2009

The Effect of a Commercially Available Caffeine Based Dietary Supplement on 40-Kilometer Time Trial Performance

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THE EFFECT OF A COMMERCIALLY AVAILABLE CAFFEINE BASED DIETARY SUPPLEMENT ON 40-KILOMETER TIME TRIAL PERFORMANCE.

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A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Health, Physical Education and Recreation

South Dakota State University

2009

The effect of a commercially available caffeine based dietary supplement on 40 kilometer time trial performance.

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department

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Acknowledgements

I would like to thank my family for their encouragement and belief in the goals I am perusing. I would especially like to thank my wife for her patience and unending support through my long hours of writing and researching. Without these important people it would have been difficult to accomplish the goals I have set for myself. I would also like to thank Dr. Matthew Vukovich for the inspiration he has given me to never stop seeking knowledge. His unwavering willingness to help me with all aspects of my project along with his genuine interest in my progress as a young professional has been remarkable. Finally, I would like to thank God for giving me the tools I need and the great people I have around me which together have all contributed to my progress.

Abstract

The effect of a commercially available caffeine based dietary supplement on 40 kilometer time trial performance.

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April 8, 2009

Purpose: To determine whether or not the nutritional supplement PreRace containing L-Taurine, Citrulline Malate, Quercetin, DiMethyl Amino Ethanol, Caffeine, Metabromine, Catechin, and Malic acid will improve performance in an indoor 40 kilometer cycling time trial.

Methods: Seven male cyclists ages 21-58 participated in a placebo-controlled investigation which consisted of two trials in which they consumed either the supplement PreRace or aloe vera juice. In each of the trials the placebo or supplement was mixed with a sports beverage. Subjects performed a 40 kilometer time trial and measurements of oxygen consumption and lactate were taken every 15 minutes as well as 3 kilometers from the end.

Results: Treatment effects were observed for average work, average watts, average velocity, oxygen consumption, heart rate, RPE, lactate, percentage of lactate threshold watts, and percentage of VO_2max. No significant difference was observed in time to complete trials even though the experimental group finished an average of 3 minutes and 17 seconds faster.

Conclusion: Ingestion of the supplement in this study results in effects that may result in an improvement in performance. A larger study is needed to determine if these results are reproducible to a statistically significant level

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Abbreviations

ANOVA	analysis of Variance
BPM	beats per minute
DCER	dynamic constant external resistance
Exp	experimental
FFA	free fatty acids
g	gram
h	hour
HR	heart rate
in	inch
kg	kilogram
km	kilometer
L	liter
lb	pound
mg	milligram
min	minute
ml	milliliter
mMol	millimol
MRS	magnetic resonance spectroscopy
NOS	nitrous oxide synthase
Pla	placebo
RER	respiratory exchange ratio

RPE	rating of perceived exertion
W	watts
VO_2	volume of oxygen
VO_2max	maximal oxygen consumption

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Chapter 1

Review of Literature

Ergogenic aids are defined as any substance that is ingested with the intention of improving performance. Many ergogenic aids are currently available and are relatively easy to attain. Conflict exists as to whether or not such aids should be legal and if they are effective. This review of literature will discuss the ingredients in a commercially available ergogenic aid which was tested in the current study.

1.1 Caffeine and its Uses

Caffeine is a methylxanthine that is contained in many beverages, foods, medications, and supplements (1,6). It is the most widely used drug in North America, and has many uses in both daily life and exercise. Many individuals utilize caffeine as a method of staying awake at work or while driving. Athletes have been using caffeine for its ergogenic effect even though some of the specifics of its benefits are still unclear. The purpose of this portion of this literature review is to examine the effects of caffeine on the body and further explain its effect on exercise performance.

A study by Costill (18) examined the effects of caffeine ingestion (330 mg) on metabolism and performance in competitive cyclists during a ride to exhaustion at 80% VO2max. Time to exhaustion was increased by 19.5% in the caffeine group. Improved endurance in this type of workload is important in

events such as a time trial where individuals must work at a high intensity for an extended period of time.

This ergogenic effect was later confirmed by Graham (29) who reported that endurance could be significantly increased with caffeine ingestion. Graham et al. (1991) studied the ergogenic effect of caffeine in male competitive runners following the consumption of 9 mg/kg of caffeine prior to exercise to exhaustion at 85% VO_2max. Time to exhaustion was increased by 22 minutes in runners and 20 minutes in cyclists following caffeine ingestion. (29) The dose in this study was almost double the dose given in the Costill (18) study, but the improvement in performance was similar which raises the question if there is an optimal dose for caffeine.

In a similar study, Graham (30) investigated the effect caffeine ingestion (4.45 mg/kg) by either capsule or coffee on a treadmill test to exhaustion at 85% VO2max. Time to exhaustion was increased by 7.5-10 minutes when caffeine was ingested through a capsule as opposed to coffee. This result is slightly surprising since the amount of caffeine ingested for each trial was equal. The results of the study imply that something in the coffee interferes with the action of the caffeine and results in a decrease in the effectiveness of the dose. (30) Caffeine ingested in the form of capsules results in a time to exhaustion increase which clarifies the results of the prior study by Graham. (29, 30)

McNaughton (46) investigated the effect of a 6 mg/kg dose of caffeine on 1 hour cycling time trial performance in well trained subjects. Subjects rode over

1.5 km farther following the ingestion. McNaughton (47) performed a follow up study to investigate the possible mechanisms behind the improvement in performance. It was reported that free fatty acid levels (FFA) in the blood were significantly higher in the caffeine trials which indicates that a glycogen sparing effect may be responsible at least in part for the improvement in performance.

French et al. (28) also investigated the effect of a high dose of caffeine (10mg/kg) on exercise to exhaustion in trained runners. Subjects completed a 45 minute run at 75% VO_2max which was followed by an increase of 2 mph per hour until exhaustion. Caffeine ingestion resulted in a significant improvement in time to exhaustion. One difference in this study was that caffeine was taken immediately prior to the onset of exercise. (28) Typically caffeine is ingested an hour prior to exercise, but the results of this study raise the question if this is necessary or not.

Cox et al. (19) investigated different types of caffeine ingestion prior to and during a 7 kJ/kg time trial to exhaustion. In the caffeine trial a 6mg/kg dose of caffeine was administered 1 hour prior to the exercise. In one of the trials subjects were given Coca-Cola during the late stages of their time trials. Cox reported that performance was improved (3.1-3.4%) following caffeine ingestion at a dose of 1.5mg/kg. Based on these results caffeine may improve performance even at low doses with similar results occurring from high doses.

Many studies investigating caffeine deal with a high dose of caffeine. Jenkins (38) investigated the effectiveness of varying doses (1mg/kg, 2mg/kg,

3mg/kg) on cycling performance consisting of a 15 minute ride at 80% VO_2max followed by a 15 minute performance ride. Performance was improved by 4% in the trials using 2mg/kg and 3mg/kg doses. The results of this study suggest that an ergogenic benefit may exist even at low doses.

Falk (26) investigated the effect of caffeine ingestion on endurance exercise performance. The exercise protocol consisted of 5mg/kg caffeine ingestion prior to the beginning of a 50km march in which 2.5mg/kg of caffeine was administered at hours 3 and 5 of the march. Following the completion of the march a cycling test to exhaustion was performed at 90% VO_2max. No significant differences were reported for time to exhaustion while there was a significant increase in blood lactate accumulation in the caffeine group.

