td>
</tr>
<tr>
<td>95% VO₂ max:</td>
<td>7.5</td>
<td>speed</td>
<td>13.0% incline</td>
</tr>
</tbody>
</table>

NOTE:
*Set treadmill to manual (protocol); run subject at 70% speed and incline for 1st 90 minutes.
AT 90 minutes, increase speed/incline to 75%; at 100 min. to 80%; at 110 to 85%; at 120 to 90%.
** At each blood draw, notate the exact time (minutes/second) when the treadmill speed begins to decline in swift preparation of blood draw and when it begins to speed back up to resume 70% VO₂ max.

***RECORD EXACT TIME OF EXHAUSTION:

Name of technician: ________________________________

<table>
<thead>
<tr>
<th>MINUTE</th>
<th>HEART RATE (Record HR and digital time)</th>
<th>BLOOD DRAW Every 20 min. (Record start and end time**)</th>
<th>Water-4 oz. (Record digital time)</th>
<th>Treadmill speed/incline changes</th>
<th>Comments on or from subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXAMPLE</td>
<td>X 110, 8:02a, 0.04</td>
<td>X 8:03 am</td>
<td></td>
<td></td>
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</table>

BLOOD DRAW IMMEDIATELY AFTER SUBJECT STOPS EXERCISE.
IMMEDIATE POST HEART RATE:
<table>
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<th>MINUTE</th>
<th>HEART RATE (Record HR and digital time)</th>
<th>BLOOD DRAW Every 20 min. (Record start and end time**)</th>
<th>Water – 4 oz. (Record digital time)</th>
<th>Treadmill speed/incline changes</th>
<th>Comments on or from subject</th>
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</thead>
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<tr>
<td>Pre warm-up</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – post warm-up (AT START)</td>
<td>X</td>
<td></td>
<td>7.5 mph at 5.0% incline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – blood</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>X</td>
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<td>40 – blood</td>
<td>X</td>
<td>X</td>
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<td></td>
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<tr>
<td>45</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 (1 hr) - blood</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>80 – blood</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>X</td>
<td></td>
<td>7.5 mph at 6.0% incline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 – blood</td>
<td>X</td>
<td>X</td>
<td>7.5 mph at 7.5% incline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td></td>
<td></td>
<td>7.5 mph at 9.0% incline</td>
<td></td>
<td></td>
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<tr>
<td>120 (2 hr) - blood</td>
<td>X</td>
<td>X</td>
<td>7.5 mph at 10.5% incline</td>
<td></td>
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</tr>
<tr>
<td>130</td>
<td></td>
<td></td>
<td>7.5 mph at 13.0% incline</td>
<td></td>
<td></td>
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<tr>
<td>Immediate post</td>
<td>X</td>
<td></td>
<td>Optional. Ad lib.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min post</td>
<td>X (manual)</td>
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</tr>
</tbody>
</table>

**BLOOD DRAW IMMEDIATELY AFTER SUBJECT STOPS EXERCISE.**
**IMMEDIATE POST HEART RATE:**

198
Appendix 3.23

General Set-Up and Testing Protocol
LLU Nutrition-Exercise Study Fall 2002 General Set Up/
Testing Protocol and Checklist

Set up:
_____ Test beverage blend and pour
_____ Metabolic cart on and warm up/tanks all unscrewed to the left
_____ Sheet on table with pillow
_____ Towels (2)
_____ Blood items out
_____ Calibration/enter data
_____ ECG: on “manual” protocol; check cords
_____ Data collection sheets out
_____ Stop watches/timers out
_____ Blood pressure equipment out
_____ Post run food
_____ CD/tape player out
_____ Measure out water into Dixie cups (6)
_____ Sugar-free gum to side table
_____ Team meeting/final questions
✓ Digital clock for time/count-up timers start together
✓ Blood on time/assist
✓ Exact time of stopping
✓ ATTENTION to subject – comfort, needs – KNOW his Name

Subject arrives 6:30 am:
_____ Initial blood draw (6:30)
_____ Resting Pulse (6:45 before food)
_____ Resting BP (6:45 before food)
_____ Test beverage (6:45) 10 minutes to consume
_____ Relax-stretch, walk around lab minimal (only if need to for warm up)
_____ Have subject remove watch if wearing one
_____ Get 24 hr recall and make 2 copies
_____ Describe test
_____ Catheter (7:40)
_____ Blood draw (7:40 before warm up)
_____ Fit headgear
_____ Heart rate monitor on and reading
_____ Bathroom break

