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Purpose: Eight high-fit (bicycle VO_{2max} = 62.4 ± 1.10 ml•kg^{-1}•min^{-1}) male cyclists, aged 28.4 ± 1.65 yrs., performed a two-hour endurance bicycle exercise to achieve depletion of skeletal muscle and liver glycogen. During recovery, Endurox R^4 Recovery Drink®, or Gatorade®, was ingested to investigate their relative restorative capacities to enable further exercise. **Methods**: Each subject performed two days of testing: one for each drink presented in random order. On each testing day, the twelve-hour fasted subject performed a two-hour cycling exercise bout at 75% VO_{2max} followed by one to three five-minute sprints at 85% VO_{2max}. At the end of the exercise blood glucose concentrations were 3.98 ± 0.138 mmol/L. A four hour recovery period ensued in which the subject was given 24-ounces of the recovery drink. A performance test at 85% VO_{2max} to exhaustion was then conducted. Ventilatory responses were collected breath-to-breath, while venous blood samples were measured for oxidation products, glucose and insulin concentrations. **Results**: The recovery phase showed significant increases in both plasma glucose and serum insulin following Endurox R^4 Recovery Drink® ingestion as compared to Gatorade®. There was a significant increase in time to exhaustion (+55%) following Endurox R^4 Recovery Drink® during the performance ride compared to Gatorade®.

Final oxidation products following Endurox R^4 Recovery Drink® ingestion were significantly decreased as compared to Gatorade® ingestion, in that Thiobarbituric Acid Reactive Substrates (T-BARS) were significantly decreased. **Conclusions**: These data indicate that the Endurox R^4 Recovery Drink®, when compared to Gatorade®, significantly enhanced recovery from glycogen-depleting exercise. In addition, Endurox R^4 Recovery® Drink decreased the formation of final oxidation products, when compared to Gatorade®.

ENDUROX R^4® & GATORADE®: EFFECTS OF RECOVERY DRINKS

AFTER PROLONGED GLYCOGEN-DEPLETING EXERCISE

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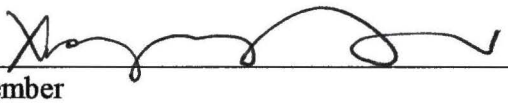

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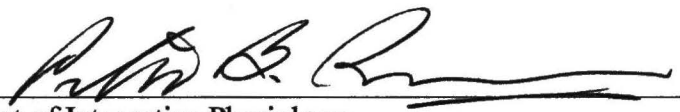

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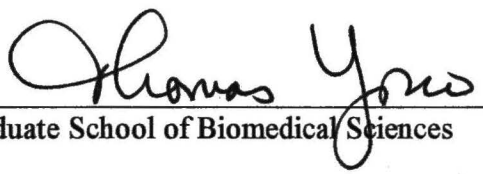

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ENDUROX R^4® & GATORADE®: EFFECTS OF RECOVERY DRINKS AFTER PROLONGED GLYCOGEN-DEPLETING EXERCISE

THESIS

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ABSTRACT PRESENTATIONS

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M. B. Williams, K.M. Gallagher, S.A. Smith, R.G. Querry, and P.B. Raven. During steady-state dynamic exercise in human decreases in blood flow are compensated by increases in oxygen extraction. *Texas Chapter of the American College of Sports Medicine*, Spring, 1998. Second place poster competition.

M. B. Williams, J. L. Ivy, and P. B. Raven. Effects of recovery drinks after prolonged glycogen-depletion exercise. *Mid-Atlantic Regional Chapter of the American College of Sports Medicine*, Fall, 1998. Oral Presentation.

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M. B. Williams, J. L. Ivy, and P. B. Raven. Effects of recovery drinks after prolonged glycogen-depletion exercise. *National meeting of the American College of Sports Medicine*, June, 1999. Poster Presentation.

PUBLISHED ABSTRACTS

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CHO	Carbohydrate
cm	Centimeter
CO_2	Carbon dioxide
ECG	Electrocardiogram
FRC	Functional Residual Capacity
g	Gram
G_s	Stimulatory G-Protein
GXT	Graded Exercise Test
h	Hour
Hb	Hemoglobin
Hct	Hematocrit
HR	Heart Rate
HR_{max}	Maximal Heart Rate
IC	Intracellular
kg	Kilogram
L	Liters
min	Minute
ml	Milliliter

n	Sampled Population
N_2	Nitrogen
O_2	Oxygen
PCO_2	Partial Pressure of Carbon Dioxide
PO_2	Partial Pressure of Oxygen
Q_c	Cardiac Output
RQ	Respiratory Quotient
RPE	Ratings of Perceived Exertion
RR	Respiratory Rate
RPM	Revolutions Per Minute
SEM	Standard Error of the Mean
T-BARS	Thiobarbituric Acid Reactive Substrate Assay
VCO2	Rate of Carbon Dioxide Elimination
V_e	Expired Minute Ventilation
VO_2	Rate of Oxygen Uptake
VO_{2max}	Maximal Rate of Oxygen Uptake

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CHAPTER I

INTRODUCTION

The purpose of this section was to introduce the research related to glycogen depletion exercise and recovery. The problem addressed by the investigation was stated along with the purpose and objectives of the study. Furthermore, the hypothesis, definition of terms, delimitations, and limitations were also presented.

During exercise, several cardiovascular events take place including increased heart rate (HR), cardiac output (Q_c), and minute ventilation (V_e). In the skeletal muscle many events occur in order to sustain the exercise workload. Of these, the breakdown of glycogen, or glycogenolysis, is crucial for maintaining the energy supply in the form of ATP. In the course of recovery from exercise, the exact opposite is true, where the formation of glycogen, or glycogenesis, is enlisted to ensure an adequate reserve for the next exercise bout. To this end, recovery drinks, such as the Endurox R^4 Recovery Drink® and Gatorade®, have been designed to further enhance the body's ability for glycogenesis in order to secure a faster and more complete recovery from exhaustive exercise.

Exercise performance has been improved by increasing pre-exercise muscle glycogen stores through the ingestion of carbohydrates (CHO) prior to exercise (12,28). Additionally, CHO ingestion during prolonged moderate exercise (i.e. 70% VO_{2max}) delays

the onset of fatigue by hypoglycemic prevention and maintenance of CHO oxidation (13,14,26). Several studies have demonstrated that muscle glycogen stores are an important factor in the muscle's ability to perform work continuously (4,5). During prolonged strenuous exercise, the decline in muscle glycogen stores closely parallels perception of fatigue, and, once substantially depleted, necessitates termination of exercise (3), or a significant reduction in exercise intensity. While the effects of CHO ingestion after glycogen depletion and recovery on reinstating the metabolic capacity of muscle have been studied extensively, studies have not been conducted to determine performance capability after glycogen depletion and immediate recovery (i.e. less than 4 hours).

During exercise bouts that are at an intensity greater than 60% VO_{2max}, epinephrine is released as a consequence of an increase in the sympathetic nervous system (2). This allows for further increases in heart rate, as well as beginning the process of glycogenolysis through increases in intracellular calcium. As seen in Figure 1, epinephrine attaches to a β-adrenergic receptor on the surface of the skeletal muscle membrane. Then, through the action of a G_s protein, it stimulates the activation of the membrane bound enzyme adenylate cyclase, which catalyzes the conversion of ATP to cAMP intracellularly. The rise in intracellular (IC) cAMP causes activation of the phosphorylase kinase enzyme, which converts phosphorylase *b* to *a* to begin glycogenolysis. It has been demonstrated that even minute increases in IC cAMP concentrations can yield the activation of phosphorylase kinase (18). Additionally, the rise in cAMP also has a negative effect on the synthesis of glycogen by inactivating glycogen synthase. This process yields an overall net reduction in carbohydrate stores (50).

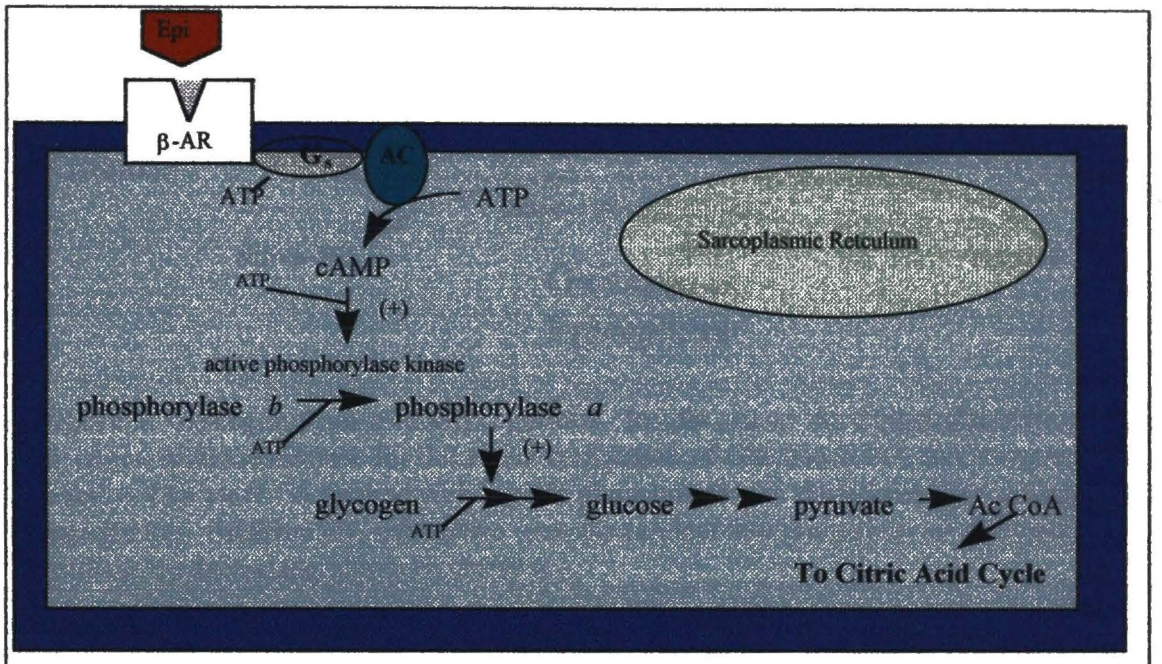

Figure 1. Epinephrine-Induced Effects on Glycogen. β-AR- adrenergic receptor; G_s- G stimulatory protein; AC- adenylate cyclase. The same cascade would be utilized if glucagon and its receptor were present in place of epinephrine and the β-AR.

In terms of glycogenesis during recovery, insulin has been demonstrated to be the key activator of glycogen synthase (15,27). In liver and adipose tissue, activation of glycogen synthase leads to a decrease in IC cAMP concentration in the tissue. In skeletal muscle, however, a consequential decrease in IC cAMP after insulin-mediated activation of glycogen synthase has not been found (24). Replenishment of glycogen stores has been demonstrated as a biphasic response with the first being rapid and the second slower (4,36,30). The rapid phase of glycogen replenishment was discovered to be the most important aspect for facilitating a rapid recovery and depended on two factors: available substrate, and increased muscle cell permeability to glucose (33).

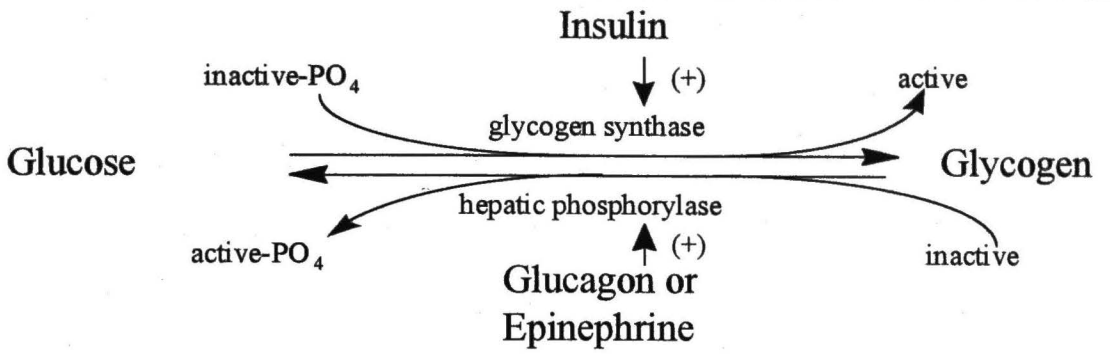

Figure 2. Glycogenolysis and Glycogenesis. Insulin causes the dephosphorylation of the glycogen synthase enzyme for the active conversion of Glucose-1-PO_4 to Glycogen. The reaction also works in reverse with hepatic phosphorylase in the presence of glucagon or epinephrine. Additionally, exhaustive exercise can increase the activity of glycogen synthase by decreasing glycogen stores (6).

Additionally, muscle contractions, such as those during exercise, have been demonstrated to have a strong insulin-like effect on muscle cell permeability to glucose, which is inversely related to muscle glycogen concentration (20,29,32,34,47) and lasts for an extended period of time (20,46). For example, as muscle glycogen decreases during exercise, muscle cell permeability to glucose increases. Further, Bergstrom *et al.* (6), has demonstrated that with decreased muscle glycogen concentration associated with exhaustive exercise, there was an increase in the intrinsic activity of the glycogen synthase enzyme. Therefore, the basis for CHO ingestion immediately post-exercise was to utilize the muscle's capacity of rapid glycogen replenishment by providing available substrate.

Two physiologic substances, protein and arginine, have been demonstrated to have a high capacity to induce the insulin response. Protein has a dose effect on the glucose response as well as the serum insulin response in that increases in protein lead to decreases

in blood glucose concentrations (50,45,17,40) and increases in serum insulin concentrations (51). Meals rich in protein yield maintenance of serum insulin at concentrations greater than normal resting values after a two hour time period (51), thus further promoting glycogen replenishment post-exercise. Arginine, one of the essential amino acids, has also been demonstrated to be an extremely potent stimulus for the release of insulin (38). It indirectly results in the increased transfer of amino acids across the cell membrane (35,48) as well as increased protein synthesis (53). Additionally, the arginine-induced insulin response is not blocked by epinephrine (19).

Finally, with endurance training, increased concentrations of skeletal muscle mitochondria have been demonstrated to yield increased generation of oxidation products, since the mitochondria is the primary source of free radicals (16). Further, endurance trained rodents on a diet inadequately supplied with vitamin E had a significant reduction in performance time as compared to those rodents on a normal vitamin E supplementation owing to increased free radical formation (43). Therefore, maintenance of cell membrane integrity is crucial to the trained athlete in terms of performance.

The specific purpose of formulating a recovery drink is to enhance the subject's ability to recover more completely in a shorter period of time from an exhaustive exercise. Metabolic recovery, as defined by Burke (10), is three pronged involving the restoration of liver and skeletal muscle glycogen stores, fluid and electrolyte replenishment, and regeneration, repair and adaptation to catabolic stresses that induce damage during the exercise. Several mechanisms can be utilized to elicit recovery including ingestion of CHO, protein, and arginine which result in stimulation of the insulin response thereby

promoting glycogenesis; ingestion of specific concentrations of electrolytes that restore the balance lost during exercise; and, ingestion of antioxidants that help to limit or negate oxidative damage caused by free radicals produced during a strenuous exercise. The Endurox R^4 Recovery Drink benefits in all areas with protein, arginine, and an increased amount of CHO to promote glycogen store replenishment, specific formulation of electrolytes for replacement, and antioxidants E & C for reduction of free radicals damage.

Statement of the Problem

The problem was to determine if a sports drink formula containing antioxidants, in the form of Vitamins E & C, and other nutritional agents known to stimulate the release of insulin, including protein & arginine, would provide a faster recovery and extend performance when compared to Gatorade®, a conventional rehydration sports drink lacking these nutritional supplements.

Purpose & Objectives of the Study

The purpose of the investigation was to determine the benefit, if any, of the use of the Endurox R^4 Recovery Drink® over that of Gatorade® in high-fit endurance cyclists during recovery and post-glycogen depletion performance exercise bouts. To accomplish these goals, the following objectives were proposed: (i) To measure significant decreases in heart rate (HR), ratings of perceived exertion (RPE), and cardiac output (Q_c) during upright bicycle ergometry. (ii) To measure significant metabolic differences between the performance exercise condition for the two drinks via oxygen uptake (VO_2), minute

ventilation (V_e), respiratory quotient (RQ), and respiratory rate (RR). (iii) To measure significant increases in time to exhaustion of the performance exercise bout. (iv) To measure the amount of oxidation product formation resulting from the exhaustive exercise.

