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EFFECTS OF THE PRBAR AND POWERBAR ON FAT METABOLISM

by

Chad F. Witt

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Health, Physical Education,
and Recreation

Western Michigan University
Kalamazoo, Michigan
April 1998

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1998

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The two years spent on this project were difficult. In time they will appear as just a glance of time, but they will always be looked back upon and cherished.

Chad F. Witt

EFFECTS OF THE PRBAR AND POWERBAR ON FAT METABOLISM

Chad F. Witt, M.A.

Western Michigan University, 1998

The purpose was to compare the effect of dietary supplementation using two different energy bars, the PRBar and the PowerBar, ingested 1 hour before exercise, on the exercise metabolism of endurance athletes. Four dependent variables were measured: respiratory exchange ratio (R), oxygen consumption (VO_2), rating of perceived exertion (RPE), and blood glucose. Independent variables were bars (2), trials (2), and sample time. Sample times occurred at 5-min intervals for R, VO_2 , and RPE. Blood glucose was sampled before exercise, at 15 min and 25 min during exercise, and after exercise. Subjects completed four 35-min training runs, two after consuming each of the energy bars, in a random order on separate days. All testing was completed within 3 weeks. Subjects ran at an exercise intensity equal to 65% to 75% of their maximal oxygen consumption. Results indicated significant differences existed (a) between the energy bars for both R and RPE, (b) between sample times for all dependent variables, and (c) between trials for RPE. Also, a significant interaction effect, Bars \times Trials, occurred for VO_2 . It was concluded that although the significant decrease in R and significant increase in RPE supported an increase in fat metabolism during exercise when subjects supplemented with the PRBar, the lack of significant changes in VO_2 and blood glucose levels across training runs indicated a mixed result. Also, the practical significance of the changes in R and RPE was questioned.

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CHAPTER I

INTRODUCTION

As an endurance athlete, this researcher has a personal interest in athletic performance improvement. In recent years the public interest in sport has grown along with the desire to improve performances through the use of viable legal methods. Aside from the limits imposed by heredity and the physical improvements associated with training, no factor plays a more dominant role in exercise performance than nutrition (Costill, 1988). Healthy nutrition has been shown in numerous published studies to have a positive effect on athletic performance (American Dietetic Association and Canadian Dietetic Association [ADA and CDA], 1993). Just 20 years ago, nutrition was not a focal point with regard to performance enhancement; however, in recent years it has become of great importance to trained individuals as well as to exercise scientists. As many individuals have become more aware of nutrition's influence on endurance performance, disagreement has arisen concerning proper nutrition for athletes. The diet of the endurance athlete must supply adequate calories to provide for the significant energy demand resulting from long hours of training. Some of the first studies on dietary carbohydrate content by Costill (1988) in the late 1970s showed that a nutritional supplement containing 60% to 70% carbohydrate is highly beneficial for athletes involved in aerobic activity.

There has been some controversy as to the most beneficial composition of the preexercise meal for endurance exercise. More recent information published without scientific support suggested that a nutritional supplement containing 40%

carbohydrates, 30% protein, and 30% fat would improve endurance performance. The theory of the manufacturers is that preexercise foods with higher fat content increases oxidation of fat stores in the body, which in turn creates a glycogen sparing effect, and performance is increased. It is thought that through increased fat consumption during exercise, triggering of elevated eicosanoid levels will create a temporary catabolic state in which the body breaks down fat for energy (Horswell, 1996). These highly promoted food supplements are available today in a ready-to-eat form called an energy bar. Originally energy bars had a nutritional content high in carbohydrate, low in protein, and low in fat, such as PowerBars[®], Powerfood Inc. Berkeley, California, and Clif Bars[®]. These bars are said to enhance endurance performance through the replenishment of glucose stores during and after exercise (PowerBar, 1997). The PRBar, made by PRNutrition Incorporated of San Diego, California, is an example of a new type of supplement. This new bar and diet plan contains more fat than the original bars and higher quantities of daily protein and fat than studies have shown to be beneficial and healthy.

Statement of the Problem

The focus of this research was to determine if the food supplement bar, PRBar, was more effective in increasing fat utilization during exercise in male and female endurance athletes, ages 18 to 40 years, than the more traditional, high-carbohydrate PowerBars. Specifically, the researcher measured RPE, blood glucose levels, maximal oxygen consumption ($\text{VO}_2 \text{ max}$), and respiratory exchange ratio (R), under two experimental conditions, after eating a PowerBar and after eating the PRBar.

Need for the Study

This study was initiated on behalf of amateur athletes who support themselves through all aspects of their training and competition. These athletes should not be encouraged to waste their money on a product that is ineffective and of unproven value. A primary reason for this study is to determine if the claims concerning the PRBar, in relation to endurance performance, are true. To be more specific, the results of the study will indicate if a moderate-carbohydrate, high-fat, and high-protein food supplement, in the form of an energy bar, truly enhances endurance performance.

Delimitations

The following delimitations were established for this study:

1. Subjects were 15 apparently healthy volunteers, both male and female between the ages of 18 to 35 years.
2. Only subjects who were capable of completing training runs of 45 min in duration at submaximal levels were recruited for the study.
3. Subjects' VO_2 max values were estimated by selecting their best performance on a graded exercise test across two trials performed on each of 2 different days.
4. Subjects performed four training runs at levels between 65% and 75% of VO_2 max. Two runs were performed after ingesting the PRBar, and two runs were performed after ingesting the PowerBar. The order of testing was randomly determined for each runner.

5. Subjects consumed the PRBar or PowerBar 1 hr prior to the training runs.
6. Prior to the consumption of the energy bar for each training run, subjects fasted for a minimum of 3 hr.
7. Subjects avoided the ingestion of alcohol; use of tobacco products or ergogenic aids, such as caffeine; and carbohydrate loading for 12 hr prior to each training run.
8. R, RPE, and oxygen uptake (VO_2) were measured every 5 min throughout the training runs.
9. Blood glucose was measured preexercise, at 15 min, 25 min, and postexercise.
10. A minimum of 24 hr occurred between training runs.
11. A maximum of 3 days separated each treadmill test.
12. All measurements were collected by the investigator or by individuals trained by the investigator.

Limitations

This study was limited by the following:

1. The sample size of the study ($n = 15$) is small. A small sample can adversely affect the external validity of the study.
2. The study was conducted in a laboratory setting on a treadmill and not in an actual training or competitive setting. This could also affect the external validity.
3. The age range of the subjects, 18 to 40 years, is narrow; this may not be truly representative of the population who may use the supplements studied.

Hypotheses

The following hypotheses were formulated based on the design of the study, the literature review, and the investigator's experience:

1. At steady state exercise, subjects will experience R values during the running trials following consumption of the PRBar that are lower than the R value after the PowerBar.
2. At steady state exercise, subjects will show greater VO_2 values during the running trials performed following supplementation with the PRBar that are greater than VO_2 values following supplementation with the PowerBar.
3. At steady state exercise, subjects will experience the same RPE during running trials for both the PRBar and PowerBar.
4. Blood glucose levels will fluctuate during the first 15 min of exercise but show little difference during the remaining exercise session.

Assumptions

It was assumed that:

1. Subjects fasted at least 3 hr prior to the supplementation for the training runs.
2. After calibration, the instruments used in the study produced accurate results.
3. Each subject gave his or her best effort when performing the graded exercise test used to estimate VO_2 max.
4. Each PRBar contained 40% carbohydrate, 30% protein, and 30% fat.
5. Each PowerBar contained 80% carbohydrate, 15% protein, and 5% fat.

Definition of Terms

The following terms were defined in relation to the context of this research project:

1. Eicosanoids: Any of the biologically active substances derived from arachidonic acid, including the prostaglandins and leukotrienes (*Dorland's Illustrated Medical Dictionary*, 1994).

2. Estimated maximum heart rate (MHR): An estimate of a subject's highest heart rate response during maximal aerobic exercise. It is calculated using the formula $MHR = 220 - \text{age}$.

3. Energy bar: A mixture of fruits, grains, and other high-energy natural ingredients. Energy bars on the market today can be divided into two main groups: (1) brands high in carbohydrates, low in proteins and fats; and (2) brands that contain a more balanced mix of proteins, carbohydrates, and fats (Lobb, 1995; PowerBar, 1997).

4. Energy balance: A metabolic state in which caloric intake equals caloric expenditure. If caloric intake is greater than caloric expenditure, a weight gain occurs. If caloric intake is less than caloric expenditure, a weight loss occurs.

5. Ergogenic aid: A substance, appliance, or procedure that improves physical performance.

6. Maximal O₂ consumption (VO₂ max): The ability to perform large muscle, dynamic, moderate to high intensity exercise for prolonged periods (American College of Sports Medicine [ACSM], 1995).

7. The Zone: A metabolic state in which the body works at peak efficiency (Sears & Lawrence, 1995). It is said to be created by consuming a diet containing

40% carbohydrates, 30% proteins, and 30% fats. The philosophy is that an individual needs to consume a balanced diet to allow the release of eicosanoids, which enable an individual to stay in the zone.

8. Carbohydrate loading: A practice in which energy expenditure is reduced and carbohydrate consumption is increased to permit “maximal” glycogen storage in muscle.