1.2 Improvement of Strength, Power and Endurance

Many mechanisms exist for improvements of strength and power as a result of caffeine ingestion. Some have been studied, and some probably have yet to be discovered. This section of the literature review will focus on two of the mechanisms which have been studied quite extensively. Several mechanisms have also been suggested for the improvement of endurance performance. In the next section, we will review increased epinephrine levels, increased plasma free fatty acid levels, and increased oxidation of fat. All of these mechanisms could possibly result in an improvement in endurance performance. Each of these mechanisms acts either on the cardio-respiratory system or on the energy systems that provide energy during long duration endurance activities. Some of

the same mechanisms that exist for an improvement of endurance performance are also areas in which strength and power are improved.

1.2.1 Central Nervous System Stimulation

Caffeine is also a known central nervous system stimulant. (55) Over stimulation of the central nervous system can cause tremors, difficulty sleeping, increased urinary output, and anxiety. Caffeine's properties as a stimulant are also a main reason why athletes choose to use caffeine before competition even if they do not understand the mechanisms of their improved performance. As mentioned above, caffeine is also used every day by many people to stay alert and awake on the road and at work. Caffeine's role as a central nervous system stimulant is also responsible for this application.

Epinephrine levels are closely related to endurance performance. Various studies exist which show an increase in epinephrine levels as a response to caffeine ingestion. Jackman et al. (36) investigated the effect of caffeine ingestion (6mg/kg) on catecholamine response to exercise to exhaustion at VO_2max. The results of this study show that plasma epinephrine levels are significantly increased (0.5-3.5nM) during exercise as a result of caffeine ingestion when compared to a placebo. This appears based on the results of the time to exhaustion testing to have an effect on endurance during this sprint type of activities.

Arciero et al. (3) investigated catecholamine response as a result of caffeine ingestion (5mg/kg) in young and old men. To begin the experiment

blood samples were taken and assessed for baseline values. The caffeine was administered and over the course of the next 180 minutes different blood samples were taken. The results of this study were concurrent with those of the Graham (29) study which was discussed in detail earlier in which norepinephrine was not affected by caffeine ingestion. (3, 29)

In the study by Graham (29) which is described in detail in section 1.3.1 metabolic responses to caffeine ingestion are investigated along with the prior discussed performance effects. The results of this study show that plasma epinephrine levels rose significantly as a results of caffeine ingestion. The levels rose both at rest and during exercise with the difference almost double. (29) That large of a difference would almost definitely improve performance in endurance and power activities.

Laurent (43) investigated the effect of 6mg/kg of caffeine on muscle glycogen utilization as well as the nueroendocrine axis during 2 hours of cycling at 65% VO_2max. No muscle glycogen sparing effect was observed following caffeine ingestion, but cortisol was increased by 10mg/mL, epinephrine was increased by167 pg/mL, and β-endorphins were increased by 23pg/mL. This data suggests that another mechanism may exist to account for the improvement in performance as a result of caffeine ingestion.

1.2.2 Increased Fat Utilization

Engels and Haymes (24) investigated the effect of caffeine (5mg/kg) on fat oxidation during exercise at 30% and 50% VO_2max. The

experiment was broken down into 4 trials: Caffeine trials at 30 and 50% VO2max and placebo trials at the same intensity. Engels and Haymes reported that caffeine caused an increase in serum glycerol which has been considered a good marker of fat oxidation. Respiratory exchange data contradicted this idea of increased fat oxidation as they were not significantly different in any of the trials. The results of this study show that fat oxidation may not increase as a result of caffeine ingestion during this type of exercise. (24)

The Arciero (3) study described in section 1.5.2 also measured free fatty acid concentrations. It was reported that both groups experienced a significant increase in free fatty acid concentrations (441 μmol/L) as a result of caffeine ingestion. There was some variation between the young and the old groups in the appearance of FFA. Younger men had significantly higher FFA levels.

Spriet (53) examined the effects of 9mg/kg dose of caffeine on performance and metabolism at the muscle during cycling exercise. Glycogenolysis was significantly decreased by 55% during the first 15 minutes which indicated that glycogen sparing may have occurred. Time to exhaustion was increased by 20.4 minutes in the caffeine group. Based on the muscle glycogen sparing it appears as glycogen sparing may have an ergogenic effect during exercise at 80% of VO_2max.

Chesley (14) investigated the effect of 9mg/kg caffeine ingestion on muscle glycogen utilization during 15 minutes of cycling at 80% VO_2max in

untrained cyclists. Results varied in that glycogen was spared by 28% in half of the subjects while in the others it was unaffected.

Costill (18) reported that while FFA levels were not significantly different, other factors existed which pointed to an increase in fat oxidation in the caffeine group. Glycerol levels were significantly higher in the caffeine group as well as the respiratory exchange ratio pointed to a significant increase in fat oxidation.

Ivy (35) investigated the effect of 250mg of caffeine fed 60 minutes prior to as well as during 15 minute intervals during the first 90 minutes of a 2 hour cycling bout. Fat oxidation was increased by 31% in the final 70 minutes of the exercise indicating a shift in substrate utilization which would favor glycogen sparing.

A meta-analysis by Doherty (23) investigated past research to determine the most critical factors related to an improvement in performance following caffeine ingestion. Doherty reported that it appears as though a shift to a greater reliance on fat as an energy source is probably the most important factor in performance improvement following caffeine ingestion.

1.2.3 Endurance Effects of Caffeine Ingestion

Caffeine acts on the body in a way that makes the body more efficient during endurance exercise, primarily due to an increase in lipid mobilization and oxidation, and decreased rate of perceived exertion. These effects will be discussed in detail in the upcoming sections.

1.2.4 Decreased Rate of Perceived Exertion

Rating of perceived exertion (RPE) is a subjective measure of how hard an individual feels they are working during an exercise bout. The most commonly used measure for this is the Borg scale (13). Perceived exertion can have a positive or negative effect on exercise depending on if the effort is perceived as difficult or not difficult. Individuals who believe they are working hard will be more likely to succumb to fatigue than individuals who do not think they are exercising hard.

Doherty (22) investigated the effect of 5mg/kg of caffeine on perceived exertion in trained cyclists during repeated high intensity cycling bouts. RPE was reported to be lower in caffeine trials than the non-caffeine trials. Many studies have been done to investigate caffeine and endurance exercise, but this study was rather unique in that it studied individuals during a short duration high intensity work bout.

Cole (15) investigated rate of perceived exertion in 10 healthy males during progressive cycling exercise following 6mg/kg caffeine ingestion. During the 30 minute trials, the subjects pedaled at 80 rpm at a work rate that they considered to be a 9 for the first 10 minutes, 12 for the second 10 minutes and a 15 for the last 10 minutes. Total work production for each stage was recorded. Cole reported that work production was significantly increased (31.1 kJ) for each of the given exertion levels. (15) This goes to show that the amount of work that is done is related to how hard individuals feel that they are working. It is

important to remember that the effect of this is variable from person to person and may be related to the regularity in which each individual consumes caffeine in their daily life.

1.2.5 Strength and Power Effects

Caffeine's role as a stimulant has led many exercise scientists to study its effects on short duration high intensity activities. Several different effects have been reported to be caused by caffeine ingestion. For this section we will cover improved power output, increased upper body strength, and improved sprint performance.

Power output is a major predictor of performance in many events both short and long duration. Power output is also commonly used measure of intensity in training because it is not affected by all of the variables that heart rate training and many other training variables are effected by. Simply put, if an individual can generate more power, they will be faster and their times in various events will improve.

A study by Jacobsen et al. (37) examined the effect of caffeine ingestion on power output in the knee flexors and extensors following 7mg/kg caffeine ingestion. Jacobsen reported that power output can be improved through caffeine ingestion in highly resistance trained athletes. The results of this study are interesting because both 1 rep maximum strength and muscular endurance were both improved. It is important to keep in mind that high doses of caffeine

may come with side effects which may negatively affect performance as much as they improve it.