Hypothesis

The following three part hypothesis was proposed:

(i) Arginine and protein, as well as the greater amount of carbohydrates provided in the Endurox R^4 Recovery Drink®, would elicit a greater insulin response,

(ii) The greater insulin response would result in promotion of glycogenesis during recovery from exhaustive exercise and be manifested by increased performance time to exhaustion; and,

(iii) Antioxidants E & C provided in the Endurox R^4 Recovery Drink® would decrease the number of oxidation products formed, an indicator of free radical formation.

Definition of Terms

1. Borborygmus- a rumbling noise produced by the movement of gas through the intestines.
2. Calorimetry- measurement of the amount of heat evolved or absorbed in a chemical reaction, change of state, or formation of a solution.

3. Ergometer- an instrument for measuring the amount of work done by a muscle or group of muscles.
4. Fitness- based on peak workload achieved during a screening cycle ergometer graded exercise test and responses to standardized medical history and nutrition questionnaire.
5. Glycogenesis- the formation or synthesis of glycogen; the investigation utilized the induction of the insulin response to stimulate the formation of glycogen.
6. Glycogenolysis- the biomedical breakdown of glycogen to glucose; the investigation utilized an endurance exercise to initiate this event.
7. Graded Exercise Test (GXT)- Testing protocol in which the subject exercised on an upright cycle ergometer at 60 revolutions per minute (rpm) with a 50 watt per two minute increase in workload until volitional fatigue was reached.
8. Insulin Response- the increase in insulin concentration following ingestion of a foodstuff (e.g. CHO, protein, etc.) that subsequently induces a rise in glycogen stores within the skeletal muscle and liver.
9. Maximal Oxygen Uptake (VO_{2max})- The maximal volume of oxygen an individual can utilize during strenuous exercise. In a GXT test with an increase in workload, the plateau of VO_2 determines the VO_{2max} when exercise intensity continues to be increased.
10. Power Test for Sampled Population (n)- The term power refers to the probability of rejecting a false null hypothesis. The probability of making a type I error (rejection of the null hypothesis when it is true) is determined by the level of significance, α, specified. For example, if α is specified as 0.05, the probability of making a type I

error is 0.05. The probability of making a type II error, (failure to reject the null hypothesis when it is false) is denoted by β, and the probability of making a correct rejection, is denoted by $1-\beta$. The latter determination is the power of a statistical test.

11. Ratings of Perceived Exertion (RPE)- rating scale of perceived effort of work from 6 to 20, 6 being very light work and 20 being very, very hard work.
12. Recovery- qualified by two items:
    a. Time- is the recovery to occur immediately (less than four-hours), over the short-term (hours to days), or over the long-term (days to weeks to months)?
    b. Type of recovery- is the recovery characterized by an injury (i.e. fractured femur, torn anterior cruciate ligament, etc.) or simple recovery from an exercise bout (i.e. marathon, decathlon, etc.).

    Additionally, as defined by Burke (10), recovery encompasses three items:

    a. "Restoration of muscle and liver glycogen stores"
    b. "Replacement of fluid and electrolytes lost in sweat"
    c. "Regeneration, repair, and adaptation processes following the catabolic stress and damage caused by the exercise."
13. Volitional Fatigue- Point in GXT test when the subject can no longer maintain 60 rpm even when encouraged verbally. Used as a marker for the termination of the cycle ergometer and performance tests.

Delimitations

Factors were incorporated into the experimental protocol to reduce the number of uncontrolled variables in an attempt to maintain feasibility and reliability of the data collected and thus obtain meaningful results:

1. Ten healthy male subjects between the ages of 18 and 35 years were asked to volunteer for the study and were screened for fitness levels prior to being accepted.
2. Subjects completed two of four exercise bouts on two separate days separated by approximately two weeks. The testing occurred on the same piece of exercise equipment for each trial at approximately the same time of day.
3. Room temperature was held at approximately 22° C during all experimental trials and recovery periods.
4. The test trials that were conducted on any given day were assigned randomly in order to eliminate any learned adaptations to the exercise protocol.
5. The subjects were asked to abstain from the consumption of any stimulants on the day of testing (i.e. caffeine, nicotine) and to begin a water-only fast twelve hours before arriving on the study day. Furthermore, the subjects were asked to abstain from alcohol consumption or strenuous physical activity for 24 hours prior to testing.

Limitations

1. It was possible that the subjects did not heed suggestions made about a substance consumption and physical activity prior to the experimental protocol which could have affected the reliability of the data.
2. It was possible that the subjects did not begin a water-only fast twelve-hours before arriving on the study day. However, this would have been recognized in the blood glucose concentrations and would only have prolonged the subject's glycogen depletion phase to assure sufficient glycogen depletion.
3. While muscle biopsies were not taken, an important factor in the investigation was assurance of glycogen depletion prior to the four-hour recovery period. This variable was accounted for by previous studies of Coyle *et al.* (14), in that depletion of liver glycogen can be appreciated if the blood glucose concentrations begin to decrease during exercise.
4. The subject's peak work load was determined when they had reached volitional fatigue. This variable may have been dependent on the subject's desire to exercise under strenuous conditions and could have varied depending on psychological (i.e. mood, etc.) and physiological (i.e. nutrition, level of rest, etc.) factors.
5. The subjects could not be blinded to the drink that was ingested, though drinks were presented in a randomized order. Further, though it was suggested that both drinks provided performance enhancement, the subjects may have expected a greater benefit with one drink over another. Additionally, due to the length of time that Gatorade®

has been on the market, subjects were more likely to be familiar with this product, possibly affecting the performance outcome.

6. Although the compositional makeup of Gatorade® is similar to that of other conventional sports rehydration drinks, data obtained from the investigation were only significant in the comparison of isovolumic amounts of Endurox R^4 Recovery Drink® and Gatorade®.

CHAPTER II

REVIEW OF RELATED LITERATURE

Chapter II represented a comprehensive review of previous research in the areas of post-exercise recovery, oxidant product formation, and glycogen-loading. A review of such work was essential in creating the design and formulating the goals of the investigation. Furthermore, an exhaustive review of related literature insured that earlier experimentation was not duplicated unknowingly.

The specific purpose of formulating a recovery drink was to enhance the subject's ability to recover more completely in a shorter amount of time from an exhaustive exercise. Several mechanisms can be utilized to elicit this type of response including carbohydrate (CHO) and protein ingestion that stimulate the insulin response, and antioxidants, that allow for recovery from oxidative damage caused by reactive oxidative products.

Insulin Response

One of the key principles in the promotion of glycogenesis following an exercise bout is the use of agents that promote the insulin response. As mentioned in the introduction, exercise promotes the breakdown of glycogen within skeletal muscle and inactivates glycogen synthesis. During recovery, however, the decrease in glycogen stores caused by the exhaustive exercise bout yields a corresponding increase in the overall

percent activity of the glycogen synthase enzyme (6). To further enhance this effect, the ingestion of a recovery drink containing CHO acts to stimulate the release of insulin from the β-islet cells of the pancreas, which directly promotes the activity of glycogen synthase. Since this mechanism can lead to more loading of glycogen within the skeletal muscle, many researchers have looked at the different contributions of various substances as well as amino acids to the promotion of this effect.

Protein & Amino Acid Effects on the Insulin Response

Fajans *et al.* (19), in 1967 published a review article that specifically looked at the various contributions, or lack thereof, of amino acids and protein to the insulin response. It was initially noted that the amino acid leucine was responsible for lowering blood glucose by stimulating the insulin response. However, upon testing the effect of leucine-derived production of insulin versus the effects of administered protein, which naturally contains an abundant amount of leucine, the insulin response was significantly greater with protein owing to the possibility that either another amino acid was working to stimulate the insulin response or amino acids in conjunction with leucine were potentiating this effect (21,22,23).

Since the discovery of the synergistic effects of amino acids, all amino acids have been tested for their ability to stimulate insulin release. Though several amino acids yield an insulin response, only arginine provided potent increases in the production of insulin (38). Further, Bratusch-Marrain *et al.* (9), using twelve- to fourteen-hour fasted human subjects, infused one gram•min^{-1} arginine for five minutes netting a hepatic venous

concentration of insulin six-fold higher than that of basal concentrations. However, arginine has been demonstrated to have additional effects on other hormones.

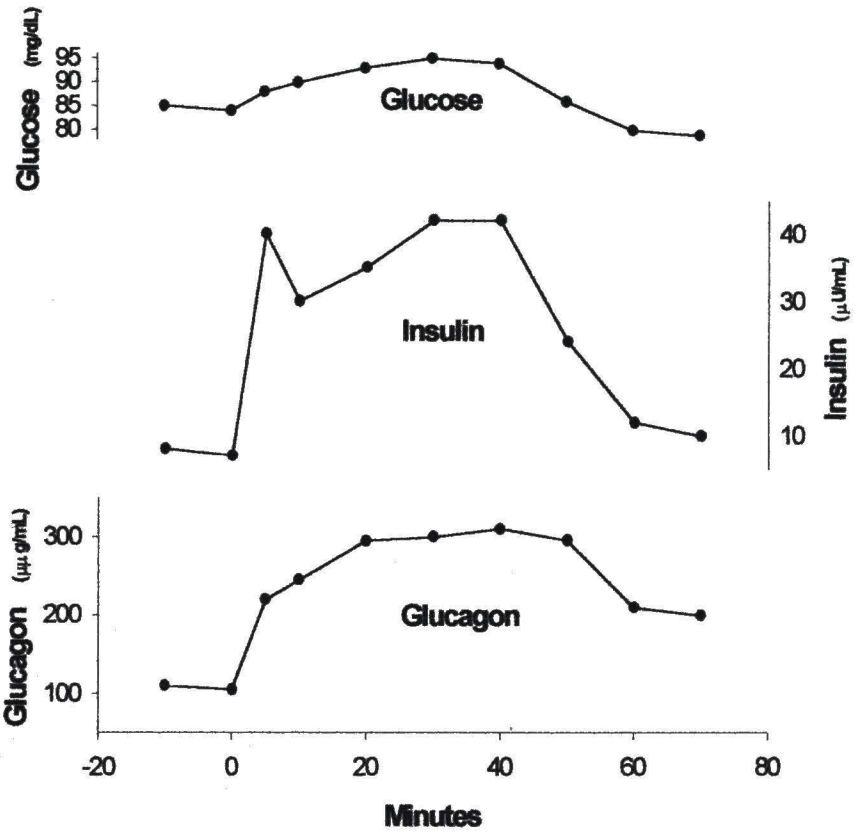

Figure 3. Arginine infusion effects. Arginine infusion (one gram•min^{-1} for five minutes) occurs at time zero. Note the increase in blood glucose concentration even though there is an increase in insulin. This end result is due to additional effect of arginine on glucagon production. Adapted from Müller *et al.* (39).

One of these, glucagon, increases blood glucose concentration by promoting gluconeogenesis in the liver. While this has beneficial effects for skeletal muscle glycogen stores, it further depletes liver glycogen.

CHO Ingestion Effects on the Insulin Response

Ivy (31) conducted an investigation that described the effects of CHO ingestion post-exhaustive exercise on glycogenesis over a four-hour recovery period. When CHO was not ingested during this time period a small glycogenesis rate of 1-2 μmol•(g wet wt)$^{-1}$•h^{-1} was found. Ingestion of a liquid CHO two-hours post-exhaustive exercise yielded a doubling in the rate to 3-4 2 μmol•(g wet wt)$^{-1}$•h^{-1}. However, ingestion of the same amount of liquid CHO immediately post-exercise produced a glycogenesis rate of 6-7 2 μmol•(g wet wt)$^{-1}$•h^{-1}, 3.5- to 6-fold over non-CHO ingestion, which is maintained for two hours followed by a 50% declination over the next two hours. The mechanism for the increase in glycogenesis after the exercise bout was produced by a translocation of more GLUT-4 receptors to the cell surface and not an increase in the intrinsic activity of the receptor itself (25). The explanation for a 50% reduction in glycogenesis rate with ingestion at two-hours post-exercise was attributed to the skeletal muscle undergoing an ever-increasing insulin resistance post-exercise, thereby reducing muscle glucose uptake and subsequent glycogenesis (31). Thus demonstrating a necessity for CHO ingestion within the first two-hours following exhaustive exercise in order to facilitate a faster recovery from glycogen store depletion.

It has also been determined that a critical amount of CHO must be ingested to yield maximum glycogenesis. Two researchers (31,7) with similar study designs of administering a particular amount of CHO and then measuring the rate of glycogenesis via muscle biopsies, demonstrated a curvilinear pattern in which increasing CHO

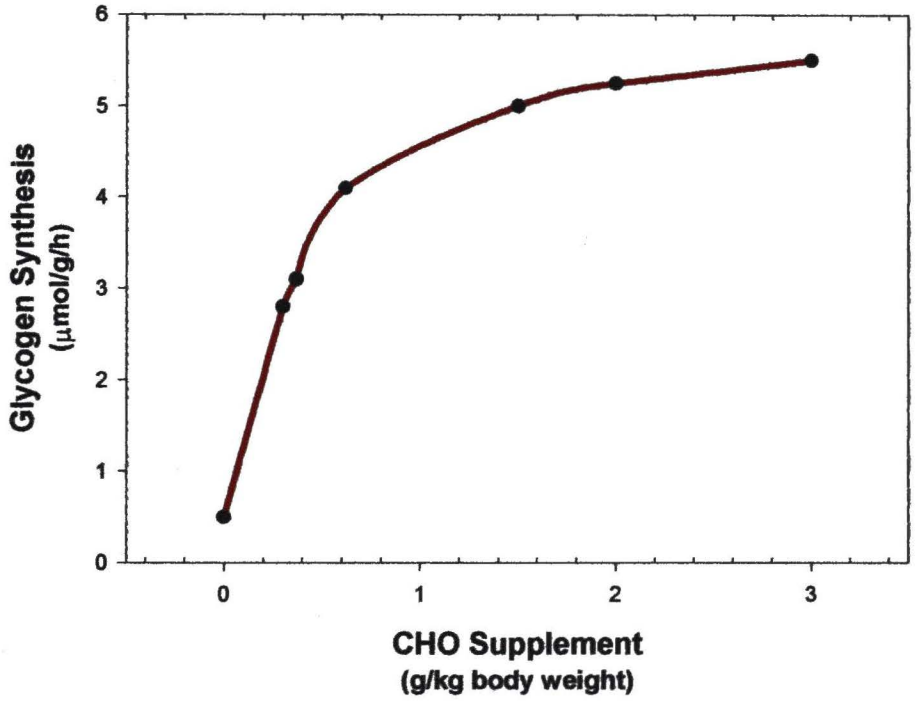

Figure 4. Glycogen-Synthesis to CHO Supplement. The amount of CHO ingested over a four-hour recovery period yields a predicted value of glycogen replenishment post-exercise. CHO was ingested at time periods immediately and two-hours post-exhaustive exercise. Adapted from Ivy (31) and Blom *et al.* (7)

consumed yielded increasing glycogenesis up to a critical point of 1.0-1.5 g CHO•(kg body weight)$^{-1}$. At this point, increases in CHO consumption did not further induce large increases in glycogenesis rates. As most conventional CHO drinks provide approximately 0.6 - 0.8 g CHO•(kg body weight)$^{-1}$ in a twenty-four ounce volume, including Gatorade®, it is necessary to note that the same volume of the Endurox R^4 Recovery Drink® provides approximately 1.7 g CHO•(kg body weight)$^{-1}$ and because of this may further increase glycogenesis over a four-hour recovery period post-exhaustive exercise.

Combining CHO, Protein, & Arginine on the Insulin Response

Combining arginine with carbohydrate yielded a five-fold increase in the insulin response over CHO alone or arginine alone with a given amount of substrate (31). Unfortunately, arginine induced such negative effects as unpalatable taste, diarrhea, and borborygmus (31). Therefore, it was determined to look at the effects of CHO in conjunction with protein since protein naturally contains an abundant amount of arginine. Zawadzki *et al.* (54), demonstrated that when compared to 112 g CHO alone, a mixture of 112g CHO and 41g protein (a 3:1 CHO:Protein ratio) yielded a significantly faster average glycogenesis [7.1 μmol•(g wet wt)$^{-1}$•h^{-1} v. 5.0 μmol•(g wet wt)$^{-1}$•h^{-1}] over a four-hour recovery period. Further, the CHO-protein combination was found to be palatable in taste without unwanted side effects (54). However these results, as suggested by Burke, demonstrated that the glycogenesis rate was not saturated. While utilizing a similar 3:1

CHO:Protein ratio, Burke (11) found a significant increase in serum insulin concentration without additional increases in glycogenesis owing to the possibility that though the insulin response is further stimulated, it may not enhance the glycogenesis rate. With this stated, the Endurox R^4 Recovery Drink® utilizes a 3.5:1 CHO:Protein ratio with the additional inclusion of arginine to provide a net further glycogenesis with palatability and without unwanted side effects.