9. Creatine kinase (CKMD): An enzyme catalyzing the transfer of phosphate from phosphocreatine to ADP, forming creatine and ATP. It is of importance in muscle contraction. CKMD is primarily found in cardiac muscle (*Steadman's Illustrated Medical Dictionary*, 1976).

CHAPTER II

REVIEW OF LITERATURE

Introduction

In recent years, athletes have been presented new alternatives to the traditional preworkout snack or energy supplements consumed during activity. These snacks and supplements are now appearing on the market in the form of a product called energy bars. A few years ago, energy bars, once the obscure foodstuff of hikers and backpackers, could be found only in health food stores (Lobb, 1995). Today, they are available in supermarkets and in sporting goods stores. The use of energy bars as an energy supplement in today's high-paced society is ideal for athletes. Traditionally, energy bars were high-carbohydrate, protein-rich, low-fat foods that were easy to digest and were reported to provide quick, sustaining energy for individuals involved in endurance training and competition (Burke, 1994). In this chapter, the researcher will provide information regarding the composition of energy bars, along with research concerning their effect on fat metabolism during exercise and other possible benefits attributed to their use.

The use of energy bars by the general public or recreational athlete is controversial. This issue will be addressed in this chapter, along with the following related topics: (a) nutrients as ergogenic aids, (b) misconceptions about nutrition and athletic performance, (c) recent trends in diets, (d) nutrient recommendations, and (e) possible complications associated with the use of the PRBar.

Nutrients as Ergogenic Aids

Athletic events are competitive by nature, thus any boost in performance, no matter how trivial, is pursued vigorously by athletes and coaches (Bucci, 1993). Several nutritional modifications have been used by athletes to try to improve performance. Recent attention has focused on diets containing more fat and branched-chain amino acids (Clarkson, 1996).

Ergogenic aids are substances or treatments that improve sport performance above and beyond the effects of physical training alone. Some aids are used to enhance physical training, and others are used to enhance performance during a competitive event (Williams, 1996). Ergogenic aids have been arbitrarily classified into four categories: (1) mechanical aids, (2) psychological aids, (3) physiological aids, and (4) nutritional aids (Williams, 1992). All categories of ergogenic aids are employed to various degrees by amateur and professional athletes alike and have become more prevalent in recent years. Several interrelated factors may have contributed to this increase, including (a) the phenomenal increase in the popularity of sports, both professional and amateur; (b) the increase in the financial and other personal benefits to successful athletes; (c) the emergence of exercise science as a respected scientific discipline; and (d) the increasingly sophisticated biomedical and nutritional advances that have led to the development of drugs and dietary supplements that athletes can use (Williams, 1996).

Carbohydrate Supplementation

The importance of adequate muscle and liver glycogen stores to performance of prolonged exercise is generally recognized in the exercise science community

(Foster, Costill, & Fink, 1979). The depletion or reduction of bodily carbohydrate reserves is associated with fatigue during endurance exercise. Consequently, it has become common practice for athletes to attempt to carbohydrate load prior to endurance competitions of 1 hr or longer in duration. Carbohydrate loading is typically practiced with decreasing training loads to enable muscle glycogen stores to rise after depletion due to intense training.

Studies in the 1980s and 1990s have unequivocally demonstrated preexercise carbohydrate feedings can significantly improve endurance performance as well as carbohydrate feedings during exercise, improving performance that is limited by carbohydrate depletion (Sherman, 1995). For carbohydrate feedings to be effective, the exercise being performed needs to be submaximal, at 70% to 80% of VO_2 max, and longer than 90 min in duration (Clark, 1996). Research has shown that performance improves due to an increased rate of carbohydrate oxidation during exercise, not because of the sparing of muscle glycogen, as once hypothesized (Sherman, 1995). The original energy bar concept was developed in an attempt to increase available glucose during exercise.

Fat Supplementation

The two main sources of energy during exercise are carbohydrates and fats. Over the past 30 years many laboratory and field researchers have demonstrated the importance of muscle and liver glycogen in reducing fatigue and improving athletic performance. The ingestion of fats as an ergogenic aid has been under study since the mid 1970s (Costill et al., 1977). In some researchers' eyes, improved endurance capability is observed following aerobic training. It is attributed to an increased oxidation of fat relative to carbohydrate; this carbohydrate sparing presumably delays

the point at which reduced carbohydrate causes fatigue (Sherman & Leenders, 1995). This leads to the suggestion that greater availability of fat during exercise can improve performance by carbohydrate sparing and “fat loading.” Supplementation with fats has been attempted in three forms: (1) long-chain triglycerides, (2) medium-chain triglycerides, and (3) intravenous lipid infusions (Coyle, 1995).

The first form of fat supplementation, long-chain triglycerides, is not possible due to the body's inability to ingest free fatty acids (FFAs). FFAs are too acidic for digestion and need a protein carrier for intestinal absorption. Coyle (1995) stated, “Although not proven, it is unlikely that ingestion of long chain triglycerides have much potential to provide significant fuel for muscle during exercise” (p. 4).

The second method, ingesting medium-chain triglyceride (MCTs), is a very rapid way of raising FFA levels. MCTs are absorbed by the liver and pass into the blood very quickly. Recent studies have shown that a larger percentage of MCT oxidation occurs when it is consumed along with carbohydrate; however, it has also been shown that gastrointestinal discomfort may arise when ingesting MCTs above 30 g per meal (Coyle, 1995; Evans, 1996).

The third method of fat supplementation is intravenous lipid infusion. This technique was used in research studies to raise plasma FFA levels within the blood. Injecting Intralipid, followed by heparin, causes the activation of a lipolytic enzyme, lipoprotein lipase, at receptor sites on adipose cells. There, the enzyme splits triglycerides into glycerol and FFA. The elevation of FFA via intravenous infusion slightly reduces the rate of muscle glycogen utilization (Costill et al., 1977; Vukovich et al., 1993). This effect is relatively small, and any benefit to performance has yet to be demonstrated (Coyle, 1995). Although fat loading is a plausible hypothesis, it is

not supported by a sufficient number of valid, credible, and replicated studies at this time (Sherman & Leenders, 1995).

Protein Supplementation

It has been shown through numerous studies that increased protein intake combined with strength training is associated with increased muscle mass (Evans, 1996). It is clear that some athletes who ingest the ADA's recommended dietary allowance of protein, $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, may show a negative nitrogen balance. At Penn State, Evans (1996) found that endurance exercise was associated with dietary protein needs greater than the current RDA, reaching levels of $0.94 \pm 0.05 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Thus, it is recommended that athletes consume quantities of protein above the RDA to maintain a positive nitrogen balance. However, the amount of protein needed to increase muscle mass is unclear. Current recommendations for protein range from 1 to $2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (Evans, 1996). Lemon and Nagle (1981) also supported the concept that athletes need increased levels of protein, having stated endurance athletes need $1.2\text{--}1.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ and strength trained athletes need $1.7\text{--}1.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.

Caffeine Supplementation

Major dietary sources of caffeine are coffee, tea, chocolate, colas, and several herbs, all of which represent important dietary items for most cultures (Bucci, 1993). Studies conducted in the 1970s indicated caffeine supplementation resulted in little or no increase in the performance of athletes. Since the initial use of caffeine as an ergogenic aid, its specific benefits to physical performance have been questioned. Since the 1970s, studies concerning caffeine have shown that it stimulated cardiac

muscle, produced diuresis, initiated epinephrine release and lipolysis of adipose tissue resulting in increased FFA levels during exercise, and enhanced muscle contractility. Several of these effects have been postulated to improve physical performance (Bucci, 1993). In studies of caffeine's effects on endurance performance, researchers found increased levels of FFA and glycerol (Graham & Spriet, 1991). Further, caffeine has been shown to enhance performance in aerobic exercise events of short duration, such as a 1,500-m run or a 5-min exercise task (Graham, Rush, & van Soeren, 1994). An area of performance enhancement that has not yet been tested in humans is the effect of caffeine and aerobic exercise on the reduction of body fat (Bucci, 1993).

Misconceptions About Nutrition and Athletic Performance

Many misconceptions exist concerning nutrition and athletic performance (van Erp-Baart, Saris, Binkhorst, Vos, & Elvers, 1989). These misconceptions about foods and their links to athletic performance have most likely been around as long as people have been eating, recent times being no exception. In the 1960s, it was thought that eating higher percentages of carbohydrates, such as pasta, rice, and bread, would make an individual fat; however, later research has shown this to be untrue to some extent. In addition, current misconceptions exist about the amount of fat and protein needed by athletes. Some people believe that endurance athletes need very little protein in comparison to weight-trained athletes, who are thought to need large quantities.

The components of energy expenditure include basal metabolic rate (BMR), thermogenesis, and physical activity (Brooks, Fahey, & White, 1996). BMR is a

measure of the minimal amount of activity associated with the continuous organic functions of respiration, circulation, and secretion.

Energy demands of a physically active individual are based on intensity, duration, frequency, and type of physical activity performed (American Dietetic Association, 1987). The quantity and duration of physical activity are probably the most significant and variable factors of energy expenditure (Brooks et al., 1996). Longer exercise durations at lower intensities improve body composition by decreasing body fat. The greater the ratio of lean body mass to fat mass, the greater the amount of energy that is expended during the activity.