As stated in the introduction to this section, cyclists often use power as a measure of training intensity. Furthermore, cyclists know that if they improve their power they will improve their performance. Wiles (58) investigated the effect of 5mg/kg caffeine ingestion on 1km time trial performance. A significant improvement in both mean power and peak power were reported as a result of caffeine ingestion. This improvement in power resulted in a 3% improvement in performance time which clarifies the aforementioned concept that improved power results in improved performance time in a 1km time trial.

A study by Doherty (22) also examined caffeine's role in increasing power output in a study which utilized a ramp exercise protocol as the experimental protocol. Subjects in this study ingested 5mg/kg of caffeine 1 hour prior to the graded exercise test. The results were consistent with the above studies as an improvement in power output was observed in the last minute of each stage in the caffeine group.

1.2.6 Caffeine's Effect on Strength

The bench press is a commonly used test to indicate improvements in weight training. Beck (10) studied the effects of 201 mg of caffeine on Wingate tests, 1 repetition max (1RM) and muscular endurance. The supplemental group consumed approximately 201mg of caffeine. (10) The results of this study vary based on body region. One-repetition maximum for the bench press was

significantly increased in the supplement group, but was unchanged in the placebo group. Also none of the other tests that were performed yielded any significant findings. Based on this, it appears as though caffeine may help individuals competing in upper body competitions, but may have no effect on lower body competitions. (10) Beck's findings contradict the findings of Wiles' study which reported an improvement in lower body performance with the consumption of caffeine. (58) One explanation for the lack of effect in this study could be the relatively low dose of caffeine that was administered. Most studies use between 3-8mg/kg. Based on subject characteristics and the information on the supplement that was provided, this study only utilized approximately 2.4mg/kg.

Astorino et al. (5) examined the effects of caffeine ingestion of 6mg/kg on 1 repetition max bench press and leg press. Subjects completed a 1 RM followed by repetitions to failure at 60% 1 RM. No significant difference in strength was observed with the placebo or the caffeine. However, muscular endurance was increased significantly following caffeine ingestion. The results of this study contradict both the Wiles (58) study and the Beck (10) study in which a strength increase was reported. Improvements in muscular endurance are not surprising since this is an effect commonly observed in endurance exercise (18,19, 28, 29, 30).

1.3 Negative Effects of Caffeine

Caffeine has several known side-effects, some of which have greater potential effects on exercise performance than others. Increased urine production as a result of caffeine ingestion is a negative because it could lead to possible electrolyte imbalance as well as dehydration. Another negative effect of caffeine that could have an adverse effect on exercise performance is the sleep difficulties that have been reported with caffeine ingestion. It is known and accepted that well rested individuals perform at a higher level of their own maximal potential than individuals who are sleep deprived.

Diuretics are drugs that increase urine output, but urine output is decreased during exercise due to decreased blood flow to the kidneys. Sweat rate is the factor most responsible for fluid loss during exercise. A decrease in fluid can results in a drop in performance due to a decrease in oxygen delivery due to a decrease in plasma volume. Caffeine is considered a diuretic and therefore its use has been questioned for endurance athletes.

Wemple (57) investigated the diuretic effect of caffeine ingestion in healthy males and females. Four days prior to each trial the subjects did not consume any caffeine. At rest, the caffeine group excreted significantly more urine than the control group. In contrast, during moderate endurance exercise the subject's urine output was not significantly different between the two trials. This suggests that the effects of exercise on urine output are enough to neutralize the effects of the caffeine as a diuretic. The results are important because they clarify that one

of the main side effects that was thought of as a possible negative for exercise was actually not a negative side effect during exercise.

Van der Merwe et al. (55) also investigated the effect of 450mg of caffeine on urinary output during endurance exercise. Subjects exercised on a treadmill at 75%VO_2max for roughly 30 minutes during each trial. Urinary output was increased significantly during hours 1-4 in subjects who did not perform any exercise. These findings are concurrent with one of the findings of a prior study in which urine flow was decreased with treadmill exercise. (54) One explanation for this could be due to water loss from sweat during exercise.

A recent review by Armstrong et al. (4) investigated the effects of caffeine ingestion on hydration and electrolyte balance from a number of different studies. They concluded that while a slight increase in urine production may be observed following caffeine ingestion, it is not significant enough to have an effect on endurance exercise.

1.4 Taurine

Taurine is a non-essential amino acid which is found in skeletal muscle. It is not involved in the synthesis of muscle cells, but it does play a vital role in the formation of proteins which are involved in muscle synthesis. Taurine is a common ingredient in many popular energy drinks because of its proposed effect as a central nervous system stimulant. Of note, is the fact that most research on taurine has been done on body builders as opposed to endurance athletes.

1.4.1 Taurine as a Cytoprotective Agent

A study by Dawson (21) utilized rats to test if taurine could serve a protective role in muscle during exercise. Rats were split into two control groups, and one group was given a taurine transport inhibitor. The experimental group exercised a total of 90 minutes utilizing intermittent bouts and was also given a 3 percent solution of taurine. One initial observation that may have been a weakness of the study was the increased energy with which the taurine supplemented rats exercised which may have resulted in an increase in oxidative damage. The results of the study suggest that taurine may protect against oxidative muscle damage.

Protection against muscular injury is crucial in all exercise competition, but it may be even more important in ultra endurance events or stage races. If muscle damage can be decreased during these races spanning a number of days, it can be postulated that performance would be significantly enhanced.

1.4.2 Taurine and Skeletal Muscle

A study by Matsuzaki (45) investigated whether the duration of the exercise bout made a difference on the taurine in the muscle. The study utilized rats exercising for durations of 30, 60, and 100 minutes. Matsuzaki measured taurine levels in the muscle and blood samples to determine if there was any change in taurine levels. It was observed that increasing duration had no effect on the plasma levels of taurine, but increased duration did result in a greater decrease in taurine in the muscle.

Yatabe (60) examined the effects of taurine supplementation on skeletal muscle of rats. The study compared the effect of taurine on skeletal muscle at rest and during exercise. Yatabe reported that while plasma levels of taurine were significantly affected by supplementation, the levels in the muscle were not significantly greater than the rats who received no taurine. It was also reported that rats who consumed taurine had a significantly greater time to exhaustion (25 minutes) than rats who had not received taurine. (60) This study further illustrates the importance of the Matsuzaki (45) study when longer duration exercise is being performed. If an individual can fend off the depletion of taurine in the muscle it appears as though they may be able to maintain their exercise intensity for longer. It is unknown if the same result observed in this study would be observed in humans.

Ward et al. (56) also reported an increase in plasma taurine in a study which examined the effect of different endurance events on taurine. They suggest that taurine levels in the blood with increasing intensity may be a result of loss of taurine from the muscle.

1.4.3 Cardiac Effects of Taurine

Baum (9) compared the effect of a drink containing both taurine and caffeine to a drink that contained only caffeine with no taurine. The study utilized echocardiographic technology to examine cardiac parameters at three points during the incremental exercise to exhaustion. Baum reported that on the trials when the subjects consumed the beverage containing taurine that maximal

exercise capacity was improved while heart rate at submaximal workloads was decreased. This may be best explained by the increase in stroke volume as well as the decrease in fractional shortening time. The literature suggests that the stroke volume is probably most affected by the increase in left ventricular end diastolic volume and the decrease in left ventricular end systolic volume. (9)

Cuisiner et al. (20) examined if a relationship exists between plasma volume control and taurine concentration in the blood during 90 minutes of exercise at 70% max power in a hydrated versus dehydrated state. Plasma osmolality was increased by 4% in the dehydrated condition but not in the hydrated condition, while plasma taurine increased 8% more in the hydrated condition. Results of this study suggest plasma taurine to be related to osmolarity, but does not appear to be the sole factor in plasma volume.