Antioxidants

With endurance training there is a subsequent increase in mitochondria within the skeletal muscle leading to increased generation of free radicals during exercise. In terms of performance, free radicals, as measured by oxidative end products, can limit an athlete's ability to compete. Therefore, antioxidants are used within recovery drinks to limit or reduce exercise-induced muscle damage. Vitamin E, or α-tocopherol, is the primary lipid-soluble antioxidant found in the mitochondria while vitamin C, or ascorbic acid, is a water-soluble antioxidant, which can reduce the oxidized vitamin E back to its original, active form; in essence recycling used vitamin E (39,41).

Primarily, oxygen radicals are formed in membrane phospholipids such as the inner mitochondrial membrane where the electron transport and cytochrome P_{450} oxidase systems are located. Two examples of radical formations in this location are ubiquinone and cytochrome P_{450} oxidation. Further, both of these oxidations yield an extremely damaging free radical known as the superoxide radical. The superoxide radical reacts to form water and various intermediates (e.g. inorganic and organic hydrogen peroxides).

Antioxidants, such as vitamins E & C, are very effective against the superoxide radical and limit its reactivity by reducing it to alcohol (43).

Packer *et al.* (43), demonstrated that endurance trained rats with differing supplementation of vitamin E yielded different performance capacities. Those rats that were fed a diet without α-tocopherol had an endurance capacity 50% lower than that of rats with normal vitamin E supplementation. Additionally, Aikwa *et al.* (1), compared rats with normal vitamin E supplementation (40 IU Vitamin E/kg body wt.) with that of deficient supplementation (10 IU Vitamin E/kg body wt.). After endurance training, the vitamin E deficient animals were subject to marked decreases in liver and muscle α-tocopherol over control animals.

In the above studies, it was demonstrated that even with normal vitamin E supplementation, there was still a noticeable decrease in endogenous α-tocopherol with exercise training. It was then suggested that vitamin E supplementation may need to be increased to accommodate and maintain normal tissue concentrations of α-tocopherol during endurance training (44). Additionally, the use of vitamin C, as mentioned previously, has been shown to interact and spare α-tocopherol *in vitro* suggesting that its use might further limit the number of free radicals produced from endurance exercise (39,41). The Endurox R^4 Recovery Drink® includes both Vitamins E & C to help expedite the recovery from exhaustive exercise and further the performance of subsequent exercises by limiting, or reducing, free radical formation.

In summary, the current literature indicated the production of the insulin response was a key factor to increasing the rate of glycogenesis. The uses of CHO, protein, and arginine have all been shown to yield stimulation of the insulin response with the combination of the three netting synergistic increases. Further, the use of antioxidants had beneficial effects on exercise performance in terms of endurance capacity. Therefore, the purpose of the investigation was to determine the benefits of the Endurox R^4 Recovery Drink®, which was designed to promote glycogenesis through stimulation of the insulin response and reduction of free radical buildup, to promote a more desired endurance capacity over Gatorade®, a conventional rehydration sports drink.

CHAPTER III

PROCEDURES AND METHODS

In order to gain admittance into the study all subjects were required to perform a baseline graded exercise test (GXT) as part of their initial screening process. Upon admittance, all subjects were asked to perform four exercises to volitional fatigue: (i) two glycogen-depletion exercises; and, (ii) two performance exercise bouts at 85% VO_{2max}. Both exercises were separated by a four-hour recovery period. Data collected from the Endurox R^4 Recovery Drink exercise day were compared to data from the Gatorade© exercise day. This section examines the experimental protocol that was executed in acquiring and analyzing the data obtained during the investigation.

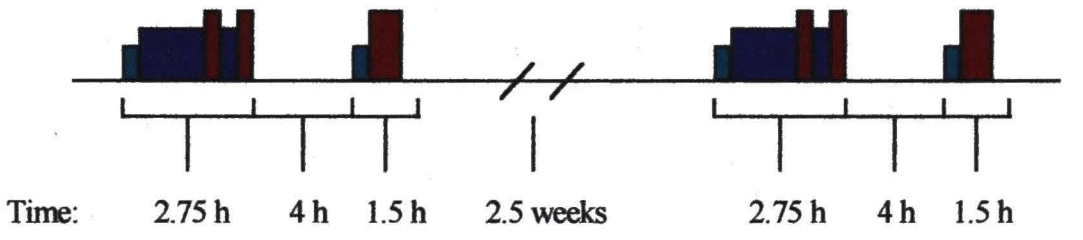

Figure 5. Time Line of Exercise Days. Eight high-fit cyclists under crossover design with drinks being presented in a random order during recovery.

Subjects

Subjects were screened prior to admittance into this investigation following guidelines established by the American College of Sports Medicine. Any potential subject that did not meet these requirements was excluded from the study. Subjects were advised of the goals of the study, its protocol, and any negative side effects that could occur as a result of their participation. They were required to sign an informed consent before participation in any testing approved by the Institutional Review Board for the Use of Human Subjects at the University of North Texas Health Science Center at Fort Worth. The criteria for selection were based on the subjects' initial GXT performance and answers from a medical history/diet questionnaire. Thirteen male volunteers between the ages of 18 and 35 years of age from the general Fort Worth area were recruited. A preliminary power test for determining sample size predicted that n = 8 were sufficient for a $p < 0.05$ probability. Subjects were accepted if they completed the GXT on an upright cycle ergometer with a $VO_{2max} \geq 60$ ml•min^{-1}•kg^{-1}, had normal heart rhythms at rest and during exercise, were asymptomatic for disease, and were non-smokers. Subjects were asked to abstain from caffeinated beverages and medications the evening before they

TABLE I. DESCRIPTIVE STATISTICS FOR SUBJECT GROUP

Age (yrs.)	Height (cm)	Weight (kg)	VO_{2max} (ml•min^{-1}•kg^{-1})	12h Fasted Blood Glucose (mmol•L^{-1})
28.4 ± 1.65	174.4 ± 1.78	73.7 ± 1.30	62.4 ± 1.10	3.98 ± 0.138

Mean ± SEM. Data obtained during screening GXT and on arrival for testing.

were scheduled for testing. Additionally, subjects were asked to limit their physical activity the day before testing and begin a water-only fast twelve hours before arriving. Upon acceptance into the investigation, the subjects were asked to complete two days of testing with four exercises to volitional fatigue. Five of the subjects were unable to complete the exercise protocol due to several mitigating factors including nausea, lack of desire, and diagnosis of an aberrant electrical pathway. Physiological descriptions of the remaining eight subjects and their initial GXT screen testing were presented in Table I.

Graded Exercise Testing Protocol

An upright bicycle ergometer (SCIFIT ID 5500) increase of grade and speed was used as an exercise stress test of maximal response. Respiratory variables were measured during the exercise test and were used to determine maximal oxygen uptake (VO_{2max}). The GXT began at a workload of 50 watts and was increased by 50 watts every two minutes until volitional fatigue. Subjects were expected to maintain an RPM of at least 60, but no greater than 90 throughout the GXT protocol. Subjective terminations of the test and objective criteria were used to qualitatively and quantitatively determine whether the subject had made a maximal effort. A determination of VO_{2max} was made by one of two criteria: a plateau of oxygen uptake (VO_2), or the point at which the VO_2 changed less than 50 $ml \cdot min^{-1}$ for a period of one-minute at the end of exercise. Oxygen uptake was determined using a dedicated breath-by-breath analysis incorporating a respiratory gas analyzer (Perkin-Elmer MGA 1100AB) to determine the partial pressure of respiratory

gases (O_2, CO_2, and N_2) in the inspired and expired breathe volumes. All variables were collected on-line using a dedicated laboratory computer (Digital Equipment Corporation MINC-23) and a customized software package to account for differences in delay and response time.

Two days, separated by 2.5 weeks, were required from each subject, to test two different performance drinks: Endurox R^4 Recovery Drink® or Gatorade®. Each testing day was divided into three phases: glycogen-depletion, recovery, and performance.

Experimental Protocol

Glycogen Depletion Exercise

The subject arrived 12-hours into a water-only fast. An intravenous cannula was placed in one of the subject's antecubital veins with an 18-gauge catheter for blood sampling. Electrocardiogram electrodes were placed on the subject for standard three-lead ECG recording. The subject was weighed, seated on the upright bike, and prepared for resting conditions.

The Glycogen-Depletion Test consisted of three parts: warm-up, endurance, and sprint. The warm-up was ten minutes in length with the subject maintaining at least 60 rpm at 50 watts increasing in 50 watt increments to a workload of 65% VO_{2max}. The endurance portion (A in Fig 6., p. 26) was two hours at a workload yielding 75% VO_{2max}. However, if the subject could not maintain the workload, the workload was decreased so

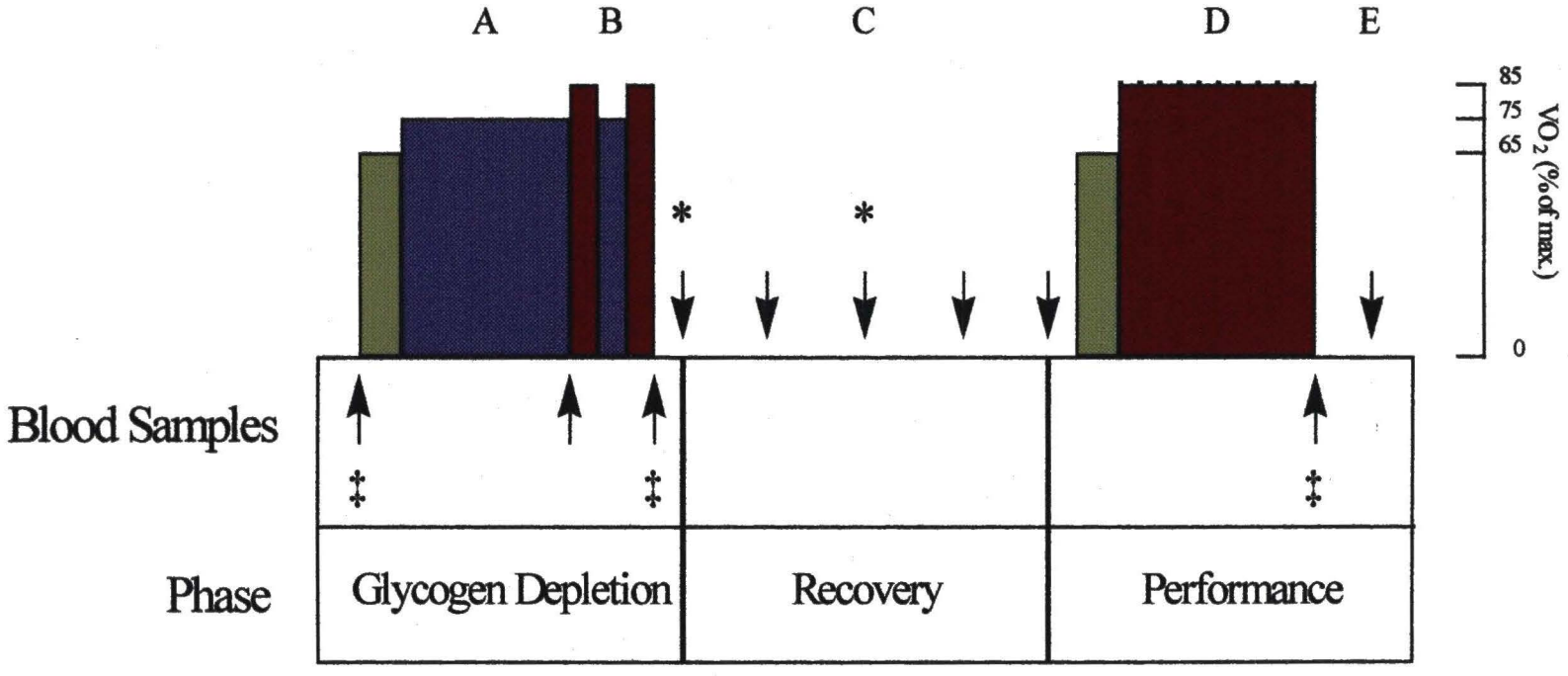

Figure 6. Study Protocol. Arrows represent time of blood sample, (‡) represent time of antioxidant sampling, and (*) represent ingestion of 12-ounces of performance drink. Dashed line (D) represents various performance time for subjects. Section A represents dynamic exercise at 75% VO_{2max} for 2-hours; Section B represents two five-minute sprints of 85% VO_{2max}; Section C represents the four-hour recovery period; Section D represents the performance evaluation to exhaustion at 85% VO_{2max}; Section E represents data collection at 1-hour post-exercise.

the subject could maintain 60 rpm for the two hour period. Sprints (B in Fig. 6, p. 26) were defined as five-minutes at a workload yielding 85% VO_{2max}, followed by a five-minute cool-down period at a workload yielding 75% VO_{2max}. If the subject could not maintain an RPM of greater than 60 for more than five seconds for a particular sprint, then the last sprint was recorded and the glycogen-depletion test was complete. A subsequent weight measurement was taken to determine water lost during this phase due to dehydration and perspiration.

Recovery

The Recovery Phase (C in Fig. 6, p. 26) began after the final blood sample of the Glycogen-Depletion Phase. The subject was asked to ingest twelve-ounces of one of the two performance drinks and was taken to a room where he was able to sleep, watch television, listen to the radio, study, etc. In addition to the supplement, a water replacement was determined by multiplying the subject's weight (lbs.) by sixteen to convert it to ounces, subtracting 24-ounces to account for the drink, and then dividing by four to determine the amount needed per hour. At the beginning of the third hour, the subject received the remaining twelve-ounces of the performance drink. Halfway through the fourth hour, the subject was taken back into the laboratory in preparation for the Performance Phase.

Performance

The Performance Phase was divided into two parts: warm-up and performance. The warm-up was ten minutes in length with the subject starting at 50 watts and increasing the wattage over the next several minutes to 65% VO_{2max}. This warm-up differed from the glycogen-depletion phase warm-up in that subjects reached 65% VO_{2max} by the end of the first five minutes. This allowed the subject to warm-up for at least five minutes at this level prior to starting the 85% VO_{2max} performance test. The performance test (D in Fig. 6, p. 26) began once the wattage had reached the 85% VO_{2max} workload level. A stopwatch was started at this time with all timing devices hidden from the subject's view, less the RPM, as to not bias their riding ability. The subject was required to follow two rules for the duration of the test:

1) He was not allowed to rise off of the bicycle seat, and,
2) Once the subject's RPM fell below 60 for more than 5 seconds, the test was over and the time marked.

During the first five to ten minutes, the VO_2 was assessed to determine if adjustments were needed to maintain an 85% VO_{2max} workload. If needed, the change in wattage was noted at the exact time that it was made in order to duplicate the changes for the next exercise day. The subject was relentlessly encouraged to go as far as possible. Once the subject reached the end of the performance test, the wattage was lowered to approximately 100 watts at which point the subject was allowed to decrease it on his own.

When the subject's heart rate was lower than 125 beats•min^{-1}, he was allowed to get off the bike. After the final blood sample one hour later (E in Fig. 6, p. 26) and the intravenous cannula was withdrawn and bandaged, the subject was released.

Techniques of Measurements

Metabolic Measurements During Exercise

In order to assess oxygen uptake (VO_2) during each exercise test, the subject was required to breathe through a ventilation recording apparatus, which consisted of a mouthpiece attached to a saliva trap and a turbine column transducer. The saliva trap was equipped with a sampling port, which allowed the collection of respired oxygen (O_2) and expired carbon dioxide (CO_2) partial pressures (PO_2 and PCO_2) which were then analyzed by a mass spectrometer (Perkin-Elmer MGA-1100A). The subject was also required to wear a nose-clip in order to prevent respiration through the nasal passages. Before the utilization of this equipment during each exercise test the mass spectrometer was calibrated with known gas concentrations of CO_2, O_2, and nitrogen (N_2). A turbine transducer was utilized to measure the expired and inspired airflow, which transmitted this information via a voltage output to the ventilation measurement module (VMM; Alpha Technologies, Inc.). The signals were analyzed using analog-to-digital conversion (Vetter Digital Model 4000A) with a laboratory computer (Dell Computers) equipped with a customized VO_2 program (Hex3) for on-line, breath-by-breath computation of VO_2,

carbon dioxide output (VCO_2), respiratory exchange ratio (RQ), and minute ventilation (V_e).