Misconceptions About Carbohydrate Use

Carbohydrates are the source of energy most favored for use by the body. They are easily digested by the body. Many people believe that if they consume too many carbohydrates they will feel fat and heavy. This feeling occurs because carbohydrate storage in the body results in water retention. For every gram of carbohydrate stored by the body as glycogen, 3 g of water is also stored. Individuals who consume the recommended amount of carbohydrate, 60% to 70% of their dietary intake, are likely to fluctuate in weight due to this water retention. Another misconception is that people who consume the majority of their daily calories from carbohydrates and protein and avoid fats will not get fat. Carbohydrates, like any other form of energy consumed in excess, will be stored in the body as fat.

Misconceptions About Fat Consumption

A common practice by misinformed individuals is the complete avoidance of fats. Stored fat is the body's most plentiful source of potential energy (McArdle,

Katch, & Katch, 1991). The body needs fat in the diet both to use as fuel and to absorb fat soluble vitamins. However, fats can be harmful to the human body when consumed in excess of basic needs.

Although complete understanding of fat metabolism during exercise does not exist, there is now enough information to cast serious doubt on recent advertising claims advocating special diets and nutritional supplements with more fats and fewer carbohydrates (Coyle, 1995). Fat is stored in fat cells in the form of triglycerides, which contain a glycerol complex with three fatty acids attached. Fatty acids are taken up by skeletal and cardiac muscle in proportion to plasma concentrations in the blood and then used as fuel. The fate of the fatty acids is either immediate metabolism within muscle mitochondria to produce energy or storage as intramuscular triglycerides (Williams, Breuer, & Patton, 1984).

Misconceptions About Protein Use

No nutrient has received more attention in relation to athletic performance than protein. In the past, protein was considered the super nutrient (van Erp-Baart et al., 1989). Protein is oxidized as an energy source during exercise. A misconception regarding protein relates to the quantity of protein needed by athletes during training. Many uninformed individuals believe that endurance athletes need less protein than strength athletes. Bodybuilders typically consume larger quantities of protein than endurance athletes. Researchers who have compared body builders and endurance athletes have shown that endurance athletes need more protein than body builders due to protein catabolism during endurance exercise (Tarnopolsky, MacDougall, & Atkinson, 1988).

A diet providing 12% to 15% protein, or $1.24 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, is usually recommended to provide adequate protein for endurance athletes. Evans (1996) found that when protein is expressed as a percentage of daily energy expenditure ($3,910 \pm 240 \text{ kcal/day}$), subjects needed only $6.9\% \pm 0.5\%$ of total calories as protein. Thus, athletes consuming 12% to 15% or more of their calories from protein are likely to get enough.

One current controversy regarding protein is the quantity of protein oxidized during extensive endurance exercise when glycogen stores are depleted. Various studies have monitored the R, which indicates that during endurance exercise, oxidation of protein from muscle can increase 5% to 15% as an energy source (van Erp-Baart et al., 1989).

Recent Trends Concerning Nutrition for Exercise

Although there is a theoretical basis for high-fat, high-protein diets to enhance performance, there is insufficient evidence to state unequivocally that high-fat, high-protein diets are effective (Clarkson, 1996). The trends discussed in this section are based on published articles, books, and informational handouts related to current energy supplements. This information is not specifically supported with published data and is very controversial.

The Fat-Burning Zone

The fat-burning zone is a concept discussed in popular articles relating to energy metabolism; however, the producer of the PRBar, PRNutrition, San Diego, California, presented information that is questionable in its scientific authenticity. To keep blood sugars stable, the PRBar manufacturers claimed that by consuming their

bar, people receive constant energy, burn fat, recover quickly, and increase metabolism and lean body mass (PRNutrition, 1994). The idea behind the PRNutrition program is to decrease the hyperglycemic condition associated with high-carbohydrate intake. According to PRNutrition, the ratio of carbohydrates, proteins, and fats eaten at every meal determines the true power of nutrition; it is the precise macronutrient composition of a meal that controls the release of insulin (the fat storage hormone) and glucagon (the fat mobilization hormone).

Carbohydrate Intake and Obesity

The current view held by some manufacturers of energy bars is that the consumption of carbohydrates will make an individual fat. It has been stated that one can control the fuel the body burns through diet (Maffetone, 1994). Although diet may be a contributing factor, the greatest contributing factor is the intensity and duration of one's training, not diet, that determines an athlete's ability to burn fat (Horswell, 1996). Maffetone (1994) stated that consuming too many carbohydrates, even if the diet is fat-free, can actually make you fat due to the way your body stores and uses the end product from carbohydrates. This philosophy about carbohydrates stems from the notion that if one consumes excess amounts of carbohydrates, the blood insulin level rises, causing glucose levels to increase, thereby storing carbohydrates as glycogen. Once glycogen stores have been repleted, excess carbohydrates are then stored as fat. Other researchers disagree. Evans (1996) and Clark (1996) stated that during a typical day very little carbohydrate is converted to fat due to the high energy cost of conversion of carbohydrates to fat. Also, the amount of time it takes the body to perform this is quite long, and the body will use the carbohydrates as its fuel before storage as fat is complete.

The Zone: 40:30:30

In the book, *Enter the Zone*, Sears and Lawrence (1995) discussed a diet containing a ratio of 40% carbohydrate, 30% protein, and 30% fat. Their theory is that an individual needs to consume a balanced diet to allow the release of eicosanoids, enabling an individual to stay in “The Zone.” The zone is defined as a metabolic state in which the body works at peak efficiency (Sears & Lawrence, 1995). The authors claimed that through the consumption of a 40:30:30 diet, people achieved optimal body function, freedom from hunger, and greater energy and physical performance, as well as improved mental focus and productivity. These claims are contrary to the high-carbohydrate diet containing carbohydrates, protein, and fats, 60:20:20, respectively, recommended by the ADA and CDA. Sears and Lawrence believe that this type of diet would continuously create an insulin rollercoaster throughout the day and not enable one to stay in the zone.

Nutrient Recommendations for Endurance Exercise

There are set nutritional guidelines formulated by the American Dietetic Association for athletes. These guidelines cover the recommendations suggested by scientific studies and the ADA and CDA for endurance athletes.

Carbohydrates

Specific information about the best composition of an athlete’s diet has become available in the last 2 decades (van Erp-Baart et al., 1989). Carbohydrates have been shown to be the preferred fuel for the body because they are easily broken down and digested rapidly by the body. Respiratory gas exchange, muscle biopsy,

and other procedures have demonstrated the overwhelming importance of glycogen as the fuel for exercise (Brooks et al., 1996). Athletes who participate in endurance sports, such as marathon running, cross country skiing, distance swimming, and cycling, need to consume adequate energy, with the majority coming from carbohydrates (Hoffman & Coleman, 1991). The current recommendation for carbohydrate intake for athletes is 60% to 70% of the diet for individuals who train exhaustively on successive days (ADA & CDA, 1993). The idea that carbohydrates can make you fat is supported by Maffetone (1994) and Sears and Lawrence (1995). Excess energy is stored as fat when the energy balance becomes positive (Brooks et al., 1996), regardless of the form in which the excess energy is consumed.

At the onset of exercise, muscle glycogen is the primary source of carbohydrate energy (Costill, 1988). It is also known that there is an increase in muscle glycogen use when work intensity approaches maximal levels. If there is a deficiency in carbohydrates, there is a reduced ability to use glycogen, which may compromise performance (Hoffman & Coleman, 1991). Therefore a high-carbohydrate diet (a minimum of 500 g) is recommended throughout training to maintain glycogen stores in both muscle and blood (Coyle & Coyle, 1993).

Fat

Bloodborne fatty acids and intramuscular triglycerides are important energy sources during prolonged exercise (Brooks et al., 1996). Further, fats are necessary in the diet, both to provide energy and to absorb fat-soluble vitamins. The question is: Should athletes consume extra fat in their diet? The answer is a resounding “NO!” (Brooks et al., 1996). Depending on the type of athlete, a diet containing 20% to 30% fat is sufficient. Fat should contribute no more than 30% of total energy to the

diet (ADA & CDA, 1993). Total fat intake is likely to be higher for individuals who must consume large quantities of energy to maintain body mass. This is typical in football or in other sports in which large mass is important. In determining a caloric breakdown in a diet, protein and carbohydrate requirements should be calculated first. Fat intake can be used to provide additional energy up to 30% of the total energy requirements. Energy needs beyond this level should be consumed in the form of high carbohydrate low-fat foods (ADA & CDA, 1993).

Protein

The Recommended Daily Allowance (RDA) for all individuals is $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. When consumed by athletes while training, this level has been shown to be insufficient and create a negative nitrogen balance in athletes (Evans, 1996). Evans stated that the dietary needs for athletic populations vary depending upon the type of training undertaken. Endurance athletes need to consume $1.24 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of protein in order to maintain positive nitrogen balance. This quantity is needed due to the increased levels of CKMD found in endurance-trained skeletal muscle. This enzyme is typically found only in cardiac muscle. Its presence in skeletal muscle is associated with muscle breakdown, most notably in athletes participating in endurance exercise and in eccentric muscle contractions performed repetitively. Eccentric muscle contractions often lead to delayed onset muscle soreness (DOMS). In comparison, strength-trained individuals need to consume $0.94 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, an amount less than that recommended for endurance athletes.