1.4.4 Taurine and Performance

Yatabe (60) reported that time to exhaustion during running was improved by 25 minutes in rats regardless of the lack of increase in muscle taurine levels. The results suggest that one of the other mechanisms of taurine supplementation may be the reason for this increase in performance. Improvement in cardiac efficiency as reported by Baum (9) is one mechanism in which performance could be enhanced. Further research on endurance athletes is needed to determine the exact mechanism for the improvement in performance as a result Taurine supplementation.

Ivy et al. (34) investigated the effect of a beverage containing taurine (2g) and caffeine (160mg) on 40 minute cycling time trial performance. An improvement in performance of 4.7% was reported. No improvement in substrate utilization was reported following ingestion of the energy drink.

1.5 2-dimethylaminoethanol (Deanol)

Prior research has suggested that the administration of Deanol has resulted in an increase in brain acetylcholine (34, 49). Until a study by Zahniser et al. (62) published in 1977 no method for the proper evaluation of whether or not this occurs was available. Analysis showed that acetylcholine in the brain was actually unaffected by the administration of Deanol. This is contrary to the prior research, but it is also the first study to use a direct measure.

1.6 Citrulline Malate

Energy production at the cellular level in muscle is a crucial process during intense exercise. Scientists are constantly searching for a method to enhance this process through the use of ergogenic aids and training techniques. Bendahan (11) investigated the effect of 6g/day citrulline malate on ATP production during finger extension exercise. Subjects reported fatigue based on a visual analog scale and finger muscle ATP production was evaluated using P MRS exploration. A significant increase in oxidative ATP production (34%) was reported while phosphocreatine was also observed to increase at a greater rate during recovery. This data suggests an improvement in the energy production during and after aerobic energy may exist.

Wu (59) investigated the effect of malate supplementation of varying doses (0.12g/kg L-malate, .21g/kg, and .63g/kg) on enzymes related to the malate-aspartate shuttle in male mice. The mice participated in a swim until exhaustion with lead rings around their tail that weighed 5 percent of their body weight. Wu reported that creatine kinase levels in the blood were decreased in the treatment groups. It may be important to determine if the same results are observed in the muscle. Evidence that these levels were down may indicate that L-malate serves some sort of protective mechanism during exhaustive exercise. Another mechanism for the improvement observed in this study could be the increased enzymatic activities associated with the malate-aspartate shuttle in the liver (59). Wu et al. (59) were focusing on the liver; therefore, this study does not support the claims made by the manufacturer.

A study by Hickner et al. (32) tested the effect of L-citrulline on endurance performance. They reported that time to exhaustion was decreased by 7.2 seconds when individuals consumed 3g of citrulline 180 minutes prior to exercise or 3 doses of 3g of citrulline 24 hours prior to the test. Prior research has reported that l-malate may have an ergogenic effect, but the research by Hickner et al. (32) raises the question as to whether citrulline is ergolytic and if it cancels any possible ergogenic benefit of malate.

A study by Koh and Tidball (41) published in 1999 hypothesized that nitric oxide synthase inhibitors would reduce sarcomere addition in the skeletal muscle of rats. The study utilized an immobilization-remobilization protocol to test the

rate of sarcomere redevelopment. Female rats were anaesthetized and fit for casts on their leg that kept them in a plantarflexed position for four weeks. During the remobilization the rats were split up into three different experimental groups. Group 1 was given a non-isoform-specific nitric oxide system (NOS) inhibitor L-nitro-arginine methyl ester in their drinking water. Group 2 was injected with 1-(2-trifluromethyl-phenyl)-imidazole in phosphate-buffered saline. Group 3 was given L-arginine which is a substrate of NOS. Rats in group 1 had a decreased sarcomere development compared to control rats. Rats in group 2 had very similar results to those in group 1. Group 3 rats had similar sarcomere numbers to those of the contralateral side which hadn't been immobilized. (41) This data suggests that nitrous oxide may have a positive effect on the development of sarcomeres. Muscle recovery could be enhanced by this effect which could in turn improve performance.

Balon and Nadler (8) investigated the hypothesis that nitric oxide increases glucose transport in skeletal muscle. Male rats participated in an incremental training program beginning at 15 meters/minute for 15 minutes per day and progressing by 5 minutes and 3 meters/minute per day every 3 workouts until they were able to run 36 meters/minute. At this time sprints were incorporated into the rats training protocol. The rats were then anesthetized and their extensor digitorum longus was stimulated by either electronic stimulation or insulin. This was done after the introduction of a NOS inhibitor as well as sodium nitroprusside.

The results indicate that sodium nitroprusside significantly improved glucose transport in the muscle. This effect is likely due to sodium nitroprusside's role as a nitric oxide donor. A negative finding of this study was that when nitric oxide levels are elevated too high they actually disrupt the metabolic processes of muscle (8). With this information, it is important that any use of nitric oxide for muscular benefit must be dosed properly. Improving glucose transport in the muscle would be extremely beneficial during exercise because of the body's increased need for energy during exercise. The information in this study makes it evident that nitric oxide may be a key in improving glucose transport.

It is important to note that there is no research to directly support the claim that citrulline-malate or taurine have an effect on the nitric oxide system.

1.7 Quercetin

Quercetin is a naturally occurring flavanoid that is found in many fruits, vegetables and teas. A study, by MacRae and Mefferd (44) tested the effect of 6 weeks of quercetin supplementation on 30 kilometer cycling performance. Investigators reported that power output was improved in the subjects who ingested the quercetin for the six weeks. As would be expected, performance on the 30k time trial also improved by 3% with a 2% improvement over the last 5k with the quercetin supplementation.

1.8 Conclusions and Summary

Endurance performance is dependent on many factors and individuals are

continually searching for methods to enhance performance. Dietary supplements and ergogenic aids use is one popular method to enhance performance. The supplement being tested in this study has made an effort at improving upon many of these factors. The neuro-stimulatory effects of caffeine serve many benefits that in theory should improve endurance performance, but no direct research is available to support this claim. Caffeine also improves the efficiency with which critical energy stores are utilized. Taurine has been reported to have a cytoprotective mechanism which may help to delay fatigue through slowing muscle damage. Taurine has also been shown to increase cardiac efficiency which makes it beneficial during exercise. Citrulline malate has been shown to improve oxidative metabolism through improved enzyme activity at the liver but it is unclear if a similar effect will be seen at the muscle. An ergolytic effect has also been observed following the ingestion of citrulline malate in which a decrease in time trial performance was observed. The current study will investigate the effectiveness of many ingredients which have been discussed and it is unclear what the ergogenic potential will be when all of the ingredients are mixed. We hypothesize that based on the information we have that performance will be enhanced by the ingestion of the PreRace, but possibly not to the same extent that is claimed.

Chapter 2

Introduction

Traditional sports drinks provided carbohydrates and electrolytes in an attempt to prepare the individual for competition and to replenish fluids and electrolytes lost through sweat, and to provide carbohydrates for metabolism. A new classification of sports drinks are available which are attempting to enhance fuel metabolism and stimulate the nervous system to enhance sports performance. These new sports drinks include a cocktail of ingredients in addition to carbohydrates. One such drink includes taurine, citrulline-malate, quercetin, caffeine, dimethyl amino ethanol, and catechin. The drink is marketed to ..."increases mental stamina, increases time to exhaustion, increases maximum workload, improves mental clarity and increases oxygenation of muscles."