Cardiovascular Measurements

Heart Rate (HR) was continuously monitored using a three-lead electrocardiogram (Hewlett-Packard 78342A) and was also recorded on the Dell computer via a customized computer interface and data acquisition program.

Measurement of Cardiac Output

Cardiac output (Q_c) was determined during rest and dynamic cycle exercise by using a non-invasive acetylene rebreathe technique (52). Acetylene gas uptake from the alveoli is proportional to pulmonary capillary blood flow and therefore can be used to accurately estimate Q_c. Concentrations of acetylene, carbon dioxide, and oxygen were analyzed using a mass spectrometer (Peking-Elmer MGA-1100A) with a sample flow rate of 60 ml/min. During experimental collection of Q_c, a nose-clip was utilized to prevent the subject from inhalation through the nasal passages. A rebreathing apparatus equipped with a mouthpiece, turbine flow meter, and rebreathing bag was placed in the subject's mouth. A customized cardiac output analysis program (Dufis) triggered the release of acetylene into the rebreathing bag at the subjects end exhalation, or functional residual capacity (FRC). The subject was instructed to take a deep inhalation once the bag had been filled with acetylene and rebreathe into the bag for a minimum of five breaths. The mixed expiratory fractions of acetylene, carbon dioxide, and oxygen were measured during

the rebreathing period as well as the flow rate. Data were collected via a customized data acquisition system and cardiac output was calculated utilizing a Fick's Principle equation after completion of the measurement on-line.

Measurement of Perceived Exertion & Performance

Ratings of perceived exertion (8) was measured using the Borg Scale. The Borg Scale started with a numerical grade of 6 (very, very light work) and continued up to a numerical grade of 20 (very, very hard work). This scale was validated against HR and was demonstrated as a cognitive assessment of the perception of workload. The subject was asked to identify their level of work by pointing to the corresponding scale number on a chart with the ratings displayed. The subject's perception of the effort of work performed by their legs was assessed using this technique.

Performance was indicated by time to complete the exhaustion phase. Each subject's effort was timed from the end of the warm-up to the end point of maintaining an RPM less than 60 for five seconds.

Measurement of Clinical Chemistry

The Instrumentation Laboratory Synthesis 35 measured the concentrations of the following variables according to the sample times represented in Figure 6, p. 26: pH, sodium ($mmol \bullet L^{-1}$), potassium ($mmol \bullet L^{-1}$), calcium ($mmol \bullet L^{-1}$), glucose ($mmol \bullet L^{-1}$), hematocrit (%), total hemoglobin (THb, $g \bullet dL^{-1}$), hemoglobin (%), and oxygen (vol. % O_2). The YSI 2300 Stat L-Lactate analyzer measured lactate (mmol/L) and glucose

(mmol/L). Insulin samples were immediately centrifuged at 1200*G in a refrigerated centrifuge (Beckman® Model TJ-6) after which the serum was withdrawn and frozen at -20°C for analysis within 60 days by radioimmunoassay (Coat-A-Count® Insulin).

Measurement of Oxidation Products

Oxidation products were measured as diene (ηmol/L), triene (ηmol/L), and Thiobarbituric Acid Reactive Substrates (T-BARS) Assay (ηmol/L) as described by Malshet *et al.* (37) and Pryor *et al.* (45). Dienes and trienes represent the first and second products of oxidation in the form of decomposition to aldehydes and ketones, while T-BARS Assay yields the final products of oxidation primarily in the form of malonyldealdehyde (37).

Statistical Analysis

The matrix of this experiment was divided into three different sections: (i) glycogen-depletion, (ii) recovery, and (iii) performance. The glycogen-depletion section required a 1 x 11 one-factor ANOVA format with repeated measures taken across points in the exercise. The recovery section required a 2 x 3 two-factor ANOVA format with repeated measures taken across points in the recovery period. The performance section required a 2 x 8 two-factor ANOVA with repeated measures across points in the exercise period. This experimental strategy allowed for comparison of changes in blood and serum measurements and exercise performance between groups during the different experimental

conditions. The dependent variables that were measured to elucidate the exercise effects and test the hypothesis were formation of oxidant products and time to exhaustion in the performance section of the test.

Other variables were measured in order to define differences between exercise conditions and substantiate oxidation product formation and time to exhaustion. These variables included heart rate (HR), ratings of perceived exertion (RPE), cardiac output (Q_c), oxygen uptake (VO_2), serum electrolytes, hematocrit, pH, blood [glucose], serum [insulin], and taste of the product.

TABLE II. EXERCISE TESTING DESIGN

1 x 11 ONE-FACTOR ANOVA		2 x 11 TWO-FACTOR ANOVA		
	Subject		Gatorade	Endurox R[4]
Glycogen-Depletion		Recovery		
Rest	1,2	1h-Post	1	1
15m	2	2h	1	1
30m	2	3h	1	1
45m	2	Performance		
60m	2	Pre-Perform.	1,2	1,2
75m	2	5m	2	2
90m	2	10m	2	2
105m	2	15m	2	2
2h	1,2	20m	2	2
Sprint	1,2	25m	2	2
5m-Post	1	Post-Perform.	1	1
		1h-Post	1	1

1-Serum Measurements; 2-HR, RPE, VO_2, V_e, Q_c, RR

Differences between the two drinks were assessed using an analysis of variance (ANOVA, SigmaStat for Windows, Jandel Scientific Software, SPSS Inc., Chicago, IL) with a repeated measures design. Data was presented as exercise condition means plus or minus the standard error of the mean (SEM). Parametric paired t-tests were utilized in the analysis of oxidation product formation, insulin, and performance time. Parametric one-way repeated measures ANOVA with Tukey multiple range test was used to analyze Endurox R^4 Recovery Drink insulin values during the Recovery Phase, while non-parametric one-way repeated measures ANOVA with Tukey multiple range test was used to analyze the Gatorade® insulin concentrations during the same time period. All other variables were statistically analyzed using parametric two-way repeated measures ANOVA. For the metabolic measurements, Days 2 and 3 were broken up into four sections: Resting conditions between the Glycogen Depletion and Performance Phases, Warm-up conditions between the Glycogen Depletion and Performance Phases, Glycogen Depletion Phase for the two-hour 75% VO_{2max} plus the last sprint for each cyclist, and the Exhaustion Phase between the first five minutes and the last five-minutes. For all blood measurements except the oxidation products, Days 2 and 3 were assigned into three sections: Glycogen Depletion, Recovery, and Performance. Oxidation products were normalized using their resting values for determining percent change for each drink, then the post-glycogen depletion phase blood sample and the post-performance phase blood sample were compared using a parametric paired t-test. Differences in dependent variables within and between exercise conditions were accepted as significant at $p < 0.05$ unless otherwise noted.

CHAPTER IV

RESULTS

Chapter IV introduced the results obtained during the investigation. Presentation of the data included the mean values of the group in each of the two experimental conditions as well as the standard error of the mean (± SEM) associated with each value. Comparisons were made were made within the group between each exercise protocol executed during the experiment. The analysis concentrated on delineating the hormonal and oxidant product adaptations associated with each drink.

Descriptive Characteristics of Subject Group

Physical characteristics of the subject group as well as the results from the initial bicycle ergometer graded exercise test to maximum (GXT), which included the maximum heart rate (HR_{max}), and maximum rate of oxygen uptake (VO_{2max}) are presented in Table I of Chapter III, p. 23. Maximal oxygen uptake, a traditionally used parameter to evaluate individual fitness, and mean age adhered to the guidelines set forth in the design of the study. All subjects were competitive bicyclists with a VO_{2max} of 60 $ml \bullet min^{-1} \bullet kg^{-1}$ or greater on an upright cycle ergometer.

Metabolic Measurements

Following several minutes of resting data, measurements of respiratory rate (RR), respiratory quotient (RQ), minute ventilation (V_e), and oxygen uptake (VO_2) were evaluated during each of the three divisions in the glycogen depletion exercise bout. One minute averages were taken at the ninth minute of warm-up, 14th, 29th, 44th, 59th, 74th, 89th, 104th, and 119th minutes during the two-hour endurance ride at 75% VO_{2max}, and during the 4th minute of each five-minute sprint at 85% VO_{2max}. Respiratory Rate was significantly increased when the workload was increased from 75% VO_{2max} to 85% VO_{2max} for both exercise days. All other results indicated no significant differences associated between different exercise days.

Measurements of the same variables were also taken during the performance exercise test at 85% VO_{2max}. One-minute averages were taken at the 9th minute of warm-up and every 5th minute of the exhaustion phase until exhaustion. A significantly decreased RQ, V_e, and VO_2 were noted from the first five-minutes of exercise to the last five-minutes for both drinks, but no differences were demonstrated between drinks.

Cardiovascular Measurements

Heart rate (HR) was continuously monitored throughout the glycogen-depletion and performance phases. One-minute averages were taken at the same times as listed in the Metabolic Measurements section above. Heart rate was significantly increased when the workload was increased from 75% VO_{2max} to 85% VO_{2max} for both exercise days.

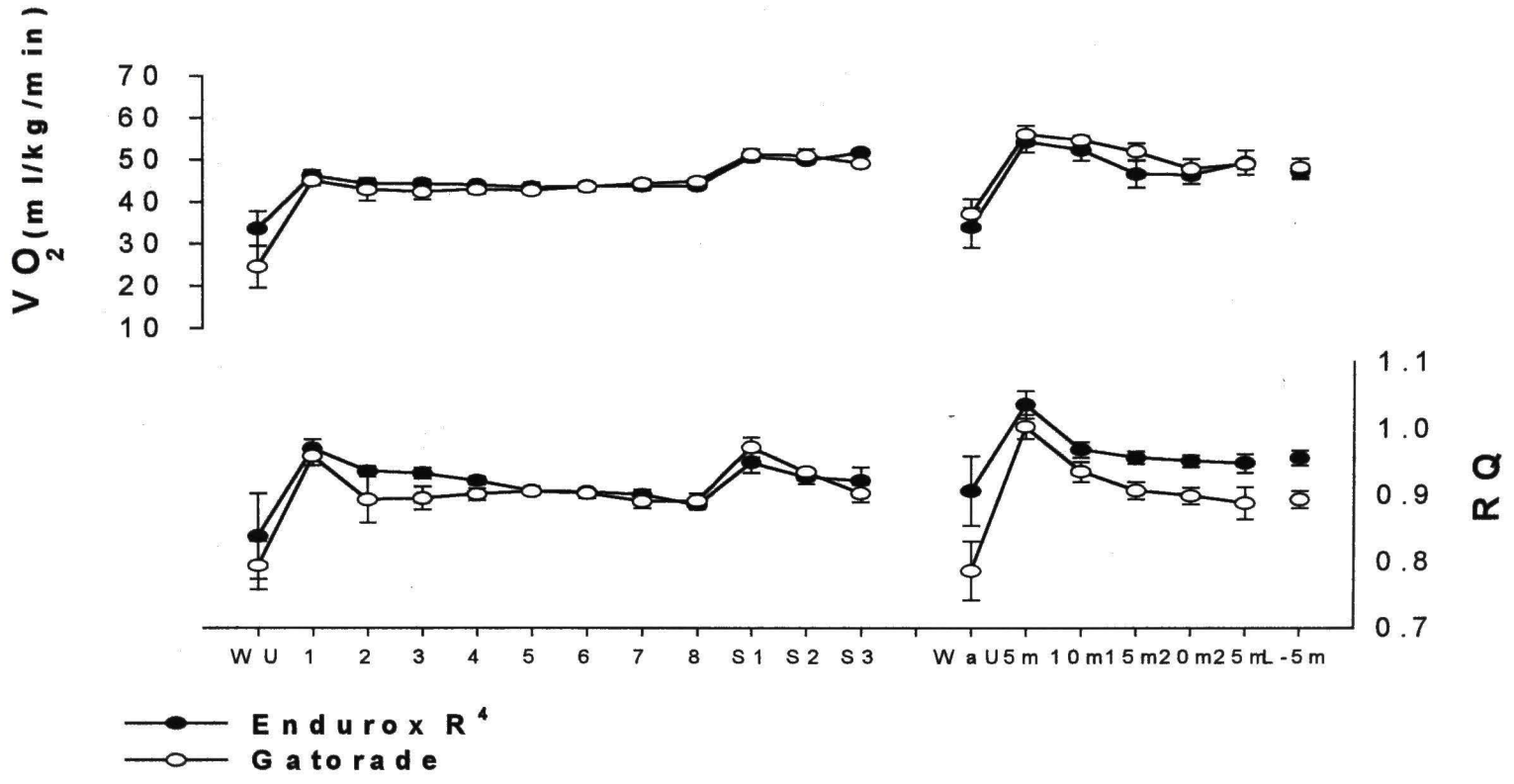

Figure 7. Respiratory Quotient and Oxygen Uptake During Glycogen-Depleting Exercise and Performance. RQ and VO_2 were similar for both Endurox R^4® and Gatorade® throughout the experimental protocol. WU/WaU-warm-up, 1-8-fifteen minute measurements during two-hour endurance exercise bout, S1-S3- sprints one through three, 5m-25m- time into performance test, L-5m- last five minutes for every subject in the performance test

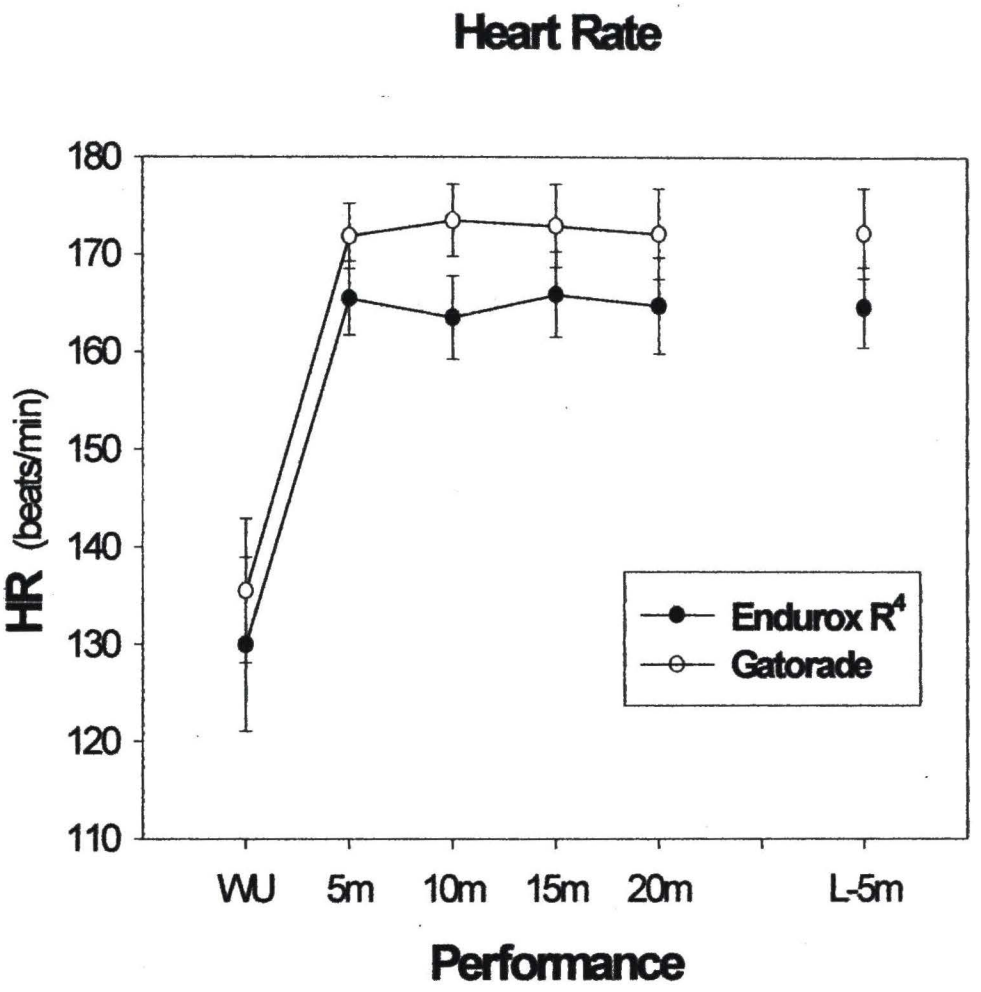

Figure 8. Heart Rate during Performance Exercise Bout. Sample size (n) begins to fall at 25 minutes. Note during the last five-minutes for all subjects a similar HR is achieved indicating a similar exercise performance to exhaustion for both drinks.