Complications Associated With Increased Fat Ingestion and Decreased Carbohydrate Consumption

Long-term use of high-fat diets may have negative consequences on health. The safety of long-term use of a diet containing the 40:30:30 ratio of carbohydrates, fats, and protein has not been established.

In combination with a low-carbohydrate diet, hard training may empty the liver glycogen stores (Costill, 1988). Studies have shown if people exercise with suboptimal glycogen levels and eat a low carbohydrate diet, they may be more susceptible to exercise-induced muscle damage (Bar-Or et al., 1993). Endurance athletes who are expending 3,000 to 4,000 kcal per day are advised to eat amounts of carbohydrates of 8 to 10 g • day • kg⁻¹ body weight. More simply, this equals 10 to 14 50-g portions of carbohydrate each day (Coyle & Coyle, 1993). Several efforts have been launched to educate Americans about the critical role of nutrition in health, such as the National Cholesterol Education Program, the National High Blood Pressure Education Program, and the Nutrition Screening Initiative (Blair et al., 1996). These programs, along with goals outlined by *Healthy People 2000* of increasing the span of healthy life for Americans (Blair et al., 1996), are health educators' efforts initiated to improve the overall health of the American public.

As indicated by Clark (1996), this dietary ratio is very similar to the diet already being consumed by the public, which contains levels of protein and fat above the ADA's recommendation of 60:20:20. There are no published scientific studies that support the use of a 40:30:30 diet for athletic populations or the improvement of health in the general public. At this time, there is no evidence that consuming this diet will improve health or athletic performance better than the ADA-recommended dietary composition of 60:20:20.

Energy Bars

A traditional energy bar is a mixture of fruits, grains, and other high energy, natural ingredients high in carbohydrate. Energy bars on the market today can be divided into two main groups: (1) the brands high in carbohydrate, low in protein and fat; and (2) brands that contain a more balanced mix of nutrients (Lobb, 1995).

The value of energy bars is their nutritional composition and ease of digestion, which depends on the consistency of the bar. Their availability and ready-to-eat form also make them convenient for consumption. Two primary reasons for consuming an energy bar are to replete glycogen stores during long exercise bouts and to serve as a postexercise source for high carbohydrates. The popular belief regarding the preexercise meal is that this meal should be 200 to 500 kcal; it should be high in easily digested carbohydrates and low in slower digested fats and proteins (Nelson-Pfab, Nethery, Gee, & Bergman, 1997). The traditional energy bar composition parallels this belief.

PowerBars

The PowerBar is a high carbohydrate energy bar. High carbohydrate energy bars are used to supply a balanced combination of simple and complex carbohydrates, a small amount of protein, and some fat to help maintain consistent energy levels and replenish the body's energy stores as they are being depleted. High carbohydrate energy bars contain 70% to 90% of their calories in carbohydrates (Burke, 1994) and as little as 2% in fats (Lobb, 1995). Some common names of high carbohydrate bars include PowerBar, Exceed, Tiger Sport, and Clif Bar. Based on scientific research,

the makers of these bars claim that the high-carbohydrate composition provides a superior source of fuel for endurance performance.

PRBars (Balanced Nutrient Concept)

Some bars that are more balanced in carbohydrate, protein, and fat are the PRBar, Balance Bar, and Carbo-crunch Bar. Manufacturers of these bars argue that the higher protein and fat content and lower carbohydrate content (40% to 60%) promote greater fat-burning during exercise (Lobb, 1995). In an article by Burke (1994), the executive vice president of one manufacturer was quoted as saying:

Our bar is based on new research which shows that carbohydrate/protein ratio in food determines to a large extent whether one burns stored fat. In the presence of the right amount of protein and dietary fat, carbohydrate entry into the system is retarded, thereby, keeping insulin levels down and fostering higher levels of the hormone glucagon. This steadies blood sugar for extended periods by encouraging the body to access body fat for energy. Steady blood sugar has clear benefits for athletes, including better energy, greater endurance and better concentration and focus. (p. 1)

The benefits associated with the PRBar and PowerBar are different. The claims are based on different theories. The original theory associated with the high carbohydrate energy bars was that their use improved the amount of carbohydrate available as an energy source during exercise. The theory for the new bars, which contain more protein and fat with less carbohydrate, is that their use stimulates the consumption of more fat for energy during exercise, thus sparing carbohydrate.

Summary

Energy bars are a relatively new concept in the field of nutritional supplementation. The original forms of energy bars were based on scientific studies supporting the concept that carbohydrates are the primary source of fuel during

exercise. New concepts in nutrition have surfaced within recent years with respect to composition of macronutrients recommended in the diet. Research supporting these new recommendations is not extensive. Additional research is needed to determine whether a diet higher in fat and protein is better for athletes than the traditional high-carbohydrate diet. The new energy bar, the PRBar, contains less carbohydrate and more fat and protein than current recommendations suggest. The claims of enhanced performance due to consumption of this product are in question at this time.

CHAPTER III

METHODOLOGY

The problem investigated in the study was to determine if the PRBar was more effective in increasing performance and fat utilization in male and female runners than the traditional higher carbohydrate bar, the PowerBar. Two VO_2 max tests were completed so that the exercise intensities for the training runs, 65% to 75% of VO_2 max could be determined. $\dot{V}\text{O}_2$, RPE, and serum blood glucose were measured during the performance of four training runs at an exercise intensity between 65% and 75% of VO_2 max and duration of 35 min. Two runs on separate days were performed after the consumption of the PRBar, and two were performed after consumption of a PowerBar. This chapter includes the following procedural steps: (a) subject selection, (b) instrumentation, (c) testing procedures, and (d) research and statistical design of the study.

Subject Selection

Subjects were 15 “apparently healthy” (ACSM, 1995) male and female volunteers, ages 18 to 35 years. Subjects were recruited from a collegiate cross-country team and a local running club. The subjects were screened for cardiovascular disease risk factors and orthopedic problems that would limit their participation in the study (see Screening Form, Appendix A). Individuals with cardiovascular disease, those with known symptoms of cardiovascular disease, or those possessing two or more known risk factors for cardiovascular disease were not allowed to participate in

the study. Individuals with orthopedic injuries that required medical treatment during the previous year or those whose injuries were considered chronic were excluded. Subjects gave written consent prior to participation in the study (see Consent Form, Appendix B). Prior approval to conduct this study was given by Western Michigan University's Human Subjects Institutional Review Board (see Approval, Appendix C).

Instrumentation

The equipment used to measure subjects' VO_2 max consisted of the following:

1. Quinton metabolic cart, model Q-plex 1, Quinton Instrument Company, Seattle, WA.
2. Bosch 501A modified lead EKG, Bosch Medical Engineering, Berlin, Germany.
3. Bosch oscilloscope, ECS 502, Berlin, Germany.
4. Borg's Rating of Perceived Exertion (RPE) scale ranging from 6 to 20 (ACSM, 1995).
5. Quinton Q65 treadmill, Quinton Instrument Company, Seattle, WA.

All equipment listed above, except the EKG and oscilloscope, were used during the training runs, along with the following:

1. Microtainer Lancets, Becta Dickinson, Rutherford, NJ.
2. Offset DX ECG electrodes, Graphic Controls, Buffalo, NY.
3. Medisense II Blood Glucose Sensor, MediSense Inc., Bedford, MA.

Testing Procedures

All testing was completed in the Exercise Physiology Laboratory in the University Recreation Center, at Western Michigan University, Kalamazoo. Prior to the study, prospective subjects completed a health screening form and signed a consent form. Subjects were asked to wear appropriate clothing and shoes for running on the treadmill. Subjects were allowed time to become comfortable with the treadmill and metabolic cart before testing and running trials began.

VO₂ Max Test

On 2 different days subjects performed a VO₂ max test on a treadmill using a graded exercise test protocol for endurance-trained athletes (Lamb, 1984). Two graded exercise tests were completed by each subject to ensure a reliable measure reflective of each subject's true VO₂ max. The better of the two trials was chosen to represent the subject's VO₂ max. Following a 3-min warm-up, the subjects performed a graded exercise test with increasing work intensity (Lamb, 1984). The graded exercise test continued until either VO₂ max was achieved, estimated MHR was reached, or volitional exhaustion was reached. MHR was calculated using the age-predicted heart rate formula, $MHR = 220 - \text{age}$. During the test, the subject's VO₂, heart rate, and electrocardiogram were monitored continuously with a modified four-lead EKG and oscilloscope. An RPE value was determined near the end of each stage.

Running Tests

During four training runs, subjects performed a 35-min bout of exercise on a treadmill at an intensity between 65% and 75% of their VO_2 max. Prior to each run, individuals were allowed to warm up for a period of 3 to 5 min. The experimental conditions of ingesting one PRBar or one PowerBar were randomized by condition. The subjects were asked to refrain from carbohydrate-loading prior to the running trials and to consume a normal food intake the day prior to each trial. In addition, the pretrial meal was consumed at least 4 hr prior to each test. The meal was restricted to typical training foods and quantities eaten by the subject. Before the appropriate training runs, subjects were asked to consume either the PRBar or a PowerBar 60 min prior to the test.

All trials were completed on separate days. Heart rates were monitored continuously during the runs while R values and VO_2 values were collected every 20 s and averaged during time blocks of 5 min. RPE was measured at 5 min intervals throughout each training run. Blood glucose levels were measured at 2 min preexercise, 15 min, and 25 min, postexercise, following Western Michigan University procedures (see Appendix D).