It is widely accepted that caffeine increases endurance performance and time to fatigue (18, 29, 53). One mechanism responsible for the improvement in performance is the increase in fat oxidation, which spares muscle glycogen (18, 34, 24, 53). Additionally, caffeine is a nervous system stimulant, which may improve performance in shorter events by enhancing mood and alertness (29, 36, 54).

The other ingredients in the sport drinks do not have the amount of research to support their use; nevertheless, many sport drinks contain

ingredients that have only been purported through indirect research to enhance performance. L-Taurine and Dimethylaminoethanol (DMAE) may promote alertness and mood (32, 49). Cirtulline-malate has been reported to increase time to exhaustion in exercising rats (59). However, L-citrulline has been reported to have a negative effect on time to exhaustion (33). Metrabromine® is a product from the Theobroma Cacao tree which contains small amounts of theobromine (6%) and caffeine (1.5%). Catechin is a polyphenol, while quercitin is a flavonoid, both with antioxidant properties.

2.1 Study Purpose

The purpose of this study is to determine whether a nutritional supplement containing a cocktail of ingredients will improve performance in an indoor 40 kilometer cycling time trial.

2.2 Research Hypothesis

It is hypothesized that individuals will perform better during a simulated 40km time trail following the ingestion of the dietary supplement.

Chapter 3

Methods

3.1 Subject Consent

Subject consent was obtained in accordance with the policy statements of Human Subjects Committee at South Dakota State University. Subjects were informed of their rights to privacy, anonymity, and confidentiality in compliance with institutional guidelines. The subjects were also informed of the purpose and possible risks and benefits of their participation in the study, and signed an informed consent form prior to participation.

3.2 Subjects

Seven male cyclists between the ages of 21-58 years of age were recruited to participate in this study. Individuals were recruited by word of mouth and through a local cycling team. Subjects were also required to have been cycling for a minimum of 2 years. Initial testing procedures were used to determine body composition utilizing air displacement plythesmography (BOD POD®, Life Measurements, Concord, CA), maximal aerobic capacity (VO_{2max}), and lactate threshold.

3.3 Experimental Procedures

Subjects made a total of three visits to the Human Performance Laboratory on the campus of South Dakota State University over a 3-week period. The initial visit included VO2max testing and lactate threshold testing as

well as body composition evaluation. The subsequent visits were two 40 kilometer simulated indoor time trials.

Performance Trials

Performance trials were randomly assigned and consisted of a simulated 40 kilometer time trial on a cycle ergometer. For the performance trials subjects were required to consume an energy bar two hours prior to the beginning of their ride. Forty-five minutes before the beginning of the ride subjects consumed either a serving of PreRace® (1^{st} Endurance, Inc. Salt Lake City, UT) mixed with 12 ounces of sports beverage or aloe vera juice mixed with 12 ounces of sports beverage. The Aloe Vera Juice was chosen as to mimic the bitter taste of the caffeine in the PreRace® drink. Variables measured during the performance trials included ratings of perceived exertion (RPE), heart rate (HR), O_2 consumption (VO_2) and blood lactate (BLa) at 15 min intervals, as well as time to complete the trial, and average power over the course of the trial.

Body Composition

Body composition was estimated by air-displacement plethysmography using the BodPod® (Life Measurement Incorporated, Concord, CA), following manufacturer's recommendations.

Oxygen Consumption

VO_2 was determined by means of indirect calorimetry (Parvo Medics TrueOne Metabolic Measurement System, Sandy, UT) during an incremental cycling test to exhaustion performed on a cycle ergometer. Subjects began the

test cycling at 95 watts (W), every 3 min the wattage increased by 35 watts until volitional exhaustion. VO_2max was determined as the highest VO_2 value recorded prior to test termination. Heart rate was monitored continuously by means of radio telemetry (Polar Electro, Lake Success, NY). Ratings of perceived exertion (RPE) were based off of the Borg 6-20 RPE scale and will be assessed at the end of each stage. Respiratory exchange ratio (RER) was also measured during the VO_2 max testing.

Blood Lactate Measurement

Samples for lactate determination were collected by finger stick at the end of each 3 min stage during the incremental cycling test to exhaustion. The skin around the site was cleansed with alcohol, followed by a small finger prick with a lancet device. A capillary tube was filled, and the wound was covered with gauze. Lactate values were determined using a Lactate Plus lactate analyzer (Nova Biomedical, Waltham, MA).

Dietary Supplement.

The 1st Endurance product, PreRace® was currently available to consumers at the time of testing, with no adverse effects had been reported by the company. The ingredients contained within this supplement (First Endurance PreRace; L-Taurine, Citrulline Malate, Quercetin, DiMethyl Amino Ethanol, Caffeine, Metabromine, Catechin, and Malic acid) are not determined to cause adverse events according to the Food and Drug Administration database. Because this product contains caffeine (200 mg), potential side effects included,

dizziness, tremors, nervousness, headaches, elevated heart rate, and tingling sensations; however, previous findings from our laboratory reported no adverse effects in subjects who consumed a single 200 mg dose of caffeine (7).

Subjects were required to keep a 24-hour diet log prior to their performance trials. Subjects were then asked to repeat the first week's diet to ensure consistency between trials.

3.4 Statistical Analysis

A two way ANOVA with time and treatment as factors was used to analyze the effect of ingestion of the supplement. The variables analyzed ratings of perceived exertion (RPE), heart rate (HR), O_2 consumption (VO_2) and blood lactate (BLa), respiratory exchange ratio (RER), average power, time to complete 10k splits, as well as time to complete the trial.

Chapter 4

Results

4.1 Subject Characteristics

The average age of subjects was 30.9 ± 14.1 years. Average height and weight were 176.9 ± 6.9cm and 79.9 ± 11.7kg, respectively. Body fat percentage was 18.1 ± 4.5%. Subjects had an average of 3.4 ± 3.0 years of cycling experience. VO_2max average for the group was 4.2 ± 0.5 L/min. Subject's absolute maximal power output was 290.5 ± 36.3 watts. Watts at lactate threshold was 191.4 ± 21.5 watts. The average percentage of VO_2max at lactate threshold was 65.6 ± 7.6%. Diet records are represented in table 1. CHO, CAF, Kcal were similar between trials.

4.2 Performance characteristics

A treatment effect ($p<.0001$) existed for work with total work being greater during the EXP (817.9 ± 50.73kJ) trail compared to the PLA (782.6 ±42.8kJ) However, individual time points were not different between the two trials. (Figure 1).

A treatment effect ($p<.0001$) existed for power output between the two trials (EXP 178.4 ± 19.2 watts, PLA 163.7 ± 15.5). Again, there was no interaction of time by treatment analysis (Figure 2).

A treatment effect ($p<.0001$) existed for velocity between the two trials (EXP 30.8 ± 1.7, PLA 29.6 ± 1.8 km/hr). No time x treatment effect was observed, thus velocity was not improved during any given time segment (Figure

3). No effect was observed for average RPM maintained during either trial (EXP 85.7 ± 15.0 rpm, PLA 88.7± 10.2 rpm).

Total time taken to complete each trial did not vary, due to the large variance among subjects. Mean performance time to complete the 40km was 78:10 minutes ± 4.42 and 81:27 ± 4.52 minutes for the experimental and placebo trials, respectively (p=0.23). Analysis of time taken to reach each 10 kilometer check point indicated no difference between trials (figure 4). Cumulative time is reported in figure 5.