During the performance test, HR was not statistically different when comparing the first five-minutes of exercise to the last five-minutes for both drinks. This indicated that the exercise to exhaustion was equivalent with the use of either drink. Also, trend analysis using one-minute averages for times 4^{th}, 9^{th}, 14^{th}, and 19^{th} minutes to predict times 35-, 40-, and 45-minutes revealed no significant differences with this sample size. Variables up to twenty minutes were used in this analysis to predict trends with the same sample size.

Cardiac output, Q_c, as measured by acetylene re-breathe technique, was obtained during the glycogen-depletion phase at times listed in the Metabolic Measurements section without significant differences between days. Due to the fact that performance time is a much more important variable in this study, Q_c measurements were obtained at irregular intervals in order to not elude the subjects as to the amount of time elapsed since beginning the test. Further, Q_c measurements at the end of the exhaustive exercise were technically difficult to obtain utilizing the acetylene rebreathe technique.

Ratings of Perceived Exertion

Measurements of RPE were obtained during the times listed in the Metabolic Measurements section for both the glycogen-depletion and performance exercise tests. During the performance test, RPE was not statistically different when comparing the first five-minutes of exercise to the last five-minutes for both drinks. This indicated that the exercise to exhaustion was equivalent with the use of either drink. Though RPE was not statistically significant, an apparent trend was noted for a longer time at a decreased RPE

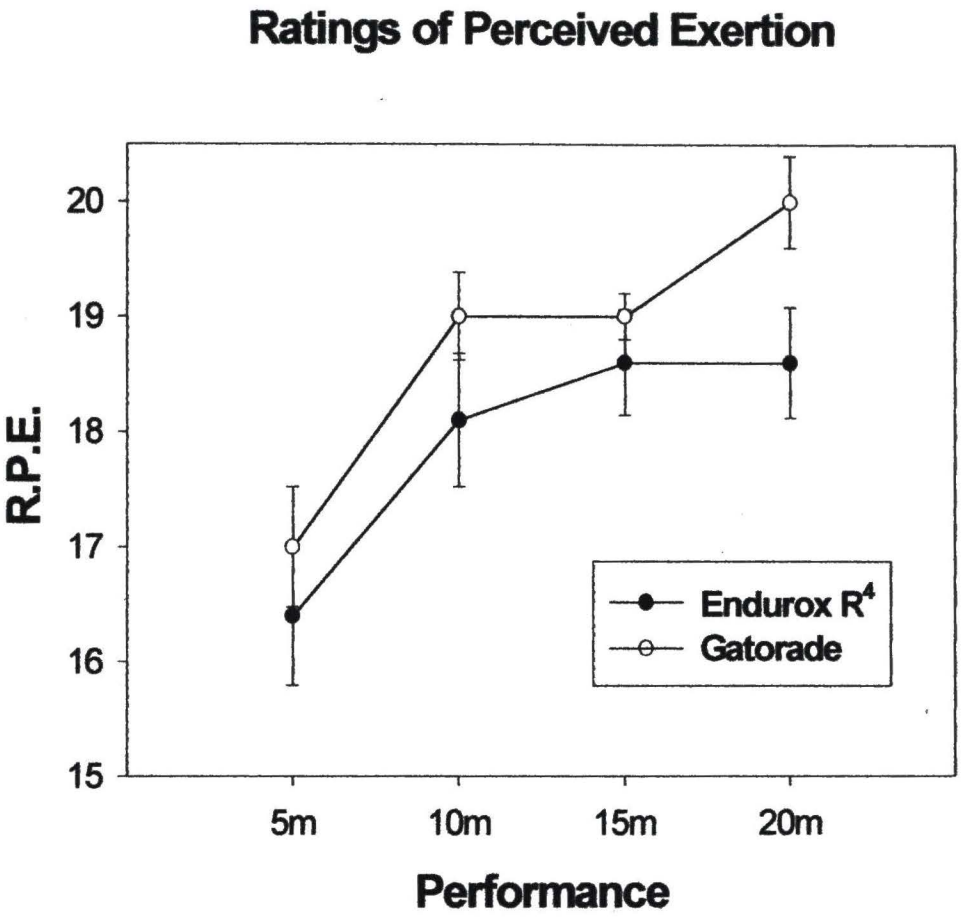

Figure 9. Ratings of Perceived Exertion During Performance Test. No significant differences were noted for the first five-minutes of the performance test and the last five-minutes between the Endurox R^4 Recovery Drink® and Gatorade® indicating that the performance test for both drinks yielded a similar endpoint. However, at time 20-minutes Gatorade® subjects are at the upper limit in RPE while Endurox R^4 Recovery Drink® subjects are continuing the performance exercise bout at a lower RPE.

with the use of the Endurox R^4 Recovery Drink®. However, trend analysis using one-minute averages for times 4^{th}, 9^{th}, 14^{th}, and 19^{th} minutes to predict times 35-, 40-, and 45-minutes revealed no significant differences with this sample size. Variables up to twenty minutes were used in this analysis to predict trends with the same sample size.

In terms of amount of CHO ingested, 24-ounces of Endurox R^4® provided 1.73 ± 0.035 g CHO•kg^{-1} body weight while an isovolumic amount of Gatorade® provided 0.584 ± 0.012 g CHO•kg^{-1} body weight yielding a significantly decreased amount of CHO ingested (paired t-test, $p < 0.001$).

Performance

The performance time was measured from immediately after warm-up in the performance phase until the subject could not maintain greater than or equal to 60 RPM for more than five seconds. The average time of performance for the Endurox R^4 Recovery Drink® was 31.1 ± 3.19 minutes while 20.0 ± 1.96 minutes was the average performance time for Gatorade®. This represented a 55% increase in performance time with the use of the Endurox R^4 Recovery Drink® over that of the same volume of Gatorade® (paired t-test, $p = 0.011$).

Measurements of Rehydration and Weight

Measurements of total hemoglobin (THb) and hematocrit (Hct) were used to determine the percent change in plasma volume as indicated by the formula:

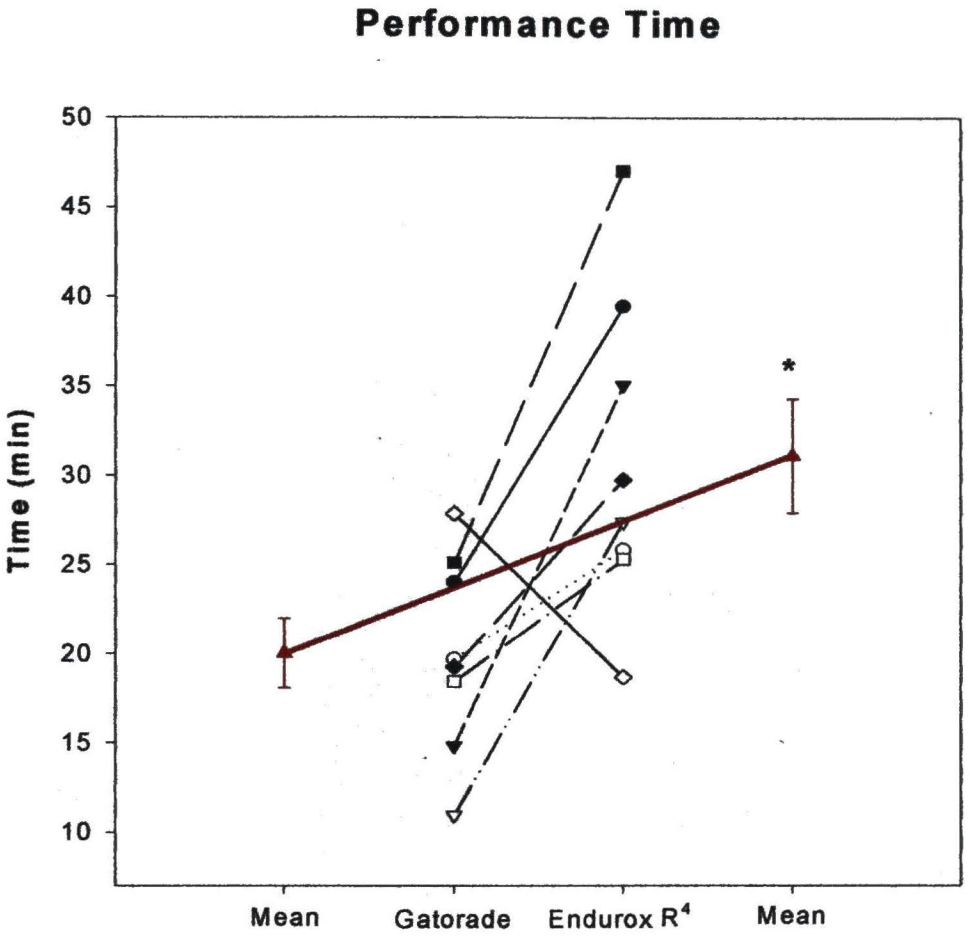

Figure 10. Performance Time. Subjects 1-8 and the mean increase in time of performance. Note that one person decreased with the use of the Endurox R^4 Recovery Drink®, but even with that decrease the mean increase represents a statistically significant 55% gain in performance time with the use of the Endurox R^4 Recovery Drink® over that of Gatorade® (*).

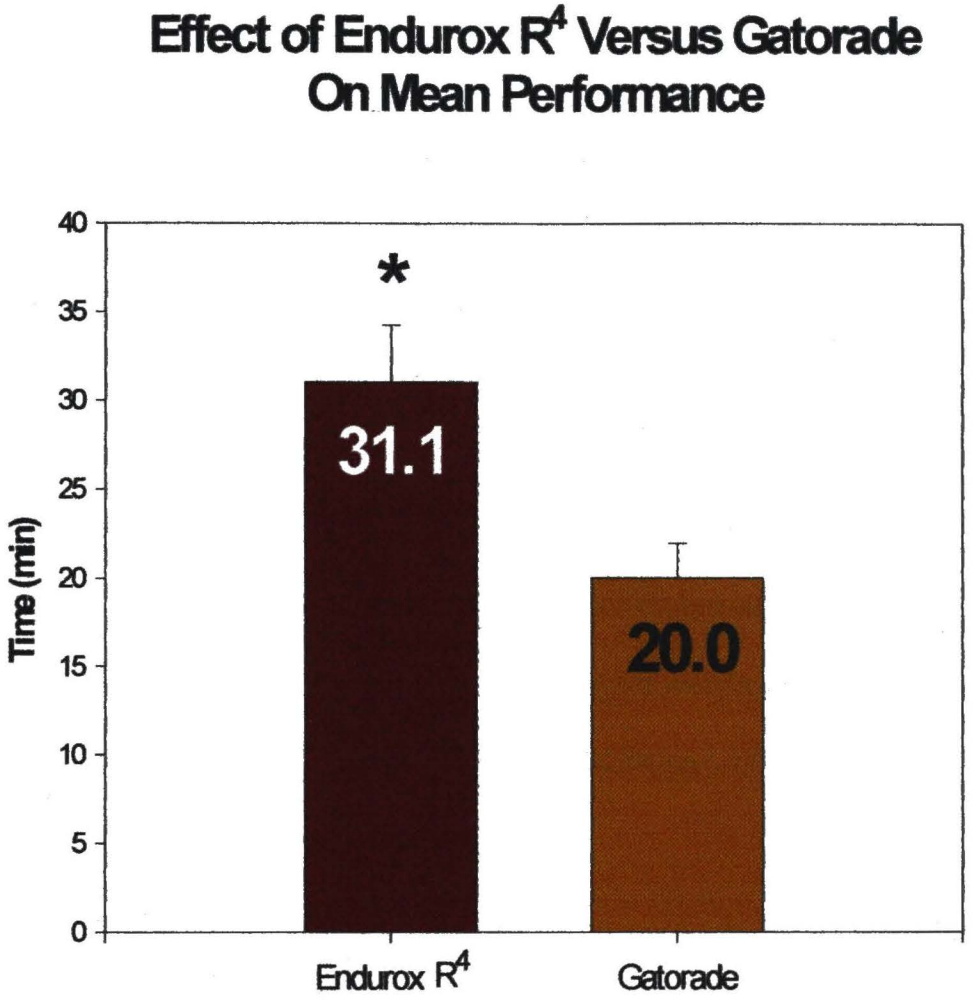

Figure 11. Mean Performance Times. The use of the Endurox R^4 Recovery Drink® yielded a significant 55% increase in performance time when compared to Gatorade® (*).

$$\% \text{ Change in Plasma Volume} = \left[\frac{(THb_a)(100\text{-}Hct_b)}{(THb_b)(100\text{-}Hct_a)} * 100 \right] - 100$$

where *a* represented blood measurements of THb and Hct prior to the glycogen depletion phase and *b* represented the blood measurement immediately post-glycogen depletion. As body weight was not significantly different between the two drinks, the percent change in plasma volume was used as an indicator of hydration status during the four-hour recovery period. As illustrated in Figure 12, p. 45, no significant differences were noted between percent changes in plasma volume resulting from ingestion of either recovery drink. Average weight loss for Endurox R^4® and Gatorade® were 2.07 ± 0.222 kg and 2.02 ± 0.334 kg, respectively, with an average weight gain, after the four-hour recovery and 24-ounces of recovery drink ingested, being 1.14 ± 0.197 kg for Endurox R^4® and 0.91 ± 0.312 kg for Gatorade®.

Measurements of Clinical Chemistry

Clinical chemistry measurements were obtained at sampling times as described in Fig. 6, p. 26, of the study protocol.