Research and Statistical Design

A randomized-block factorial ANOVA design was calculated for each of the dependent variables: (a) R, (b) VO_2 , (c) RPE, and (d) blood glucose. A research variable for the design was supplementation consumption of two different bars. The PRBar or the PowerBar was ingested before each trial. A second research variable for the design was sample time, which had six levels: (1) 10 min, (2) 15 min, (3) 20

min, (4) 25 min, (5) 30 min, and (6) 35 min. Sample time with six levels was used in the ANOVAs for RPE, VO_2 , and R. The values for VO_2 and R were averaged over the 2 min prior to the sample time. VO_2 and R were sampled every 20 s. Sample time for blood glucose had four levels: (1) preexercise, (2) 15 min, (3) 25 min, and (4) postexercise.

CHAPTER IV

RESULTS AND DISCUSSION

The problem of the study was to determine if the food supplement bar, PRBar, was more effective in increasing performance and fat utilization in male and female endurance athletes, ages 18 to 35 years, than the more traditional, high-carbohydrate energy bar, the PowerBar. A finger stick blood sample was collected to determine blood glucose levels preexercise, at 15 and 25 min during the exercise, and postexercise. R, VO_2 , and RPE were measured at 5-min intervals throughout the running trials during the two dietary treatments. This chapter was organized into the following results sections: (a) subject characteristics, (b) R analysis, (c) VO_2 analysis, (d) RPE, (e) blood glucose analysis, and (f) discussion.

Results

Subject Characteristics

Characteristics of the subjects are presented in Table 1. Fifteen subjects, 9 males and 6 females, participated in the study. The mean age of the subjects was 21.4 years with a range of 18–27 years. The subjects were all trained distance runners capable of completing a 45-min training run at submaximal levels. Seven of the male subjects were collegiate-level cross-country runners. The other 2 males and 4 of the females were competitive recreational runners. The remaining 2 females were trained marathoners. All subjects completed two VO_2 max tests and four training runs within

a 3-week period. This was done to avoid deconditioning effects or significant changes in fitness level. The subjects had a mean height of 172.3 ± 9.9 cm and a mean mass of 60.7 ± 7.3 kg. The mean VO_2 max of the subjects was 70.98 ± 7.42 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ with males and females having respective means of 77.78 ± 5.58 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ and 60.48 ± 6.68 ml \cdot kg $^{-1}$ \cdot min $^{-1}$. Subjects performed the training runs at an average VO_2 of 52.44 ± 5.49 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ for the males and 43.64 ± 4.34 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ for females. The mean VO_2 for all subjects was 48.92 ± 6.48 ml \cdot kg $^{-1}$ \cdot min $^{-1}$. These intensities equate to the males running at $67.3\% \pm 5.3\%$ of VO_2 max and the females at $70.0\% \pm 5.3\%$, with a combined mean of $68.4\% \pm 5.8\%$ of VO_2 max.

Table 1
Descriptive Characteristics of the 9 Male and 6 Female Subjects

Variables	M		F		Combined	
	Mean	<u>SD</u>	Mean	<u>SD</u>	Mean	<u>SD</u>
Age (<u>N</u> = 15)	20.40	3.00	22.70	3.40	21.50	3.10
Height (cm)	178.50	6.10	162.80	5.90	172.30	9.90
Mass (kg)	65.30	6.20	55.80	3.80	60.70	7.30
VO_2 max (ml \cdot kg $^{-1}$ \cdot min $^{-1}$)	77.78	5.85	60.48	6.68	70.98	7.42
Training VO_2 (ml \cdot kg $^{-1}$ \cdot min $^{-1}$)	52.44	3.92	43.43	5.78	48.84	6.45
Training VO_2 (% of VO_2 max)	68.20	5.10	67.30	3.90	69.70	5.80

R Analysis

A randomized-block factorial ANOVA was calculated on the dependent variable, R. Bars (2), trials (2), and time (6) were the three research variables in the ANOVA design. The ANOVA for R is presented in Table 2, and the results are summarized below:

Table 2
ANOVA Summary Table for R Values

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Bars (B)	0.0072	1	0.0072	18.56*
Trials (Tr)	0.0001	1	0.0001	0.21
Times (Ti)	0.0208	5	0.0042	10.72*
B × Tr	0.0007	1	0.0007	1.86
B × Ti	0.0005	5	0.0001	0.28
Tr × Ti	0.0005	5	0.0001	0.23
B × Tr × Ti	0.0007	5	0.0001	0.36
Residual	0.1249	322	0.0004	

* $p < .05$.

1. A significant difference was found between bars, $\bar{M} = 0.91$ and $\bar{M} = 0.92$ for the PRBar and PowerBar, respectively, $F(1, 322) = 18.56$, $p < .05$.

2. A significant difference was found between sample times, $F(5, 322) = 10.72$, $p < .05$.

3. A Tukey HSD test was calculated to make all pairwise comparisons among the means for sample time. The differences for all pairwise comparisons are presented in Table 3. Significant differences were found between the following sample time means: (a) 25 and 30 min, (b) 15 and 30 min, (c) 10 and 30 min, (d) 15 and 20 min, (e) 10 and 20 min, and (f) 10 and 35 min.

Table 3
Differences Among the Means for R Values

Sample Time Means	20	35	25	15	10
30 min = 0.8891	0.0092	0.0012	0.0170*	0.0204*	0.0225*
20 min = 0.9083		0.0020	0.0078	0.0112*	0.0133*
35 min = 0.9103			0.0058	0.0092	0.0113*
25 min = 0.9161				0.0034	0.0055
15 min = 0.9195					0.0021
10 min = 0.9216					

* $p < .05$.

VO₂ Analysis

A randomized-block factorial ANOVA was calculated on the dependent variable, VO₂. Bars (2), trials (2), and times (6) were the three research variables in the ANOVA design. The ANOVA for VO₂ is presented in Table 4, and the results are summarized below:

Table 4
ANOVA Summary Table for VO_2 ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Bars (B)	1.50	1	1.50	0.32
Trials (Tr)	1.68	1	1.68	0.36
Times (Ti)	166.93	5	33.39	7.12*
B \times Tr	9.51	1	19.51	4.16*
B \times Ti	3.80	5	0.76	0.16
Tr \times Ti	6.15	5	1.23	0.26
B \times Tr \times Ti	15.10	5	3.02	0.64
Residual	1509.53	322	4.69	

* $p < .05$.

1. A significant difference was found between sample times, $F(5, 322) = 7.12$, $p < .05$.

2. A Tukey HSD test was calculated to make all pairwise comparisons among the means for sample time. The differences for all pairwise comparisons are presented in Table 5. Significant differences were found between the following sample time means: (a) 25 and 20 min, (b) 15 and 20 min, (c) 35 and 20 min, (d) 25 and 30 min, (e) 15 and 30 min, and (f) 35 and 30 min.

3. The interaction effect for Bars \times Trials was significant, $F(1, 322) = 4.16$ at $p < .05$. This interaction effect is illustrated in Figure 1. The analysis for simple main effects indicated no significant differences.

Table 5
Differences Among the Means for VO_2 ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)

Sample Time Means	30	10	25	15	35
20 min = 47.83	0.45	0.92	1.60*	1.62*	1.88*
30 min = 48.28		0.47	1.15*	1.17*	1.43*
10 min = 48.75			0.68	0.70	0.96
25 min = 49.43				0.02	0.28
15 min = 49.45					0.26
35 min = 49.71					

* $p < .05$.

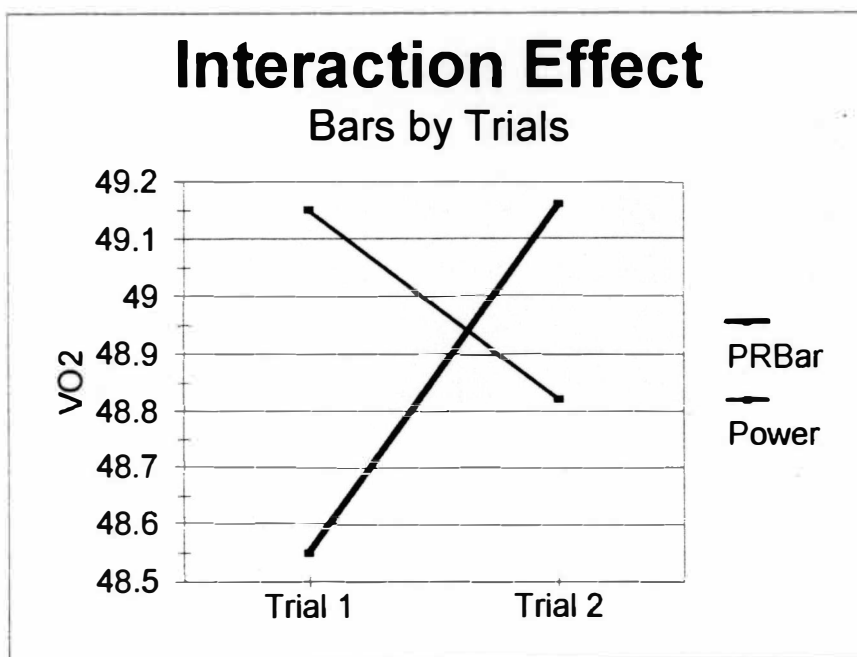


Figure 1. Bars \times Trials Interaction Effect.