Oxygen consumption (VO2) was higher in the experimental trial than the control trial (EXP, 2.7 ± 0.2 L/min, PLA, 2.3 ± 0.2 L/min) (p<.0001). There was no significant time x treatment interaction for VO2 (Figure 6). No differences existed for RER, and also for grams of fat and carbohydrate oxidized as a result of the treatment (Figure 7).

A treatment effect (p<.0001) was observed for average heart rate between the two trials (EXP 167.0 ± 10.2bpm, PLA 163.3 ± 8.6bpm). There was no significant time x treatment interaction for HR (Figure 8).

A treatment effect (p<.0001) existed for lactate accumulation between the two trials (EXP 7.6 ± 0.3 mmol/L, PLA 6.6 ± 0.3 mmol/L). No time by treatment interaction was observed (Figure 9). Percentage of lactate threshold was higher in the experimental group than the placebo group (EXP 96.3 ± 1.4% watts at LT, PLA 87.4 ± 1.3% watts at LT). Again, no time x treatment interaction was observed. A difference in percentage of VO_2max between trials (EXP 65.8 ±

12.5%VO_2max, PLA 56.2 ± 10.6%VO_2max) was also observed. Once again, no time x treatment interaction was observed (Figure 8).

Rating of perceived exertion was affected by the treatment (EXP 14.5 ± 0.8, PLA 12.4 ± 0.9). Subjects did not have a significant variation in their RPE during any specific time point (Figure 10).

Chapter 5

Discussion

Dietary supplement manufacturers are seeking to set themselves apart from other companies by providing athletes with ergogenic aids that are "different". The companies attempt to combine ingredients to further enhance caffeine's ergogenic effect. The supplement tested in the present study contained ingredients [L-Taurine, Citrulline-malate, Quercetin, DiMethyl Amino Ethanol (DMAE), Caffeine, Metabromine, Catechin, and Malic acid], which may or may not provide an ergogenic benefit. The purpose of the present study was to determine whether or not the nutritional supplement PreRace® (1st Endurance Salt Lake City, UT) containing the above ingredients would improve performance in an indoor 40-kilometer cycling time trial. The results of this study indicate a small treatment effect for work, watts, velocity with the participants producing more work and power at a higher velocity during the EXP trial. Despite the EXP group experiencing the improved work, the time to complete the 40km time trial was not significantly different between the two groups. Furthermore, the EXP treatment did not enhance fat oxidation as measured by indirect calorimetry. The higher VO2's and HR's during the EXP trial are more than likely the result of the higher work rate the cyclists were able to maintain during the EXP trial.

Caffeine is a well researched substance that has a clear and well accepted ergogenic effect. Several researchers have reported caffeine to

significantly improve time to exhaustion (18, 19, 28,29) and spare muscle glycogen (14, 18, 23, 24, 35, 53) Of interest to the use of commercially available caffeine products as opposed to simply drinking coffee is another study by Graham et al. (30) which found that time to exhaustion was improved to a greater extent following ingestion of a caffeine capsule as opposed to 2 cups of coffee which contained the same amount of caffeine (4.45mg/kg/body weight).

Power output was improved in our study following the ingestion of the supplement (200mg Caffeine). This study is in agreement with prior research which found an improvement in power output following the ingestion of 5 to 7 mg/kg of caffeine (22, 37, 58). All three studies utilized different protocols with one being leg extension (37), another utilized a 1 kilometer time (58), and the other utilized a ramp protocol on a cycle ergometer used to determine maximal power output (22). While these studies are all in agreement with the results of the current study, it is important to consider the effect of the other substances contained within this supplement. The possibility exists that any of the substances contained could have a negative effect on another one of the substances. The opposite reaction could also be true in which the substances contained actual accentuate the effect of the other substances.

Quercetin supplementation lasting 6 weeks has been shown to improve power output during cycling in a study by MacRae and Mefferd (44). There is no research available investigating the acute effect of quercetin by itself or in combination with other ingredients on endurance performance.

Taurine ingestion has been found to reduce heart rate at submaximal levels at a given work rate along with an increase in stroke volume. Baum (9) attributed the change in stroke volume to higher end diastolic volume, lower-end systolic volume, and slightly increased fractional shortening following taurine supplementation of 1g in humans. This would appear to make it possible for an individual to work at a higher work rate without reaching fatigue because their cardiac response to exercise would be similar to a lesser work rate. It is of note that in the Baum study the substance that was consumed contained 80mg of caffeine. Ivy (34) investigated the same beverage containing 80mg of caffeine and 1g of taurine per serving. In the Ivy study, subjects consumed 2 servings (160mg caffeine, 2g taurine) prior to completing a time trial of pre-determined length (.75 x max watts x 3600s). Subjects who consumed the energy drink completed the time trial 184 seconds faster which was accompanied by an increase in HR and epinephrine levels. It is unclear whether it is the caffeine, the taurine, or the combination of the two that resulted in the improvement in performance in the Baum and Ivy studies (9, 34).

Of particular interest in this study is the fact that time to complete the 40km time trial was not different between trials. As mentioned earlier, subjects completed the time trial in an average of 3 minutes and 17 seconds faster following the consumption of the supplement, the large variability among participants and the small sample size accounting for the lack of significance. We also considered if there may have been something contained in the

supplement that did not effectively produce an ergogenic effect. A study by Hickner et al. (33) reported that L-citrulline actually had an ergolytic effect during an incremental treadmill test to exhaustion. Time to exhaustion was significantly decreased when L-citrulline was ingested. The study also suggests that L-citrulline may have a negative effect on nitric oxide mediated insulin secretion and insulin clearance following exercise, which could have a negative Impact on glycogen resynthesis following exercise.

Another possible explanation for the non-significant results is the dose of caffeine in the current study was on average 2.5mg/kg. Doses in many other caffeine studies have been over 4mg/kg with many studies utilizing 5-10mg/kg (15, 24, 28, 29, 30, 36, 37). However, one study reported improvement in performance with as little as 2mg/kg caffeine. (38)

Caffeine has a significant effect on lipid and carbohydrate metabolism as well. Costill (18) and Spriet (53) have reported significant increases in fat oxidation and reductions in CHO oxidation following the ingestion of 330mg and 9mg/kg of caffeine. An effect of caffeine which has an effect on performance is increased fat oxidation (24). RER data in the current study indicates that the dose of caffeine was not high enough to increase fat oxidation. Evidence that the dose of caffeine was not sufficient to result in a change in substrate utilization was reported by Titlow (54) who found that RER and free fatty acid levels were unaffected by the ingestion of 200mg of caffeine prior to 60 minutes of cycling exercise at 60% VO_2max.

In our study we observed that heart rate was higher in the EXP trials. A possibility for the elevated HR may be due to the higher work rate that was observed during the EXP trail. The higher heart rate is contradictory to the purpose of one ingredient, taurine. Baum (9) reported a decrease in heart rate as well as an increase in stroke volume during an exhaustive bout of endurance exercise following taurine supplementation. The addition of taurine to the PreRace ® formula does not appear to serve its purpose of improving cardiac function by increasing stroke volume and lowering heart rate

In the current study, subjects had an increased rating of perceived exertion during the EXP compared to the placebo trial. This is contrary to prior research involving caffeine which has showed that rating of perceived exertion is reduced following caffeine ingestion in a preloaded effort requiring maximal power output for 2 minutes (22). The current study varies from the Doherty (22) study in that the current study involved a longer trial. Another explanation of this difference is that subjects had a feeling that they were able to push harder for longer which resulted in their perceived exertion being higher. This could be a result of the effects of caffeine as a central nervous system stimulant. (55) Our results for lactate concentration would also explain the greater perceived exertion seen following supplement ingestion. Lactate accumulation occurs when exercise is performed at an intensity in which lactate production exceeds clearance. Subjects in the current study cycled at a greater intensity which resulted in the higher lactate concentration readings. All of these factors together

may explain why subjects reported a greater perceived exertion in the experimental trial.