200
Warm-up official 5 min
Begin test
(Borg, Water, HR, Gas, Blood)
At 90 min – increase treadmill every 10 minutes until exhaustion.
Blood draw immediate post
Record stop time (EXACT)
Remove Polar heart rate monitor
No food until 30 min. post blood draw (use timer to count-up time)

After 30 minutes post:
Final blood draw
Food for subject to consume in lab or to go
Have subject drink at least 1 juice box before leaving
Attend to subject

Clean-up:
Turn off treadmill
Print out test results (10-second intervals)
Compile all data test sheets (to Wendy)
Rinse out Polar heart rate monitor elastic (not the hard band)
Help with general clean up
Set up for next day
Hang sampling tubes
Close gas tanks
Clean flow-sensor/mouth pieces
Put boom box (carefully) into back closet and out of sight
Make sure to lock and close side door
Appendix 3.24

Blood Draw Protocol
Blood Draw Protocol

Set-up

1. Remove contents from covered green bin and spread on stainless steel movable blood cart.
2. Re-check supplies for day (restocked from day before.)
3. Cut tape strips.
4. Re-check boxes of disposable gloves, assure back-up box is available.
5. Make sure biohazards bags and Sharps containers are in place.
6. Check that blood-processing protocol set up is complete.

Blood Draw

Follow Universal Procedures for all blood draws.

Fasting and Final Blood Draw

1. Use direct stick method.
2. Clean area around the elbow crease.
3. Assist with supplies, as needed.
4. Use 21-gauge Vacutainer™ needle, splashguard, and 10 cc Vacutainer™ for collection.
5. Follow blood-processing protocol.

Serial measurements

1. Use catheter for serial measurements.
2. Insertion of catheter (20 gauge) should be into prominent vein in the forearm.
3. Secure tourniquet on biceps region.
4. Assist in holding supplies, re-taping, cleaning, and transferring of supplies following instruction.
5. After, blood draw and saline flush, cap of catheter is re-secured.
6. Re-wrap forearm lightly with elastic bandage.
7. Help expedite athlete’s return to treadmill and follow blood-processing protocol.
Avoiding/Handling Hemolysis

1. Always use tourniquet.
2. Do not pump syringe; change syringe if necessary.
3. Collect 10 cc blood sample. If 10 cc is difficult, get as close to 10 cc as possible within 1 minute.
4. Transfer syringe slowly with minimal movement.
5. Transfer blood into opened Vacutainer™, using slow drip method.
6. Follow blood-processing protocol.
7. If 2 of more hemolyzed samples arise during run, do 1 direct stick method draw during run at one of the 20 minute time points.

Clean-up/Preparation for Next Day

1. Return all sterile, unused contents to green covered bin.
2. Restock items as needed for following day.
3. Spray down and wipe all surfaces with antibacterial, hospital cleaner.
4. Close biohazards containers and return Sharps container to pick-up location.
Appendix 3.25

Blood Processing Protocol
Blood Processing Protocol

Set-up
1. The centrifuge should be placed on the copy machine table and set at 10 min. Leave the centrifuge open until the tubes for centrifuging are placed inside.
2. Spread a white sheet or paper towels on the worktable.
3. Keep a Styrofoam holder for micro centrifuge tubes (storage vials). Also have a smaller one ready for storing them in the refrigerator.
4. Keep a pipette, pipette tips, labels, biohazard garbage bag and container, box of gloves, glass beaker (to place Vacutainer™ tubes), a glass of water, individual Vacutainers™ with water to balance in the centrifuge, a pen, and numbers and dated log sheet for each subject in a binder for reporting of collection times, values, special notations and volume of serum collected.