RPE

Statistical comparisons were done using a randomized-block factorial ANOVA on the dependent variable, RPE. Bars (2), trials (2), and times (6) were the three research variables in the ANOVA design. The ANOVA for RPE is presented in Table 6 and the results are summarized below. Collections for RPE during the trials were done at 5-min intervals.

Table 6
ANOVA Summary Table for RPE

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Bars (B)	5.3	1	5.3	9.91*
Trials (Tr)	2.8	1	2.8	5.24*
Times (Ti)	36.6	5	7.3	13.51*
B × Tr	0.0	1	0.0	0.00
B × Ti	0.9	5	0.1	0.34
Tr × Ti	0.3	5	0.0	0.09
B × Tr × Ti	0.4	5	0.0	0.16
Residual	174.8	322	0.5	

* $p < .05$.

1. A significant difference was found between bars, $\underline{M} = 10.83$ and $\underline{M} = 10.59$ for the PRBar and PowerBar, respectively, $\underline{F}(1, 322) = 9.91$, $p < .05$.

2. A significant difference was found between Trials 1 and 2, $\bar{M} = 10.79$ and $\bar{M} = 10.61$, respectively, $F(1,322) = 5.24$, $p < .05$.

3. A significant difference was found among sample times, $F(5, 322) = 13.51$, $p < .05$.

4. A Tukey HSD test was calculated to make all pairwise comparisons among the means for sample times. The differences for all pairwise comparisons are presented in Table 7. Significant differences were found between the following sample time means: (a) 20 and 10 min, (b) 25 and 10 min, (c) 30 and 10 min, (d) 35 and 10 min, (e) 30 and 15 min, (f) 35 and 15 min, (g) 30 and 20 min, and (h) 35 and 20 min.

Table 7
Differences Among Means for RPE Values

Sample Time Means	15	20	25	30	35
10 min = 10.17	0.37	0.45*	0.63*	0.88*	0.93*
15 min = 10.54		0.08	0.27	0.52*	0.57*
20 min = 10.62			0.18	0.43*	0.48*
25 min = 10.80				0.25	0.30
30 min = 11.05					0.05
35 min = 11.10					

* $p < .05$.

Blood Glucose Analysis

A randomized-block factorial ANOVA was calculated on the dependent variable, blood glucose. Bars (2), trials (2), and times (4) were the three research variables in the ANOVA design. The ANOVA for blood glucose is presented in Table 8, and the results are summarized below. Blood samples were collected for the trials: Preexercise, 15 min, 25 min, and postexercise.

Table 8
ANOVA Summary Table for Serum Blood Glucose (mg/dl)

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Bar (B)	400.42	1	400.42	1.74
Trials (Tr)	104.02	1	104.02	0.45
Time (Ti)	2993.20	3	997.73	4.33*
B × Tr	209.07	1	209.07	0.91
B × Ti	849.52	3	283.17	1.23
Tr × Ti	1312.58	3	437.53	1.89
B × Tr × Ti	715.07	3	238.36	1.03
Residual	48442.51	210	230.68	

* $p < .05$.

1. A significant difference was found between sample times, $F(3, 210) = 4.33$, $p < .05$.

2. A Tukey HSD test was calculated to make all pairwise comparisons among the means for sample times. The differences for all pairwise comparisons are

presented in Table 9. Significant differences were found between the following sample time means: (a) postexercise and 15 min, and (b) 25 and 15 min.

Table 9
Differences Among Means for Serum Blood Glucose (mg/dl)

Sample Time Means	Preexercise	Postexercise	25
15 min = 76.13	4.83	7.50*	9.40*
Preexercise = 80.96		2.67	4.57
Postexercise = 83.63			1.90
25 min = 85.53			

* $p < .05$.

Discussion

In this section the results of the present investigation are compared to information gleaned from similar studies completed by other investigators and to the experience of acknowledged experts in exercise physiology. A basic premise of the present investigation was that a higher fat utilization would occur when subjects ingested the PRBar rather than the PowerBar. The discussion was organized into the following sections: R value, VO_2 , RPE, and blood glucose.

R Value

It was hypothesized in this study that subjects would show a lower mean R value when supplementing with the PRBar than when supplementing with the

PowerBar. This reasoning was based on the fact that the chemical composition differs between fats and carbohydrates. Fats contain considerably fewer oxygen atoms in proportion to carbon and hydrogen atoms. When fat is used in the body as a fuel source, more oxygen is required to oxidize the fat to carbon dioxide and water than when carbohydrates are used. As more fat is used in metabolism in the body, the value of R approaches 0.70. When more carbohydrates than fats are used, the value of R rises toward 1.00 (McArdle et al., 1991). By monitoring R during exercise, the relative contribution of fats and carbohydrates can be estimated. It is thought that as exercise begins, carbohydrates contribute the majority of the fuel used. This use increases as the intensity of exercise increases (McArdle et al., 1991). With endurance exercise, R rises initially then falls and stabilizes at a steady-state value. If the endurance exercise is performed at a high steady state ($> 60\%$ of VO_2 max), an oxygen drift may occur.

The oxygen drift represents an increase in oxygen consumption without an increase in exercise intensity. An oxygen drift would result in a decreased value of R (Brooks et al., 1996). In the present investigation, a significant difference occurred between the mean R values measured during the PRBar- and PowerBar-supplemented training runs. The means were 0.91 and 0.92 for the PRBar and PowerBar, respectively. Furthermore, significant differences were found in mean R values at the six time intervals selected during the training runs for both supplement conditions. The mean R values generally decreased from the 5-min through the 35-min sample times. A possible explanation for this would be an oxygen drift. Although significant differences did occur in VO_2 from the 5-min through the 35-min sample times, results were inconsistent. Therefore, evidence of an oxygen drift is not conclusive.

In a previous study (Hoeger, Kolkhorst, & Thurman, 1995), no significant differences in mean R values were found during training runs performed at 67.2% of VO_2 max. Subjects consumed an Access Fat Conversion Bar with water or consumed only water 15 min prior to a training run lasting 45 min. During two different trials, VO_2 , R, blood lactate, and blood glycerol were measured at 30 and 45 min. No significant differences were found at the two times for any of the variables measured.

VO_2

Theoretically, an increase in fat metabolism results in an increased utilization of oxygen. In the present study, it was hypothesized that the VO_2 during the training runs would be higher after ingesting the PRBar than after ingesting the PowerBar. This was based on the claim that ingesting the PRBar results in an increased utilization of fats as a fuel during endurance exercise.

No significant difference in the mean VO_2 occurred in this investigation between the PRBar- and PowerBar-supplemented training runs. Also, although VO_2 fluctuated across the training runs, no significant difference in VO_2 occurred when 5 min was compared to 35 min in any of the training runs. It is thought that in the initial minutes of exercise, oxygen consumption rises. A plateau in VO_2 should occur in 3 to 4 min as a steady state is achieved. A steady state is described as a balance between energy requirements of the exercise and energy production using aerobic metabolic pathways (McArdle et al., 1991). However, at a high steady state (> 60% of VO_2 max) there is evidence that an oxygen drift may occur (Robergs & Roberts, 1997).

In a study conducted by Nelson-Pfab et al. (1997), no significant differences in VO_2 occurred between conditions during a 60-km bicycle ride following four different test meals consumed 1 hr prior to the exercise. The four test meals consisted of a control (no food); 12 oz of an artificially sweetened drink; a high-carbohydrate energy bar containing 76% carbohydrate, 15% protein, and 8% fat; a moderate-carbohydrate energy bar containing 40% carbohydrate, 30% protein, and 30% fat; or a high-fat candy bar containing 34% carbohydrate, 8% protein, and 58% fat. Significant differences in mean VO_2 occurred at 10, 20, 30, 40, and 50 km when compared to the mean VO_2 at 60 km. However, no significant difference in mean VO_2 occurred among the test meals.

In a similar study (Hoeger et al., 1995), the investigators compared the effect of supplementation with the Access Bar with a control (no supplementation) during endurance exercise. In trained endurance athletes, no significant differences were reported for VO_2 across the conditions or the time of the training runs, indicating no oxygen drift. These athletes exercised at an average intensity of 69.7% of VO_2 max (Hoeger et al., 1995). Although in the present investigation, no significant main effect in mean VO_2 occurred for bars or times, a significant interaction effect did occur between bars and trials. When using the PRBar, VO_2 increased between trials, but when using the PowerBar, VO_2 decreased between trials. Neither change was statistically significant.

RPE

It was thought that RPE levels during the training runs would remain the same regardless of the type of supplement bar consumed. RPE measures are expected to increase linearly with an increase in intensity of exercise. However, during the

current investigation, subjects performed steady-state exercise at a constant workload during all trials, so no difference in mean RPE for the trials or conditions was expected. In a related study by Nelson-Pfab et al. (1997), in which varying percentages of carbohydrate, protein, and fat were consumed as supplements before endurance exercise, no significant effects on performance or RPE were found. In another study by Horowitz and Coyle (1993), no differences in the RPE occurred among trials after subjects consumed high- and moderate-glycemic meals 30 min prior to a 1-hr exercise trial. They concluded that subjects were able to perform the 1-hr exercise tasks with sensations of discomfort and fatigue that were independent of the glycemic index of the meal.