1. Glycogen-Depletion: rest, post-endurance, post-sprint, and five-minutes post-sprint;
2. Recovery: one-hour, two-hour, three-hours, and four-hours; and

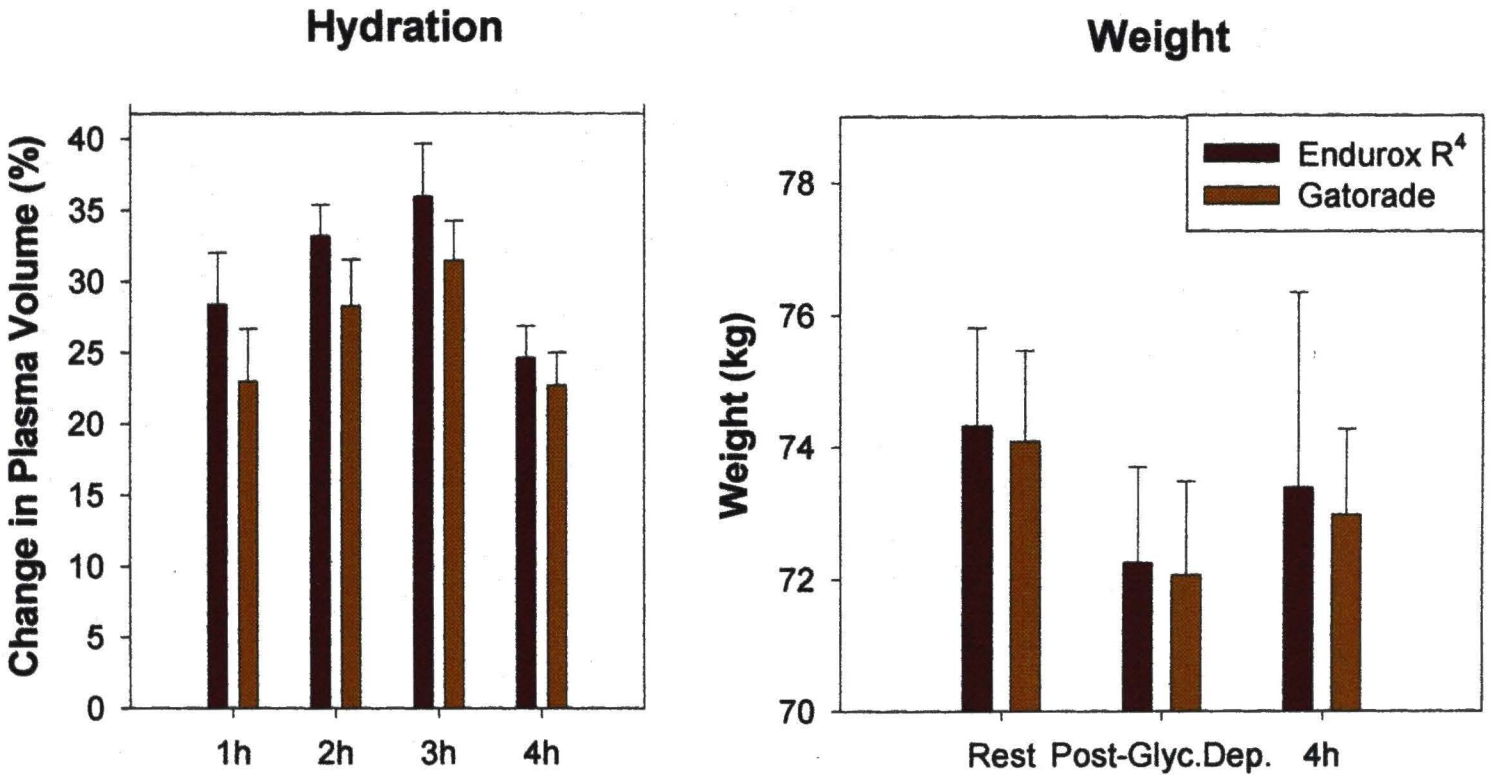

Figure 12. Effect of Endurox R^4 ® versus Gatorade® on Hydration and Weight. No significant differences were noted between drinks for hydration, in terms of percent change in plasma volume, and weight indicating similar rehydration characteristics between Endurox R^4 Recovery Drink® and Gatorade®. Additionally, as indicated by

3. Performance: post-performance and one-hour post-performance.

No statistically significant differences were noted for the following variables during the three test sections: pH, sodium, potassium, hematocrit, total hemoglobin, oxygen, and lactate. Special attention should be given to both sodium and potassium yielding similar results between drinks indicating similar electrolyte replenishment. Additionally, blood lactate concentrations for both drinks yielded similar results.

During recovery, as visualized by Section C, Fig. 6, p. 26, glucose was significantly increased using the Endurox R^4 Recovery Drink® compared to Gatorade® at one-hour into recovery, while insulin was significantly increased with Endurox R^4® over Gatorade® at three-hours into recovery. Both glucose and insulin concentrations were increased at one-, and three-hours when compared to post-glycogen-depletion. Interestingly, blood calcium concentrations were also increased during recovery and maintained a statistical elevation during the performance phase with the use of the R^4 Recovery Drink®. It was discovered that the exact amount needed to maintain this significance was due to the type of protein used in the drink formula.

Measurements of Oxidation Products

Diene, triene, and T-BARS Assay measurements were obtained at rest, immediately post-glycogen-depletion, and immediately post-performance. To measure the effects of the drink on these variables, measurements were noted as the change in the

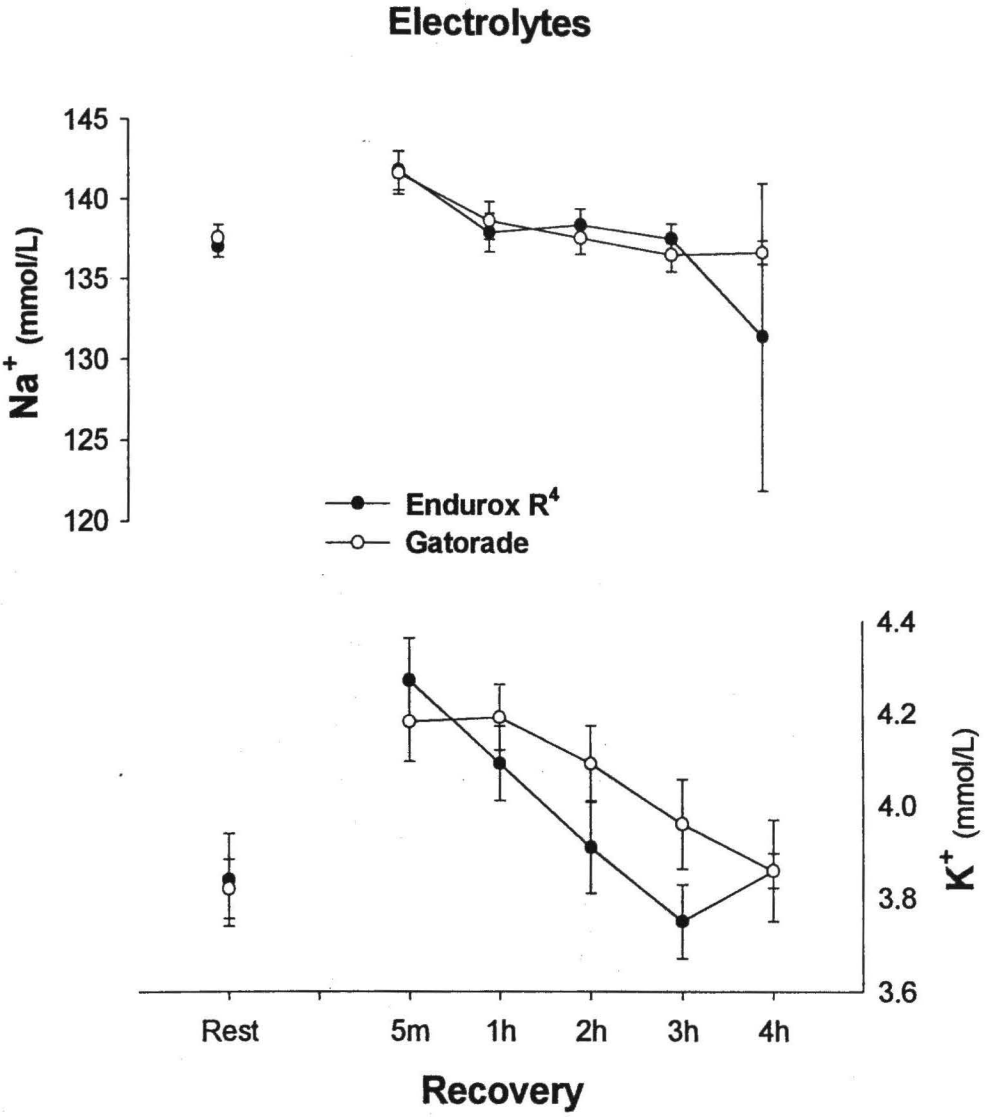

Figure 13. Electrolyte Replenishment During Recovery. No significant differences for sodium and potassium were yielded between drinks indicating similar electrolyte replenishment characteristics. Note, however, the decreased plasma potassium with Endurox R^4®. This was attributed to an increased release of insulin resulting in increased cellular uptake of potassium.

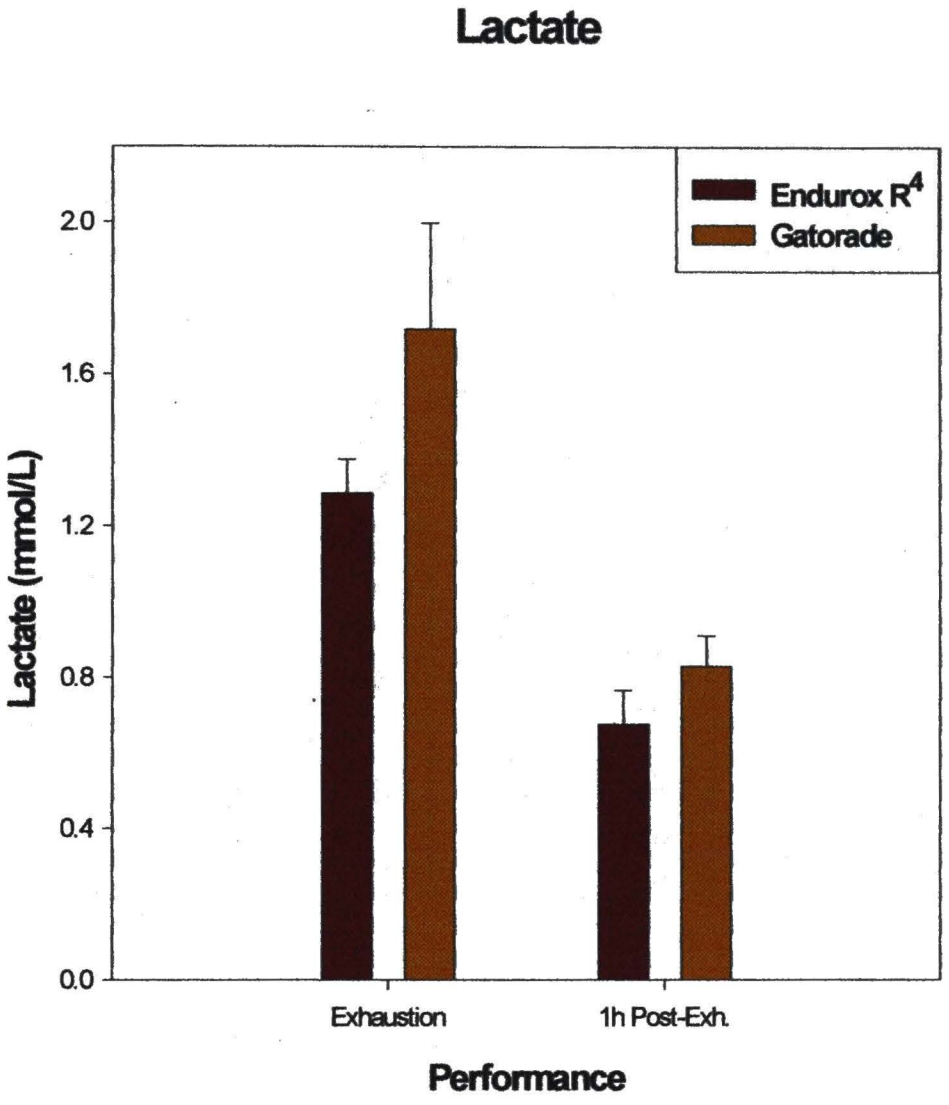

Figure 14. Drink Effects on Mean Lactate Concentrations. Though no significant differences were noted between drinks on lactate concentrations, five of eight subjects yielded a decreased blood lactate concentration with the use of Endurox R^4 Recovery Drink®.

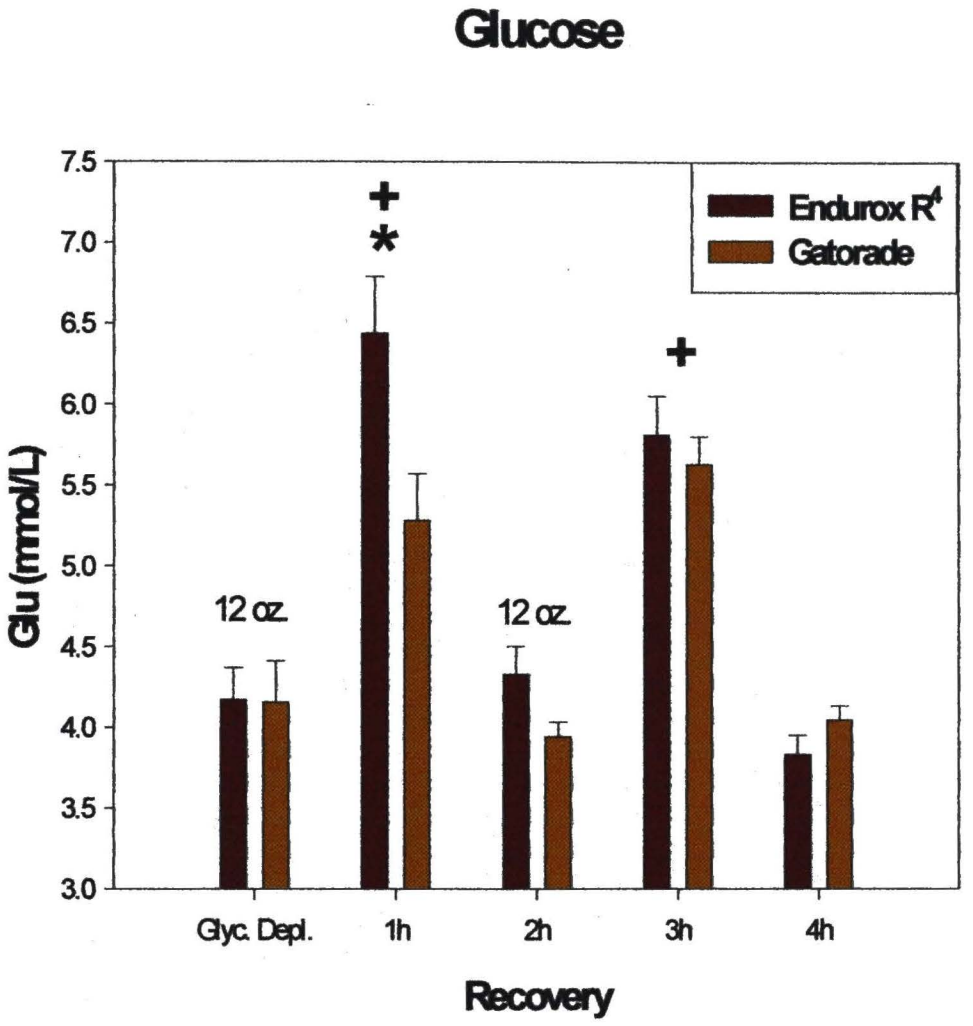

Figure 15. Blood Glucose During Recovery. Blood glucose concentrations were significantly increased one- and three- hours after drink ingestion. Endurox R^4® ingestion produced a 23% increase in blood glucose concentrations compared to Gatorade® at one-hour into recovery. (*) denotes significant difference between drinks. (+) denotes significant difference from post-glycogen depletion.

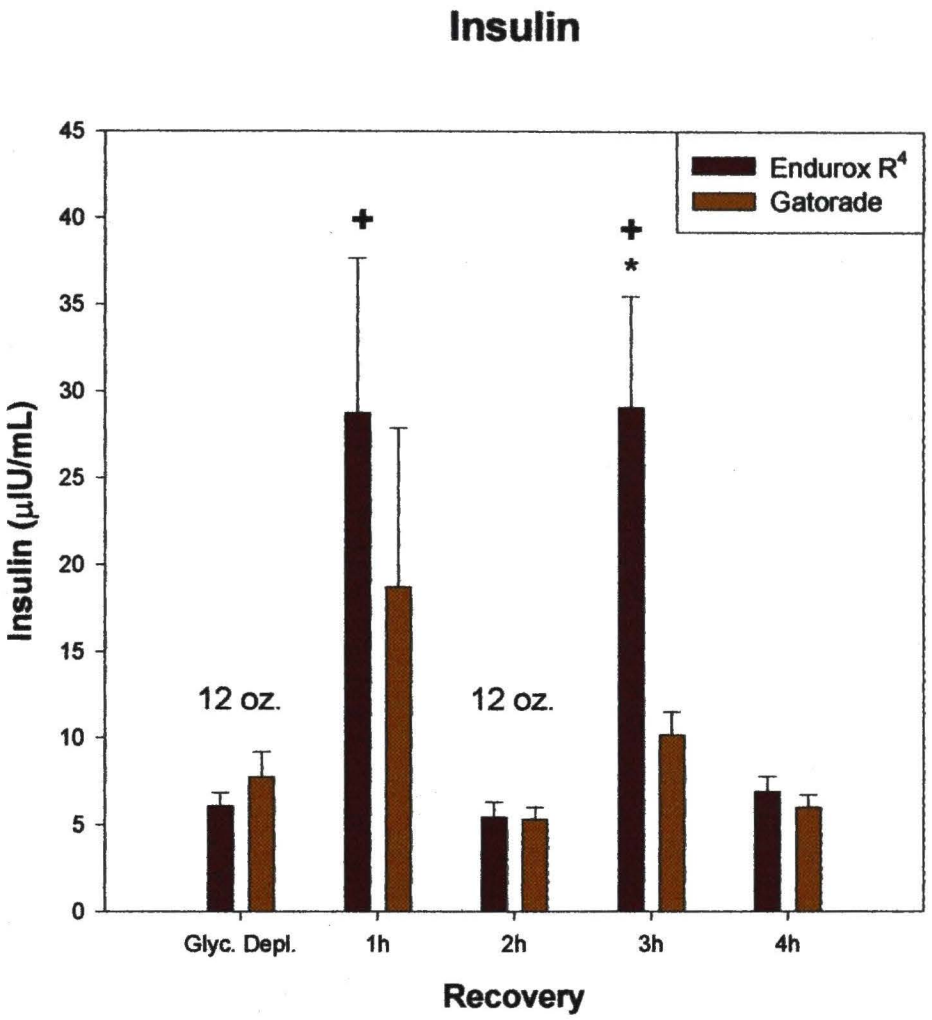

Figure 16. Plasma Insulin During Recovery. Plasma insulin concentrations were significantly increased one- and three-hours after drink ingestion. Endurox R^4® ingestion produced a 2.86 fold increase in plasma insulin concentrations compared to Gatorade® at three-hours into the recovery. (*) denotes significant difference between drinks. (+) denotes significant difference from post-glycogen depletion.