Processing
1. Place the Vacutainer™ tube with the collected blood in a glass beaker, set the timer for 20 minutes, and leave it at room temperature for 20 min.
2. At exactly 20-minute mark, place this blood sample in the centrifuge and counter balance the sample with another Vacutainer™ with an equal volume of water.
3. Shut the centrifuge, press the white button and let the sample spin for 10 min.
4. Once the centrifuge comes to a complete stop, open the lid and remove the Vacutainer™ carefully without unnecessary movement or shaking.
5. The serum is now separated from the red blood cells.
6. Carefully, carry the sample to the working bench.
7. Set the pipette to 0.5 ml.
8. Pipette the serum and aliquot into the storage vials. (2 vials with 1 ml each, 3 vials with 0.5 ml each).
9. Immediately take the vials to storage in the refrigerator in the Nurse’s Station.
10. Record in the designated log sheet whether the sample is clean, slightly, or very hemolyzed, along with the number of aliquots actually obtained on the log sheet.
11. Discard the Vacutainer™ with the red cells in the Sharps biohazard container. Dispose the rest of the supplies used for processing in the biohazard bag.
12. After all of the blood samples are separated and aliquoted, remove the storage vials from the refrigerator and immediately place them in an icebox containing dry ice packs. Take the samples immediately to the Department of Nutrition laboratory to be stored in the –80°C freezer.
13. Transfer the storage vials from the Styrofoam container to the storage box in the laboratory. Label the box on the top with subject identification number and the date of collection.
14. The key to the freezer is available from department faculty or secretary.
15. Store the dated, numbered storage box with single subject blood samples on the third shelf from the bottom.
Appendix 3.26

Borg Rating of Perceived Exertion Scale
## Borg Rating of Perceived Exertion Scale

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Rating</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Light Intensity</strong></td>
<td>6</td>
<td>No Exertion at All</td>
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<tr>
<td></td>
<td>7</td>
<td>Extremely Light</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Very Light</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Light</td>
</tr>
<tr>
<td><strong>Moderate Intensity</strong></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Somewhat Hard</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Vigorous Intensity</strong></td>
<td>15</td>
<td>Hard (Heavy)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Very Hard</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Extremely Hard</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Maximal Exertion</td>
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</table>
Appendix 4.1

Nutrition-Exercise Study Design
Nutrition-Exercise Study Design

Randomized, balanced cross-over experimental design.

\[
\begin{array}{c}
\text{TRIAL 1} \\
\text{CE} \quad \text{AR=Almond-Raisin} \\
\text{1 day diet} \\
\text{TRIAL 2} \\
\text{CE} \quad \text{CE=Carbohydrate-Electrolyte} \\
\text{1 day diet} \\
\end{array}
\]

* Trials separated by 2 weeks
Appendix 4.2

Running Performance Times on the Motorized Treadmill Between AR and CE Trials
RUNNING PERFORMANCE TIMES ON THE MOTORIZED TREADMILL BETWEEN AR AND CE TRIALS

<table>
<thead>
<tr>
<th>Trial beverage</th>
<th>Running time (min)</th>
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<tbody>
<tr>
<td>AR</td>
<td>106.4 ± 4.9</td>
</tr>
<tr>
<td>CE</td>
<td>105.9 ± 5.2</td>
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</tbody>
</table>

Table 4.2—Running performance times on the motorized treadmill between trials. Values are means ± SEM, N = 10. AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage.
Appendix 4.3

Respiratory Exchange Ratios (RER) Line Graph
Figure 4.3—Respiratory exchange ratios (RER) at 20-min time intervals during exercise for AR and CE treatments (mean ± SEM). RER was similar between groups at all time points during exercise. AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage.
Appendix 4.4

Respiratory Exchange Ratios (RER) Bar Chart
Figure 4.4—Respiratory exchange ratios (RER) at 20-min intervals during exercise for AR and CE treatments (mean ± SEM). RER was similar between trials at all time points during exercise. AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage.
Appendix 4.5

Oxygen Consumption (VO₂)
OXYGEN CONSUMPTION (VO₂)

Figure 4.5— Oxygen consumption (VO₂) at start of exercise and 20-min time intervals throughout exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. Trials were not statistically different at any time point throughout exercise.
Appendix 4.6

Heart Rates
HEART RATES

Figure 4.6—Heart rates (HR) at start of exercise, 15-min time intervals throughout exercise, at exhaustion and 30-min post exercise (mean ± SEM). AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. Trials were not statistically different at any time point throughout exercise.
Appendix 4.7

Ratings of Perceived Exertion
Figure 4.7—Ratings of perceived exertion (RPE) at start of exercise, 20-min time intervals throughout exercise, and near exhaustion (mean ± SEM). 20-point scale (range 6-20) AR = Almond-Raisin beverage, CE = Carbohydrate-Electrolyte beverage. Trials were not statistically different at any time point throughout exercise.