The results of the current investigation were contrary to these expected results. A significant difference in RPE occurred between the PRBar and PowerBar, with means of $\bar{M} = 10.8$ and $\bar{M} = 10.6$, respectively. The difference in RPE between the bars indicated that subjects perceived greater discomfort during running trials after ingestion of the PRBar than after the ingestion of the PowerBar. As discussed previously, when an individual uses fat as a fuel source, oxygen consumption increases. Consequently, subjects experience a greater sensation of discomfort and report a higher RPE. This explains why the PRBar trials were associated with a greater RPE value than those with the PowerBar. Subjects required more effort to run at the same speed during the PRBar trials.

Blood Glucose

A hypothesis for this study was that blood glucose levels during the first 15 min of exercise fluctuate due to nutrient composition of the bar and acclimation to steady-state exercise. After 15 min, differences in blood glucose levels should be

minimal. Plasma glucose and insulin levels typically peak around 30–60 min after carbohydrate ingestion. If exercise begins during this time, plasma glucose levels can be expected to fall below normal levels due to the insulin response and muscle contractions occurring simultaneously. The lowest plasma glucose concentration usually occurs about 15 min after the onset of exercise, after which concentrations may remain suppressed, return to normal, or even exceed preexercise levels (Coggan & Swanson, 1992). It is thought that this state of hypoglycemia impairs performance in some cases, although in most instances, the decrease in glucose level is too small or too brief to result in decrements in performance (Coggan & Swanson, 1992).

Results of this study show significant differences among trial times for the dependent variable blood glucose, but significant differences were not found between bars or trials. An initial drop in glucose concentration was present but it was not statistically significant. This drop was followed by a gradual rise in glucose with significant differences between the 15-min and 25-min sample times and between the 25-min and postexercise sample times.

Under normal dietary conditions, the availability of carbohydrate stores influences the pattern of substrate utilization during exercise. Studies showed that at rates of 45% of VO_2 max, hepatic glucose release can provide approximately 25% of the total energy requirement, but this declines to about 10% at an intensity of 70% of VO_2 max (Shepard & Astrand, 1992). At work rates above 60% of VO_2 max, there is a linear relationship between exercise and intensity and the rate of muscle glycogen utilization (Shepard & Astrand, 1992). The rise in blood glucose concentration found in the current study helps to explain the responses in R that were discussed earlier. A higher R value can be expected when carbohydrate is the predominant fuel; therefore, the R value is expected to be higher when greater amounts of carbohydrates are

consumed prior to exercise. Therefore, the expected scenario would be for the PowerBar to elicit a higher R value than the PRBar. However, the results in the present study were mixed. Although the R value for the PowerBar was higher than the value for the PRBar, no significant differences were found in blood glucose between the bars.

In a comparable study by Nelson-Pfab et al. (1997), significant differences occurred among exercise means for blood glucose following a high-carbohydrate meal, a nonenergy-providing placebo, a moderate-carbohydrate, and a high-fat meal. The findings showed a significant disordinal interaction in plasma glucose between tests and over distances. The significant difference among sample times for the current study parallels the Nelson-Pfab et al. results.

In Horowitz and Coyle's (1993) study, in which six different meals were consumed and all feeding trials were compared, a difference between high-glycemic and moderate-glycemic conditions was not apparent. Horowitz and Coyle found that plasma glucose for all meals declined sharply during the initial 20 min and that all the feeding trials remained below the control glucose levels, when no supplementation occurred. This finding supports the hypothesis that the consumption of a small carbohydrate meal results in the reduction of plasma glucose concentrations during initial exercise. Despite this, it is known that subsequent glucose levels will increase as the exercise duration continues, thus improving performance. Different glycemic levels in meals either high or moderate, and meals containing up to 35% of energy from fat were not shown to produce a variation in the glycemic response after 20 min of moderate-intensity exercise (Horowitz & Coyle, 1993). This helps to explain the nonsignificant findings in blood glucose for the two bars in this study. The findings of this investigation agree with findings of related studies and with the theory of no

difference in blood glucose concentrations for the supplement bars. The likely explanation for this is that the nutrient composition of the two bars was similar enough to not show significant differences in blood glucose levels during the 35 min of exercise at 69% of VO_2 max.

CHAPTER V

SUMMARY, FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The problem of this study was to investigate whether the PRBar was more effective in increasing performance and fat utilization during endurance exercise in male and female runners than the traditional higher-carbohydrate bar, the PowerBar. Fifteen subjects, 9 male and 6 female, performed two VO_2 max tests determining peak VO_2 , followed by four training runs at an intensity between 65% and 75% of VO_2 max. The subjects consumed one PRBar or one PowerBar 1 hr before each training run. R, VO_2 , and RPE were measured and compared at the following times: 10 min, 15 min, 20 min, 25 min, 30 min, and 35 min. Blood glucose was measure at preexercise, 15 min, 25 min, and postexercise. Results of the ANOVAs and Tukey HSD tests for R and RPE showed significant differences indicating potentially greater fat utilization for the PRBar. The results of the VO_2 and blood glucose ANOVAs show no significant differences between bars. A rational explanation supported by related studies was that the nutrient compositions of the two bars were similar enough that differences in blood glucose and VO_2 measures were not significant.

Findings

R

Significant differences were found for R between bars, $\underline{M} = 0.9081$ and $\underline{M} = 0.9168$ for the PR and PowerBar, respectively, $\underline{F}(1, 322) = 18.56$, $p < .05$. Significant differences were found among sample times for R, $\underline{F}(5, 322) = 10.72$, $p < .05$. The significant differences were found between the following sample time means: (a) 25 and 30 min, (b) 15 and 30 min, (c) 10 and 30 min, (d) 15 and 20 min, (e) 10 and 20 min, and (f) 10 and 35 min. This was expected based on physiological function of the body, but it contradicted results of related studies in which similar nutritional percentages were consumed.

VO₂

A significant difference was found between sample times for VO₂, $\underline{F}(5, 322) = 7.12$, $p < .05$. An interaction effect for bars by trials was found to be significant, $\underline{F}(1, 322) = 4.16$ at $p < .05$. The interaction effect between bars and trials indicated a lack of a consistent pattern in VO₂ between the trials. VO₂ fluctuated across the training runs; no significant difference in VO₂ occurred when 10 min was compared to 35 min in any of the training runs.

RPE

A significant difference was found for RPE between bars, $\underline{M} = 10.8$ and $\underline{M} = 10.6$ for the PRBar and PowerBar, respectively, $\underline{F}(1, 322) = 9.91$, $p < .05$. Subjects reported a higher RPE during trials with the PRBar than with the PowerBar. A significant difference was found for RPE between Trials 1 and 2, $\underline{M} = 10.8$ and

$\underline{M} = 10.6$, respectively, $\underline{F}(1,322) = 5.24$, $p < .05$. Significant differences were found between sample times for RPE, $\underline{F}(5, 322) = 13.51$, $p < .05$. A gradual rise in RPE value occurred over time.

Blood Glucose

A significant difference in blood glucose was found among sample times, $\underline{F}(3, 210) = 4.33$, at $p < .05$. No significant difference between bars or trials was found for blood glucose. An initial drop in glucose concentration was present, but it was not significant. The drop was followed by a gradual rise, showing a significant difference between the 15-min and 25-min sample times and between the 15-min and postexercise times, $\underline{M} = 76.13$ mg/dl, $\underline{M} = 85.53$ mg/dl, and $\underline{M} = 83.63$ mg/dl, respectively. However, when preexercise blood glucose level was compared to postexercise glucose level, no significant difference was seen. Therefore, there was no evidence of glucose depletion as a result of the training runs. This pattern occurred for both bars.

Conclusions

Results of this study demonstrated that during endurance exercise, when subjects consumed the PRBar, they produced slightly lower, but statistically significant, R values than when they consumed the PowerBar. This indicated a greater reliance on fat as a fuel during training runs. Findings also showed higher RPE ratings for subjects after consuming the PRBar than after consuming the PowerBar, indicating that steady-state exercise was more difficult after consumption of the PRBar. Although the significant decrease in R and significant increase in RPE supported an increase in fat metabolism during exercise when subjects supplemented

with the PRBar, the lack of significant changes in VO_2 and blood glucose levels across the training runs indicated a mixed result. Also, the practical significance of the changes in R and RPE was questioned.

Recommendations

After completion of this study, several recommendations can be made for future research:

1. Increase the duration of the training run to 60 min, allowing for longer monitoring of blood glucose concentrations and observation of peaks or plateaus during training runs.
2. Include a control condition (no food), allowing comparison of results from the supplement bars and a fasting state during exercise of 35 min in duration and 70% of VO_2 max.
3. Analyze other blood variables such as blood glycerol levels, insulin levels, and lactate levels throughout exercise.
4. Control subjects' diet and fluids for 24–36 hr prior to data collection to ensure that carbohydrate and liquid consumption before each trial are identical for all trials, which would allow for better internal validity when comparing differences between bars.

Appendix A
Subject Screening Form

SUBJECT SCREENING FORM

Last Name _____ Age: _____ Date: ____/____/____

First Name _____ Code number _____

The purpose of this form is to explore possible medical conditions that might increase your risk of undesirable consequences associated with physical activity. Please read each question carefully and check a YES or NO line to indicate your answer.