5.1 Limitations

The current study hypothesized that subjects ingesting a supplement containing multiple ingredients would improve time trial performance. However, the only differences we are able to detect was an increase in average watts but not in fat oxidation or time to complete 40km. The major limitation in the current study is the small sample size. A sample size calculation using the time to complete 40km, a standard deviation of 4.5 minutes, and a power of 0.8 indicates that 57 subjects would be needed to detect a difference between the two trials.

The inability to test the individual ingredients as well as the combination of the ingredients is a problem as some of the ingredients could counteract the benefit of others.

5.2 Conclusion

The results of this study suggest that the consumption of PreRace® prior to a indoor 40km time trial did not result in a faster time to completion despite the treatment effect of higher power output and work.

5.3 Future Research and Considerations

While it is evident that some treatment effects exist as a result of supplementation, future studies involving more subjects is necessary to determine if replication of these results is possible. Another possibility would be

to test a different group of athletes such as runners in a simulated time trial of 5 or 10 kilometers in distance.

Future studies are also needed to determine the interactions of all the substances contained in the supplement. Each of the substances contained in the supplement has its own research which indicates a possible benefit, but the effectiveness of all of them together cannot be fully determined. These studies should focus on pairing substances and testing if their previously studied effects are affected by the other substance.

Figures

Figure 1. Work (kJ) performed during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 2. Power out (W) which was averaged during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 3. Velocity (km/h) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 4. Time taken to reach each 10 kilometer checkpoint of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 5. Cumulative time to complete the 40 kilometer trial with data points at each 10 kilometer checkpoint following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 6. Oxygen consumption (L/min) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 7. Respiratory exchange ratio (RER) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 8. Heart rate (BPM) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 9. Plasma lactate (mmol/L) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 10. Rating of perceived exertion (RPE) during each 15 minute interval of the 40 kilometer time trial following the consumption of either a caffeine based nutritional supplement or a placebo. Values represent mean ±SD.