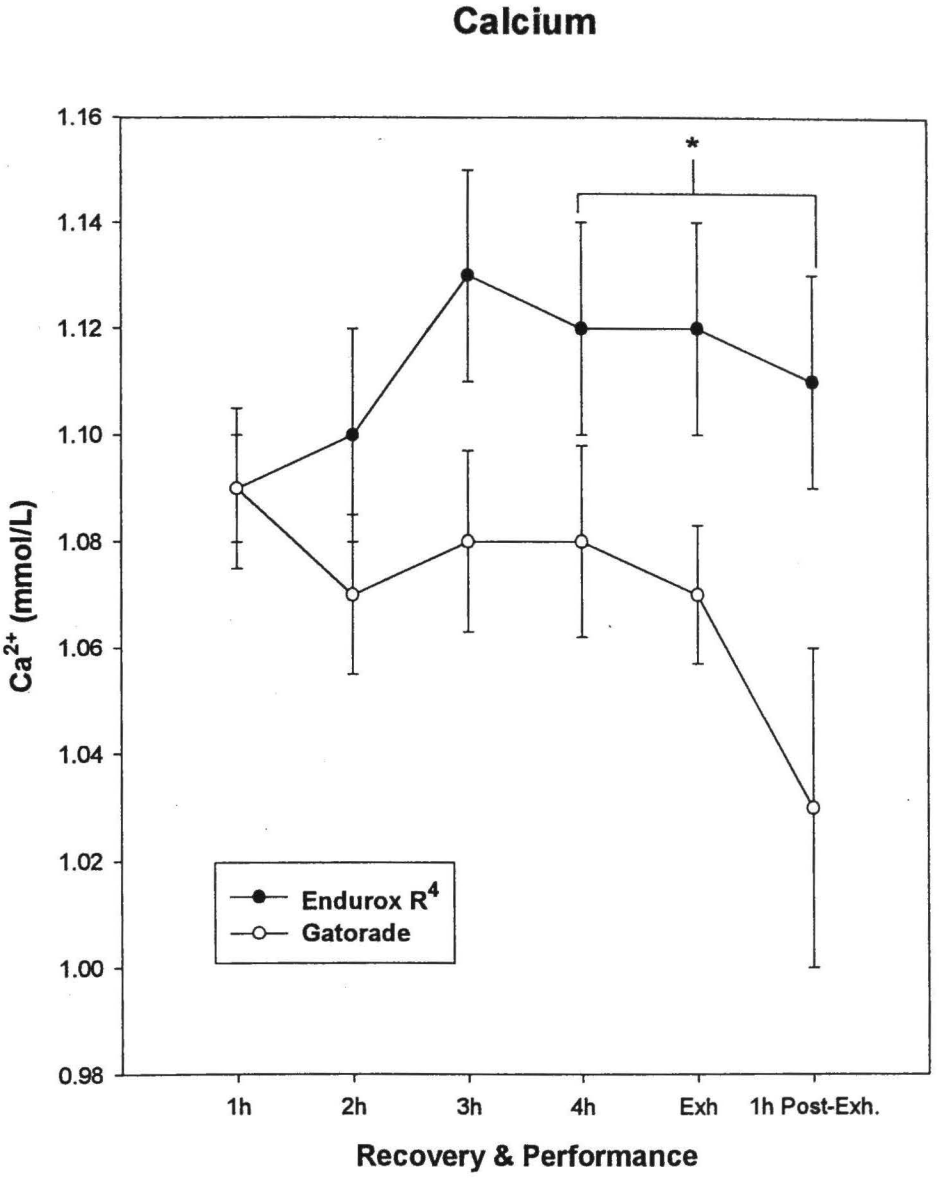

Figure 17. Drink Effects on Calcium. Drink was administered immediately post-glycogen-depletion and after measurement obtained two-hours into the recovery period. The increased plasma calcium concentration was accounted for by the formula use of calcium-casseinate as the protein base in the Endurox R^4 Recovery Drink®. (*) denotes significance between drinks.

variable from post-glycogen-depletion to post-performance. A statistically significant difference was not found for the triene variable. However, diene, the first product of oxidation, was significantly increased with the use of Gatorade® ($p = 0.006$). The thiobarbituric acid reactive substrate assay (T-BARS), a measure of the final products of oxidation, revealed a statistically significant decrease in free radical formation with the use of the Endurox R^4 Recovery Drink® while Gatorade® measurements remained similar.

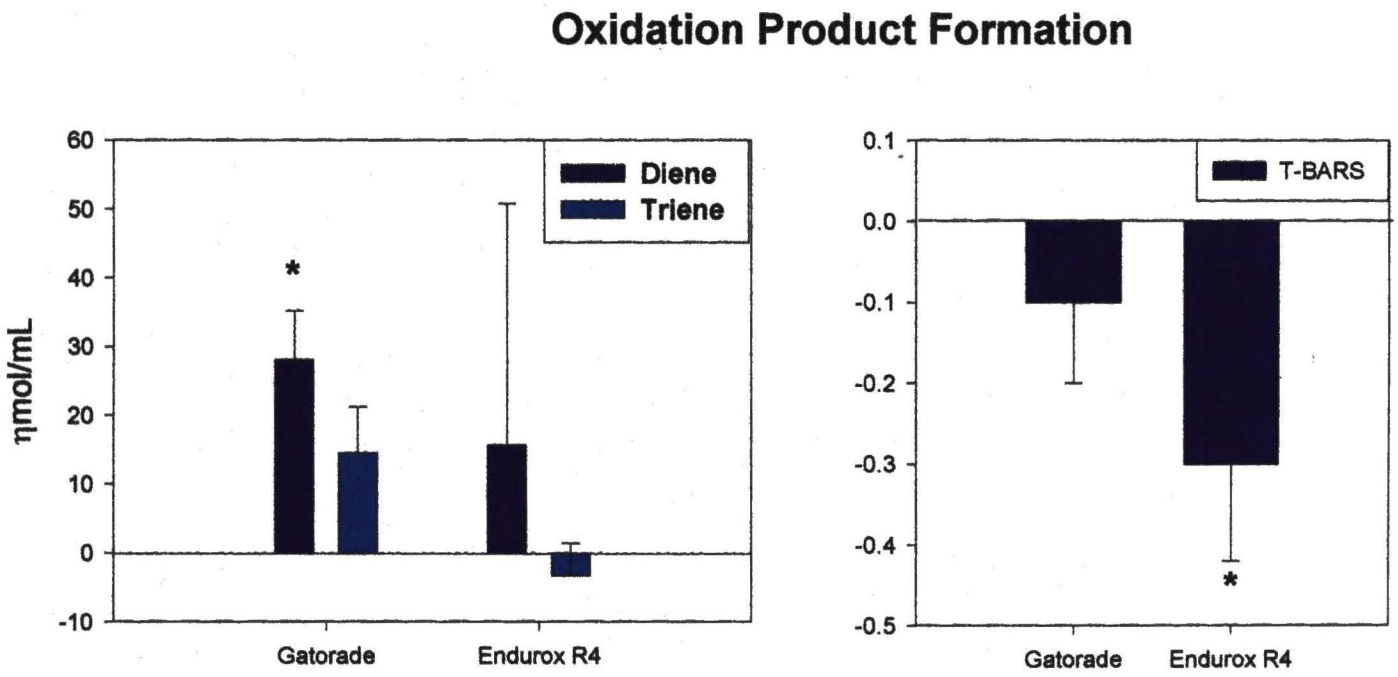

Figure 18. Free Radical Formation Effects with Differing Drinks. When comparing the amount of oxidation products that are formed from the time period immediately after the glycogen-depletion phase to immediately after the performance test, two areas were statistically significant. First, diene formation with the use of Gatorade® is significantly increased and, second, a significant decrease in final oxidation products with the use of Endurox R[4] Recovery Drink®.

CHAPTER V

DISCUSSION & SUMMARY

The purpose of Chapter V was to review the relevant findings elicited from the investigation and to determine their importance in relation to previous research. The major findings in the investigation when comparing the Endurox R^4 Recovery Drink® to Gatorade® were significantly increased performance time, increased insulin response, and decreased final oxidation products with the use of Endurox R^4® as well as equal hydration and electrolyte replenishment for both drinks. Increased serum insulin concentrations with Endurox R^4® were consistent with the first hypothesis presented in Chapter I p. 7, where protein, arginine, and increased CHO found in Endurox R^4® would elicit a significantly greater insulin response. Further, the second hypothesis, presented on p. 7, was demonstrated in that the performance time was significantly increased with Endurox R^4® owing to the increased glycogen store replenishment. Lastly, the reduction in oxidation products with Endurox R^4® ingestion yielded a reduction in markers of free radical damage as well as decreased oxidative stress, which was consistent with the third hypothesis presented on p. 7. The results of the investigation were explained and conclusions drawn based upon these findings along with the extrapolation of results from other relevant studies to this research.

Subject Analysis

Based on the results of the initial graded cycle ergometry test and medical history and diet questionnaire, thirteen subjects met the criteria for inclusion in this study (Table I., p. 23), with five subjects withdrawing from the study at various time points. Since a high physical fitness level was critical to evaluate the research findings, it was essential that all subjects have a $VO_{2max} \geq 60$ ml•kg^{-1}•min^{-1}.

Glycogen-Depleting Exercise

To ensure that both exercise days yielded the same extent of exercise performance during the glycogen-depletion bout, it was essential to ensure that the cardiovascular and metabolic responses were similar. The results indicated that HR, RR, Q_c, VO_2, RQ, and V_e at the completion of the glycogen-depletion exercise bout were all similar for both drink days, indicating the glycogen-depletion exercise resulted in a comparable endpoint. Therefore, measurements determined during recovery and performance phases would not be hindered.

As to the assurance of glycogen-depletion, Coyle *et al.* (14), demonstrated in the rat model that decreases in blood glucose concentrations during exercise were indicative of liver glycogen depletion. Since the current protocol did not utilize muscle biopsies, this conclusion was critical to the evaluation of the glycogen-depletion exercise. Though not significant, blood glucose concentrations during the glycogen-depletion exercise continued to decrease. A possible explanation for the rise in blood glucose concentrations five-

minutes after the test was completed was due to the increased exercise intensity (85% VO_{2max}) during the last minutes of the glycogen-depletion test (12,13).

Recovery

The overall decrease in glycogen stores due to the exhaustive exercise bout, as indicated by the reduction in blood glucose concentration (14), yielded a corresponding increase in the overall percent activity of the glycogen synthase enzyme (6). Ingestion of CHO immediately post-exercise has been demonstrated to produce a glycogenesis rate of 6-7 $\mu mol \bullet (g\ wet\ wt)^{-1} \bullet h^{-1}$, which was maintained for two hours (31). This was attributed to the increased serum insulin concentration resulting from CHO ingestion, which yielded a greater activity of the glycogen synthase enzyme.

As demonstrated by numerous investigators (31,53,6,38,9), the insulin response had a significant role in the promotion of glycogenesis. Since insulin concentrations increase with increases in glucose concentrations, it is important to note that both drinks yielded increased blood glucose concentrations and serum insulin concentrations one-hour and three-hours post glycogen-depletion, or one-hour post-ingestion of twelve-ounces of either drink, with no difference between the one-hour and three-hour values. However, blood glucose concentrations and serum insulin concentrations at one- and three-hour post glycogen-depletion, respectively, increased with the ingestion of Endurox R^4®, but not with Gatorade®.

Several explanations can be made to account for the increase in blood glucose concentrations and serum insulin concentrations and probable increased glycogen replenishment with the ingestion of Endurox R^4®. First, Endurox R^4® had three-times the amount of CHO (63g CHO/24 oz) than Gatorade® (20g CHO/24 oz). Due to the glycogen-depleting exercise, the skeletal muscle was primed for rebuilding its glycogen stores and a critical amount of CHO, 1.0-1.5 g CHO•(kg body weight)$^{-1}$, must be ingested to yield a maximum rate of glycogenesis (31,7). Therefore, the recovery drink that provided substrate closer to the indicated critical amount should replenish glycogen stores more completely. The Endurox R^4 Recovery Drink® provided 1.73 ± 0.035 g CHO•(kg body weight)$^{-1}$ in the immediate minutes following glycogen-depletion whereas Gatorade® provided a significantly decreased amount of 0.58 ± 0.012 g CHO•(kg body weight)$^{-1}$.

Second, Endurox R^4® utilized a combination of CHO and protein in a 3.5:1 ration. Zawadzki *et al.* (54) and Burke *et al.* (11), both demonstrated that the addition of protein to CHO in a 3:1 ratio yielded a significant increase in the insulin response over CHO alone. However, whereas Burke did not find increased glycogenesis, Zawadzki *et al.* (54), demonstrated a significantly faster glycogenesis over a four-hour recovery period with the 3:1 CHO:Protein [7.1 μmol•(g wet wt)$^{-1}$•h^{-1}] than that of CHO alone [5.0 μmol•(g wet wt)$^{-1}$•h^{-1}].

Third, the amino acid arginine has been implicated as a potent substance for inducing increased insulin release (38,31). Further, in experiments where a baseline of

substrate was provided, animals administered CHO alone or arginine alone increased glycogenesis three-to-four fold over non-feeding; where arginine combined with CHO has been demonstrated to yield a five-fold increase in the insulin response (31). Fourteen hundred milligrams of the amino acid arginine are provided in Endurox R^4®. The most probable explanation for the increased serum insulin concentrations yielded with Endurox R^4® was a synergistic effect of all three where the increased amount of CHO played a primary role.

Looking at Figure 15, p. 49, four items need to be discussed. First, Endurox R^4® demonstrated significantly increased blood glucose concentrations in the first hour of recovery as compared to Gatorade®, but resulted in a similar second-hour measurement. Therefore, it can be concluded that a greater amount of glucose was provided for skeletal muscle uptake with utilization of the Endurox R^4 Recovery Drink®. It could then be hypothesized that the additional blood glucose concentrations in hour one was transported into the liver and/or skeletal muscle cell by hour two, yielding a net faster glycogenesis.

Second, though the second twelve-ounce ingestion of Gatorade® and Endurox R^4® resulted in similar increases in blood glucose concentrations at the third-hour, respectively, when compared to the first twelve-ounce dose, the second twelve-ounce dose of Endurox R^4® had a noticeable attenuation in the rise in blood glucose concentrations as compared to the first. The conclusion for this effect was attributed to the first twelve-ounces of Endurox R^4®, which provided for the initial increase in activity of the glycogen synthase enzyme to stimulate glycogenesis. Further, since the glycogen synthase enzyme

was efficiently promoting the conversion of glucose to glycogen, the second twelve-ounce ingestion provided more substrate for an already vigorous process. Therefore, the initial "peak" concentration yielded at one-hour into recovery was apt to occur earlier than the third-hour blood measurement, though the same serum insulin concentrations were noted.

Third, the arginine provided in Endurox R^4® not only may have increased the serum insulin concentrations by stimulating the β-islets cells of the pancreas, but also caused α-islet stimulation releasing glucagon. Glucagon, while adding to the net increase in blood glucose concentrations via gluconeogenesis and providing more substrate for skeletal muscle uptake, resulted in further depletion of liver glycogen stores. This would be especially evident in the second ingestion of Endurox R^4®, when liver glycogen may have been partially restored by previously ingested CHO. Therefore, though the extent of its role in blood glucose increase and depletion of liver glycogen was not known, the primary benefit of arginine in recovery drinks was questioned.