- | | | |
|------------------------------|--|--|
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 1. Do you smoke? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 2. Do you have diabetes? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 3. Have you ever been told your blood lipids (cholesterol and/ or triglycerides) are too high? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 4. Have you ever been told you have high blood pressure or are you currently taking blood pressure medication? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 5. Have any of your blood relatives had a heart attack or stroke under the age of 55? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 6. Do you have a history of unexplained chest pains? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 7. Have you ever had "fits", convulsions, or epilepsy? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 8. Do you have a bone or joint problem that could be made worse through exercise? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 9. Do you ever experience chest pain or tightness in your chest? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 10. Do you have asthma? If yes, are your symptoms well controlled with medication? |
| | <input type="checkbox"/> yes <input type="checkbox"/> no | |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 11. Does your heart ever beat unevenly or irregularly? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 12. Have you ever had a heart attack? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 13. Do your ankles swell? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 14. Do you have varicose veins? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 15. Do you have mononucleosis? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 16. Do you have trouble with dizziness or lightheadedness? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 17. Do you ever wheeze or have to gasp to breath? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 18. Have you been recently ill? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 19. Is there a physical reason, not mentioned, why you could not participate in vigorous activity? |
| <input type="checkbox"/> yes | <input type="checkbox"/> no | 20. Have you ever had a fainting spell? |

- ☐ yes ☐ no 21. Are you currently taking any of the following? Beta Blockers, Alpha Blockers, Amphetamines, Antiadrenergic Agents, Nitrates and Nitroglycerin, Calcium Channel Blockers, Cocaine, Digitalis, Diuretics, Peripheral Vasodilators, Marijuana, Angiotension-Converting Enzyme, Antiarrhythmic Agents, Sympathomimetic or Antihyperlipidemic Agents?
- ☐ yes ☐ no 22. Are you currently taking any over-the-counter medicines such as aspirin, Tylenol, ibuprofen, or antihistamines?
- ☐ yes ☐ no 23. Do you have any limitations because of a sports injury?
- ☐ yes ☐ no 24. Women only – Is there possibility that you could be pregnant?

I have carefully answered all the above questions and affirm the accuracy of my answers.

Failure to answer any of these questions will result in elimination from the study. If a potential subject answers "yes" to two or more items, 1–5, he or she does not qualify as apparently healthy according to the American College of Sports Medicine (ACSM, 1995). Only "apparently healthy" subjects will be eligible to participate in the study. An individual judgment will be made concerning participation of potential subjects answering "yes" to items 6–24. The judgment will be based on the potential impact of exercise on that particular individual. Individuals with cardiovascular disease, or those possessing more than two known major factors or orthopedic injuries that require medical treatment during the last year or chronic enough to warrant exclusion will be eliminated.

Signature _____ Date: ____ / ____ / ____

Appendix B
Consent Form

Western Michigan University
Department of Health, Physical Education, and Recreation

Principal Investigator: Dr. Roger Zabik

Research Associate: Chad F. Witt

I have been invited to participate in a research project entitled "The effects of the PRBar on fat metabolism during endurance exercise." I understand that this research is intended to determine the effects consumption of the PRBar has on fat metabolism during distance running. I further understand that this project is Chad Witt's master's thesis in the Department of Health, Physical Education and Recreation at Western Michigan University.

My consent to participate in this project indicates that I will attend six 50 min sessions. These sessions will take place in the Exercise Physiology Laboratory, room 1055, in the Student Recreation Center. These sessions will involve two running maximal oxygen consumption tests, and four 35 min running trials, two with the experimental condition and two without. In the first session, I will be familiarized with Borg's rating of perceived exertion (RPE) scale and all testing procedures, and I will participate in the first of two maximal oxygen consumption tests.

As in all research, there may be unforeseen risks to the participant. If an accidental injury occurs, appropriate emergency measures will be taken; however, no compensation or treatment will be made available to me except as otherwise specified in this consent form. I understand there may be some potential risk of injury, such as muscle soreness or possible heart attack. However, appropriate measures will be taken to minimize these risks. These investigators and assistants in the data collection are all CPR and First Aid trained. Emergency response procedures are also posted in the Exercise Physiology Laboratory, where all the testing will take place. I also understand that I may terminate my involvement with this research for any reason at any time without prejudice or without affecting my academic evaluation in any way.

I may benefit from my participation by knowing my maximal oxygen consumption ($\text{VO}_2 \text{ max}$) on the treadmill. I may also gain insight as to the time and equipment involved for taking a maximal graded exercise test. I may also gain knowledge as to the effects the PRBar has on fat metabolism during submaximal running.

I understand that all the information collected from me is confidential. My name will only appear on this form and on a list of identification codes, and no individual names will be printed on any papers or reports other than this form, which will be seen only by the investigators and those helping to test. All data will be retained for a period of 3 years in a locked file controlled by the principal investigator. At the conclusion of the study, I will be able to receive a copy of my results upon request.

If I have any questions or concerns about this study I may contact Chad Witt at 387-7390 or Dr. Zabik at 387-2720. I may also contact the chair of the Human Subjects Institutional Review Board at 387-8293 or the Vice President for Research at 387-5926. I affirm that I am between the ages 18 and 35 years old and free of any known cardiorespiratory disease. My signature below indicates that I understand the purpose and requirements of the study and that I agree to participate.

Signature

Date

Appendix C
Human Subjects Institutional
Review Board Approval

Human Subjects Institutional Review Board



Kalamazoo Michigan 49008-3899

WESTERN MICHIGAN UNIVERSITY

Date: 1 October 1997

To: Roger Zabik, Principal Investigator
Chad Witt, Student Investigator

From: Richard Wright, Chair

A handwritten signature in cursive script, reading "Richard A. Wright".

Re: HSIRB Project Number 97-08-07

This letter will serve as confirmation that your research project entitled "Effect of the PRBar and Power Bar on Fat Metabolism During Endurance Exercise" has been **approved** under the **full** category of review by the Human Subjects Institutional Review Board. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

Please note that you may **only** conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: 1 October 1998

Appendix D

Western Michigan University Blood Collection Procedures

Western Michigan University

Procedures for Collection of Blood and Blood Products

1. COLLECTION OF THE BLOOD PRODUCT:

Blood will be collected in the HPER exercise physiology laboratory in the University Recreation Center. Blood samples by finger prick are taken each semester in laboratory activities associated with exercise physiology classes taught by the department. These samples are collected by Dr. Roger M. Zabik and Dr. Mary L. Dawson, faculty members in the department who work in the lab facility.

Samples are taken by the finger prick method. The sample site is first sterilized with a disposable alcohol prep pad. A disposable, Microtainer safety flow lancet is then used to produce a prick on the finger pad. The initial blood drop is wiped away with a disposable alcohol prep pad. The next drop is collected using a disposable heparinized blood capillary tube. The sample site is then cleaned with a disposable alcohol prep pad and a band-aid is then applied.

Because the investigators do not have access to confidential medical information about participating subjects, extreme caution will be taken in the handling of the collected samples.

No direct contact should occur between the blood sample and the investigator collecting the sample. The investigators collecting the samples will wear a lab coat, disposable rubber gloves, and safety glasses. The disposable rubber gloves will be changed between subjects. Blood samples will be taken only by Dr. Zabik and Dr. Dawson. Both have been trained by Western's environmental safety division in the handling of blood products and the Sindecuse Health Center medical lab was consulted concerning procedures for taking the blood sample.

The student investigator in this study has been vaccinated for Hepatitis B. Both Dr. Zabik and Dr. Dawson were presented the opportunity to receive the vaccination and both declined. A waiver was signed. Any accidental direct contact with the blood sample by a subject with another subject's blood will be reported to the Sindecuse Health Center staff and HSIRB. If, during blood collection, an accidental wound to the subject occurs, emergency first aid will be administered and

the subject then taken to the Sindecuse Health Center for further evaluation. The incident will be reported to HSIRB.

The blood samples will be transferred from the blood capillary tube to the glucose test blots and the blood lactate analyzer immediately after they are taken using a microsyringe. No blood samples will be stored.

2. TREATMENT OF THE MATERIAL USED TO DRAW THE BLOOD:

The blood capillary tube, glucose test strip, microtainer lancet, alcohol prep pads, rubber gloves and any other disposable materials that come in contact with blood will be placed in a Sharps container for disposal by the Sindecuse Health Center. The blood lactate instrument contains a waste collection container internally. When full, this container will be emptied by Dr. Zabik into a plastic container with a secure lid. The plastic container will then be transported to Sindecuse Health Center for disposal. If an accidental spill of the waste occurs, the spill will be absorbed using cotton pads with a plastic backing and the spill area cleaned using a bleach solution. The cotton pads any other materials used in the clean-up will be placed in a Sharps container. The waste collection container will then be sterilized using a Cydex solution. Dr. Zabik will wear a lab coat, rubber gloves, and safety glasses during these procedures. If contaminated, the lab coat will be washed and bleached. The rubber gloves will be placed in a Sharps container.

3. STORAGE AND DISPOSITION OF THE BLOOD SAMPLE:

No blood will be stored in the lab after collection. As each sample is taken, it will be immediately analyzed and then disposed of using procedures previously described and recommended by Sindecuse Health Center.

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