Finally, a limitation to measurement of blood glucose and serum insulin concentrations in the manner described was the length of time between determinations. Since an hour was used to separate measurements, the probability that the actual peak concentration of either glucose or insulin was ascertained was minimal. Though conclusions on the figure were valid, peak time measurements would be indicated in order to determine the overall effectiveness of one drink compared to the other in promoting increased blood glucose and serum insulin concentrations within a desired time window.

Electrolyte and fluid replenishment were important determinants in terms of recovery. Endurox R^4® and Gatorade® were similar in terms of electrolyte and fluid replenishment with no significant differences in percent change in plasma volume or body weight. However, plasma potassium, as demonstrated in Figure 13, p. 47, was notably lower with Endurox R^4® ingestion than with Gatorade®. This was especially discernible three-hours into recovery. A possible explanation for this effect was attributed to the increased serum insulin concentrations, yielded with Endurox R^4®, which have been demonstrated to promote the cellular uptake of potassium.

Performance

Following four-hours of recovery from glycogen-depleting exercise and twenty-four-ounces of either drink, results for the 85% VO_{2max} performance test revealed a fifty-five percent increase in performance time with the use of Endurox R^4® over Gatorade®. As stated in hypothesis *ii.* on p. 7, the increased insulin response (initiated by CHO, protein, and arginine found in Endurox R^4) led to an increased glycogenesis rate as described by the mechanisms in the preceding section. The increased glycogen store replenishment during the four-hour recovery phase was a significant factor contributing to the increased performance time.

Measurement of metabolic rate was estimated by use of indirect calorimetry via RQ. As seen in Figure 7, p. 37, the respiratory quotient obtained during the performance exercise was similar for both drinks. Since the RQ is equal to the molar ratio of the CO_2

production rate to the corresponding O_2 consumption rate, a similar RQ indicates that the ratio of CHO to fat, and, to a lesser degree protein, oxidized for both drinks was similar.

As seen in Figure 18, p. 53, indicators of final free radicals formed with the use of Endurox R^4 Recovery Drink®, as measured by the T-BARS Assay, were significantly decreased over those formed with the use of Gatorade®. As skeletal muscle was worked under aerobic conditions, the mitochondria processed, through oxidation, an increased quantity of free radicals. Further, it has been demonstrated that with endurance training there is a subsequent increased amount of mitochondria within the skeletal muscle yielding additional production of free radicals during exercise (43). Packer *et al.* (43), demonstrated that endurance trained rats on decreased vitamin E supplementation had an endurance capacity fifty-percent lower than that of rats with normal vitamin E supplementation. In terms of performance, therefore, free radicals can limit an athlete's ability to compete. The use of antioxidants (e.g. vitamins E & C) limit the reactivity of the free radicals by reducing them to alcohols.. Additionally, the use of vitamin C has been demonstrated to interact and spare α-tocopherol *in vitro* suggesting that its use might further limit the numbers of free radicals produced from endurance exercise (39,41). Indicators of free radical formation, that are sensitive to antioxidants E & C, were utilized as part of the study protocol where diene concentrations measured the first products of oxidation, triene concentrations measured the second, and the T-BARS Assay measured the final products of oxidation, primarily consisting of malonyldealdehyde and its downstream oxidation products. With this stated, the increased diene formation found

with ingestion of Gatorade® combined with the decreased final oxidation products formed with Endurox R^4® goes to support hypothesis *iii* stated on p. 7, and may further be evidence to support that the decreased free radical generation, found with the use of Endurox R^4®, provided significant benefits to performance.

Limitations to Interpretation of Results

In terms of the performance results, however, two items need to be discussed. First, subjects could not be blinded to the drinks that they were administered due to many factors including:

1. Familiarity with the taste of Gatorade®;
2. The thicker consistency and increased concentration of Endurox R^4®; and,
3. The knowledge, due to informed consent, of the name of the drinks that were to be ingested.

Though it was suggested that both drinks provided performance enhancement, the subjects may have expected a greater benefit with one drink over another.

Second, a specific focus of the investigation was a product comparison of equal volume consumption and the results herein should not be misconstrued as otherwise. For example, in order to delineate whether the consumption of 24-ounces of Endurox R^4®, with its protein, arginine, and CHO ratios, yielded the significant increase in insulin

response to further enhance glycogen stores, it would be necessary to make the drinks isocaloric, requiring 72-ounces of Gatorade®! As indicated, the exercise paradigm that was tested was comparing two drinks inherently different in their formulas. To change the formula by dilution, with Endurox R^4®, or concentration, with Gatorade®, to make the comparison isocaloric would be testing the product under conditions in a way for which it was not designed. Hence, the results would not provide the valuable information needed by the consumer, who would utilize the product as suggested by the manufacturer. Therefore, performance results should be considered within the entire context of equal volume consumption and with this additional information.

In terms of cost difference, Gatorade® ($4 / container) was lower than Endurox R^4® ($24 / container). However, when the cost per gram of CHO was accounted, Gatorade®, at 90 cents per gram CHO, was higher in cost to the consumer than Endurox R^4®, at 62 cents per gram CHO, while additionally providing additional drink components (i.e. protein, arginine, vitamins E & C) to elicit a faster recovery from exhaustive exercise.

Summary

The specific purpose of formulating a recovery drink is to enhance the subject's ability to recover more completely in a shorter period of time (i.e. less than four-hours) from an exhaustive exercise. Recovery, as defined by Burke (10), contains three specific aims involving the restoration of liver and skeletal muscle glycogen stores, fluid and electrolyte replenishment, and regeneration, repair and adaptation to catabolic stresses that

induce damage during the exercise. Several mechanisms can be utilized to enhance recovery including ingestion of CHO, protein, and arginine which result in stimulation of the insulin response thereby promoting liver and skeletal muscle glycogenesis and increasing muscle amino acid uptake; ingestion of specific concentrations of electrolytes that restore the balance lost during exercise; and, ingestion of antioxidants that help to limit or negate oxidative damage caused by free radicals produced during a strenuous exercise.

The combination of the increased insulin response (yielded protein, arginine, and an increased quantity of CHO) to promote faster glycogenesis, and the use of antioxidants E & C to decrease free radical formation give the Endurox R^4 Recovery Drink® a significant advantage when compared to an equal volume consumption of Gatorade®, a conventional sports rehydration drink, without losing the benefits of hydration and electrolyte replenishment.

REFERENCES

1. Aikawa, K. M., A. T. Quintanilha, B. O. de Lumen, G. A. Brooks, and L. Packer. Exercise endurance-training alters vitamin E tissue levels and red- blood-cell hemolysis in rodents. *Biosci Rep* 4: 253-7, 1984.

2. Banister, E. W., and J. Griffiths. Blood levels of adrenergic amines during exercise. *J Appl Physiol* 33: 674-6, 1972.

3. Bergstrom, J., G. Guarnieri, and E. Hultman. Carbohydrate metabolism and electrolyte changes in human muscle tissue during heavy work. *J Appl Physiol* 30: 122-5, 1971.

4. Bergstrom, J., L. Hermansen, E. Hultman, and B. Saltin. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140-50, 1967.

5. Bergstrom, J., and E. Hultman. A study of the glycogen metabolism during exercise in man. *Scand J Clin Lab Invest* 19: 218-28, 1967.

6. Bergstrom, J., E. Hultman, and A. E. Roch-Norlund. Muscle glycogen synthetase in normal subjects. Basal values, effect of glycogen depletion by exercise and of a carbohydrate-rich diet following exercise. *Scand J Clin Lab Invest* 29: 231-6, 1972.

7. Blom, P. C., A. T. Hostmark, O. Vaage, K. R. Kardel, and S. Maehlum. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med Sci Sports Exerc* 19: 491-6, 1987.

8. Borg, G. A. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377-81, 1982.

9. Bratusch-Marrain, P., O. Bjorkman, L. Hagenfeldt, W. Waldhausl, and J. Wahren. Influence of arginine on splanchnic glucose metabolism in man. *Diabetes* 28: 126-31, 1979.

10. Burke, L. M. Nutrition for post-exercise recovery. *Aust J Sci Med Sport* 29: 3-10, 1997.

11. Burke, L. M., G. R. Collier, S. K. Beasley, P. G. Davis, P. A. Fricker, P. Heeley, K. Walder, and M. Hargreaves. Effect of coingestion of fat and protein with carbohydrate feedings on muscle glycogen storage. *J Appl Physiol* 78: 2187-92, 1995.

12. Coggan, A. R., and E. F. Coyle. Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exerc Sport Sci Rev* 19: 1-40, 1991.

13. Coggan, A. R., and E. F. Coyle. Effect of carbohydrate feedings during high-intensity exercise. *J Appl Physiol* 65: 1703-9, 1988.

14. Coyle, E. F., A. R. Coggan, M. K. Hemmert, and J. L. Ivy. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61: 165-72, 1986.

15. Danforth, W. H., and P. Harvey. Glycogen synthetase and control of glycogen synthesis in muscle. *Biochem Biophys Res Commun* 16: 466-71, 1964.

16. Davies, K. J., L. Packer, and G. A. Brooks. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch Biochem Biophys* 209: 539-54, 1981.

17. Day, J. L., K. Johansen, O. P. Ganda, J. S. Soeldner, R. E. Gleason, and W. Midgley. Factors governing insulin and glucagon responses during normal meals. *Clin Endocrinol (Oxf)* 9: 443-54, 1978.

18. Drummond, G. I., J. P. Harwood, and C. A. Powell. Studies on the activation of phosphorylase in skeletal muscle by contraction and by epinephrine. *J Biol Chem* 244: 4235-40, 1969.

19. Fajans, S. S., J. C. Floyd, Jr., R. F. Knopf, and F. W. Conn. Effect of amino acids and proteins on insulin secretion in man. *Recent Prog Horm Res* 23: 617-62, 1967.

20. Fell, R. D., S. E. Terblanche, J. L. Ivy, J. C. Young, and J. O. Holloszy. Effect of muscle glycogen content on glucose uptake following exercise. *J Appl Physiol* 52: 434-7, 1982.

21. Floyd, J. C., Jr., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. Insulin secretion in response to protein ingestion. *J Clin Invest* 45: 1479-86, 1966.

22. Floyd, J. C., Jr., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. Stimulation of insulin secretion by amino acids. *J Clin Invest* 45: 1487-502, 1966.

23. Floyd, J. C., Jr., S. S. Fajans, S. Pek, R. F. Knopf, P. Weissman, and J. W. Conn. Adrenergic effects which modify arginine-induced insulin release in man. *J Lab Clin Med* 78: 990-1, 1971.

24. Goldberg, N. D., J. Larner, H. Sasko, and A. G. O'Toole. Enzymic analysis of cyclic 3', 5'-AMP in mammalian tissues and urine. *Anal Biochem* 28: 523-44, 1969.

25. Hansen, P. A., L. A. Nolte, M. M. Chen, and J. O. Holloszy. Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J Appl Physiol* 85: 1218-22, 1998.

26. Hargreaves, M., and C. A. Briggs. Effect of carbohydrate ingestion on exercise metabolism. *J Appl Physiol* 65: 1553-5, 1988.

27. Haugaard, N., R. A. Mickel, and E. S. Haugaard. Studies of the actions of thiols, lithium ions and insulin on carbohydrate metabolism of the isolated rat diaphragm. *Acta Endocrinol Suppl* 191: 145-52, 1974.

28. Hawley, J. A., S. C. Dennis, and T. D. Noakes. Oxidation of carbohydrate ingested during prolonged endurance exercise. *Sports Med* 14: 27-42, 1992.

29. Holloszy, J. O., and H. T. Narahara. Studies of tissue permeability. X. Changes in permeability to 3- methylglucose associated with contraction of isolated frog muscle. *J Biol Chem* 240: 3493-500, 1965.

30. Iakovlev, N. N. [The influence of regular muscle activity on the activity of the enzymes operating the conversion of glycogen and glucose-6-phosphate in muscles and liver]. *Biokhimiia* 33: 737-44, 1968.

31. Ivy, J. L. Glycogen resynthesis after exercise: effect of carbohydrate intake. *Int J Sports Med* 19 Suppl 2: S142-5, 1998.

32. Ivy, J. L. The insulin-like effect of muscle contraction. *Exerc Sport Sci Rev* 15: 29-51, 1987.

33. Ivy, J. L. Muscle glycogen synthesis before and after exercise. *Sports Med* 11: 6-19, 1991.

34. Ivy, J. L., and J. O. Holloszy. Persistent increase in glucose uptake by rat skeletal muscle following exercise. *Am J Physiol* 241: C200-3, 1981.

35. Kipnis, D., and N. MW. Stimulation of amino acid transport by insulin in the isolated rat diaphragm. *Biochim. Biophys. Acta* 28: 226-227, 1958.

36. Kochan, R. G., D. R. Lamb, S. A. Lutz, C. V. Perrill, E. M. Reimann, and K. K. Schlender. Glycogen synthase activation in human skeletal muscle: effects of diet and exercise. *Am J Physiol* 236: E660-6, 1979.

37. Malshet, V. G., A. L. Tappel, and V. M. Burns. Fluorescent products of lipid peroxidation. II. Methods for analysis and characterization. *Lipids* 9: 328-32, 1974.

38. Merimee, T. J., D. A. Lillicrap, and D. Rabinowitz. Effect of arginine on serum-levels of human growth-hormone. *Lancet* 2: 668-70, 1965.

39. Muller, W. A., G. R. Faloona, E. Aguilar-Parada, and R. H. Unger. Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. *N Engl J Med* 283: 109-15, 1970.

40. Niki, E., T. Saito, A. Kawakami, and Y. Kamiya. Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. *J Biol Chem* 259: 4177-82, 1984.

41. Nuttall, F. Q., A. D. Mooradian, M. C. Gannon, C. Billington, and P. Krezowski. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care* 7: 465-70, 1984.

42. Packer, J. E., T. F. Slater, and R. L. Willson. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 278: 737-8, 1979.

43. Packer, L. Vitamin E, physical exercise and tissue damage in animals. *Med Biol* 62: 105-9, 1984.

44. Packer, L., A. L. Almada, L. M. Rothfuss, and D. S. Wilson. Modulation of tissue vitamin E levels by physical exercise. *Ann N Y Acad Sci* 570: 311-21, 1989.

45. Pryor, W. A., J. P. Stanley, and E. Blair. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. *Lipids* 11: 370-9, 1976.

46. Rabinowitz, D., T. J. Merimee, R. Maffezzoli, and J. A. Burgess. Patterns of hormonal release after glucose, protein, and glucose plus protein. *Lancet* 2: 454-6, 1966.

47. Richter, E. A., L. P. Garetto, M. N. Goodman, and N. B. Ruderman. Enhanced muscle glucose metabolism after exercise: modulation by local factors. *Am J Physiol* 246: E476-82, 1984.

48. Richter, E. A., L. P. Garetto, M. N. Goodman, and N. B. Ruderman. Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. *J Clin Invest* 69: 785-93, 1982.

49. Scharff, R., and I. G. Wool. Effect of diabetes on the concentration of amino acids in plasma and heart muscle of rats. *Biochem J* 99: 173-8, 1966.

50. Soderling, T. R., J. P. Hickenbottom, E. M. Reimann, F. L. Hunkeler, D. A. Walsh, and E. G. Krebs. Inactivation of glycogen synthetase and activation of phosphorylase kinase by muscle adenosine 3',5'-monophosphate-dependent protein kinases. *J Biol Chem* 245: 6317-28, 1970.

51. Spiller, G. A., C. D. Jensen, T. S. Pattison, C. S. Chuck, J. H. Whittam, and J. Scala. Effect of protein dose on serum glucose and insulin response to sugars. *Am J Clin Nutr* 46: 474-80, 1987.

52. Triebwasser, J. H., R. L. Johnson, R. P. Burpo, J. C. Campbell, W. C. Reardon, and C. G. Blomqvist. Noninvasive determination of cardiac output by a modified acetylene rebreathing procedure utilizing mass spectrometer measurements. *Aviat Space Environ Med* 48: 203-9, 1977.

53. Wool, I. G. Relation of effects of insulin on amino acid transport and on protein synthesis. *Fed Proc* 24: 1060-70, 1965.

54. Zawadzki, K. M., B. B. d. Yaspelkis, and J. L. Ivy. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J Appl Physiol* 72: 1854-9, 1992.