Figure 1

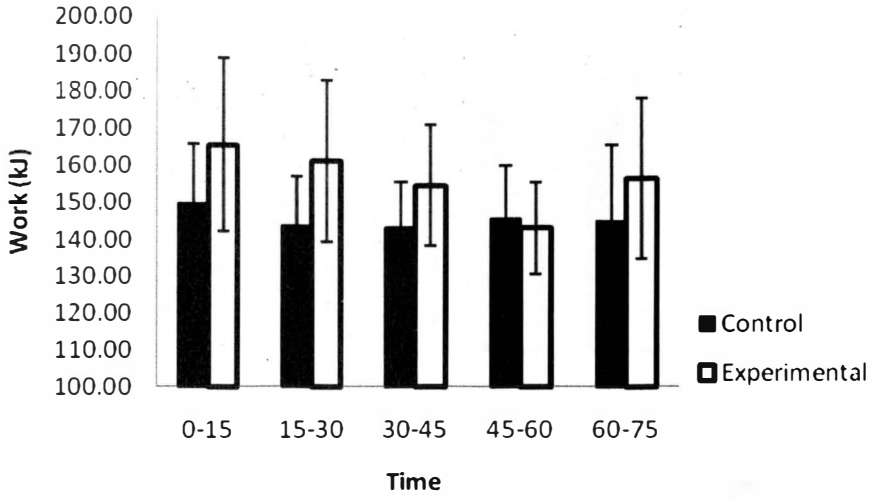


Figure 2

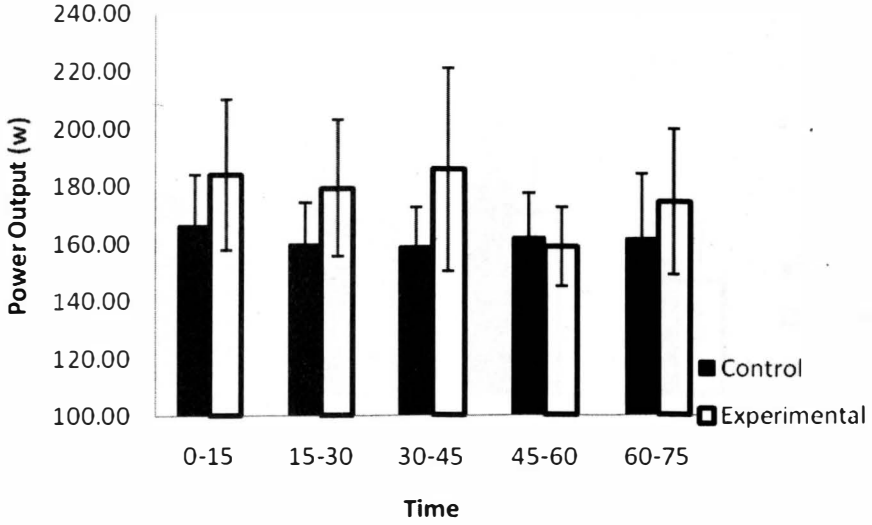


Figure 3

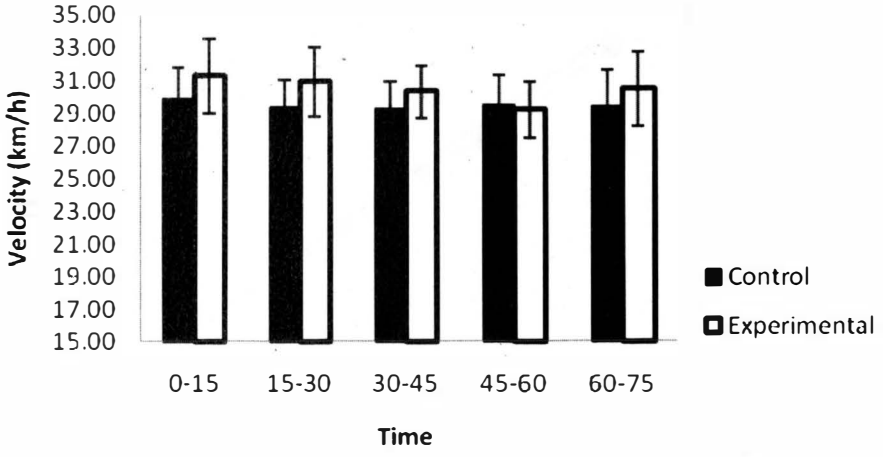


Figure 4

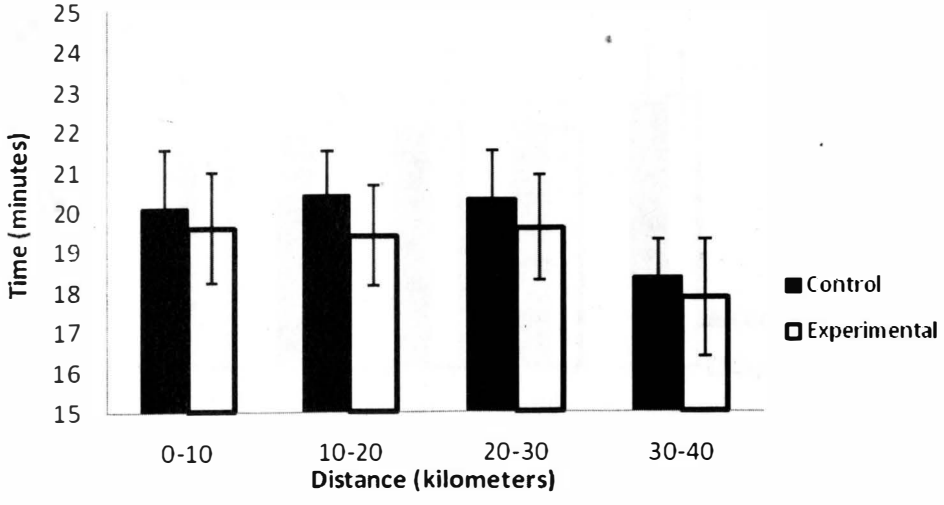


Figure 5

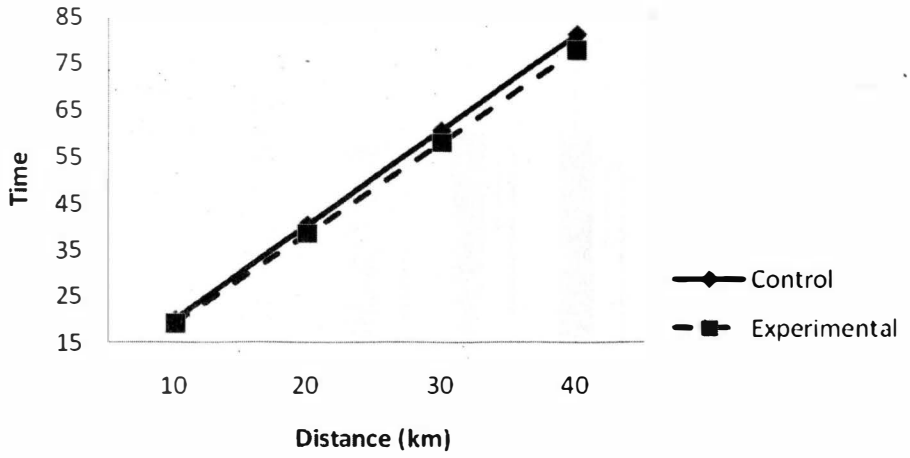
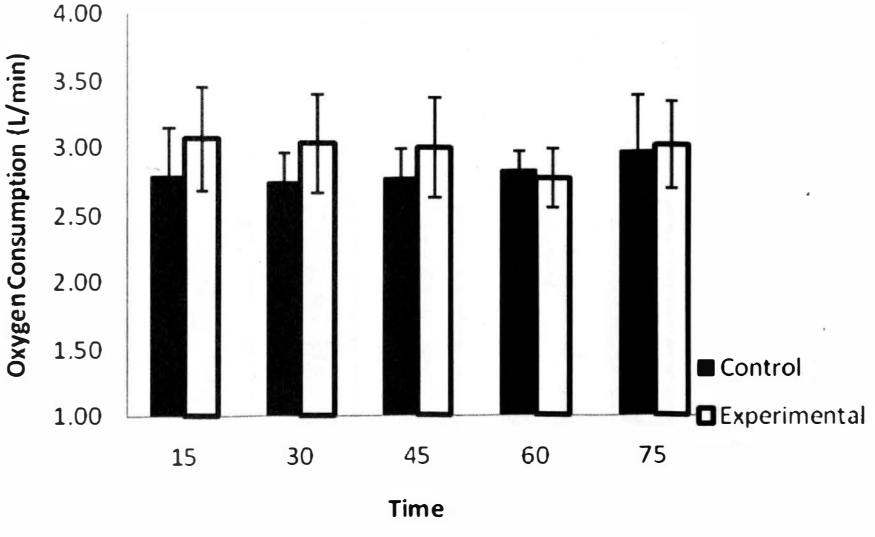


Figure 6



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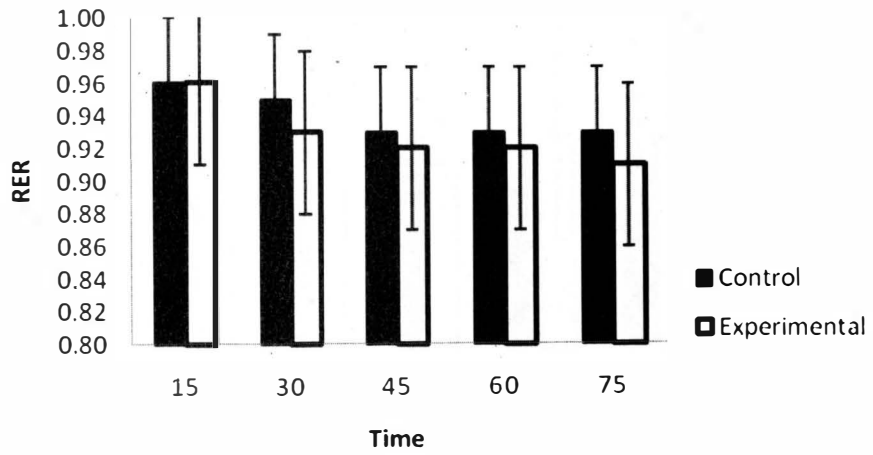


Figure 8

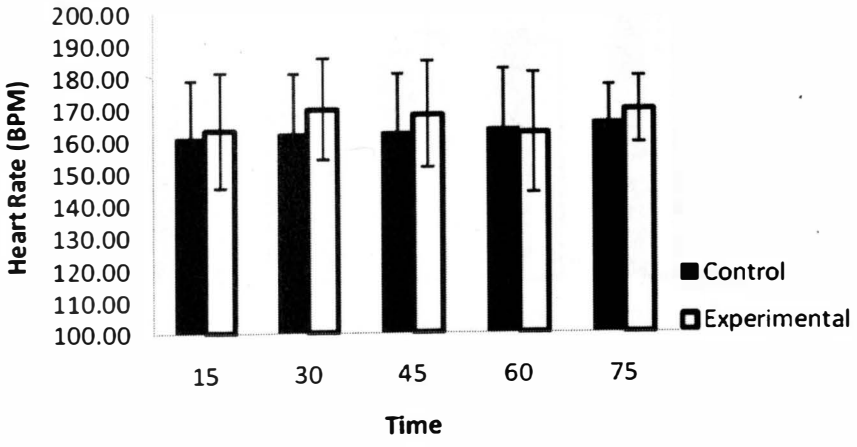


Figure 9

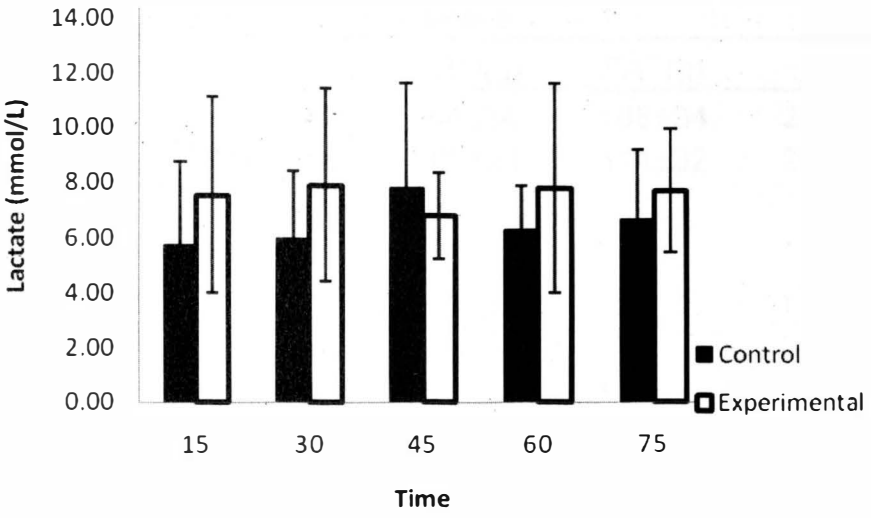
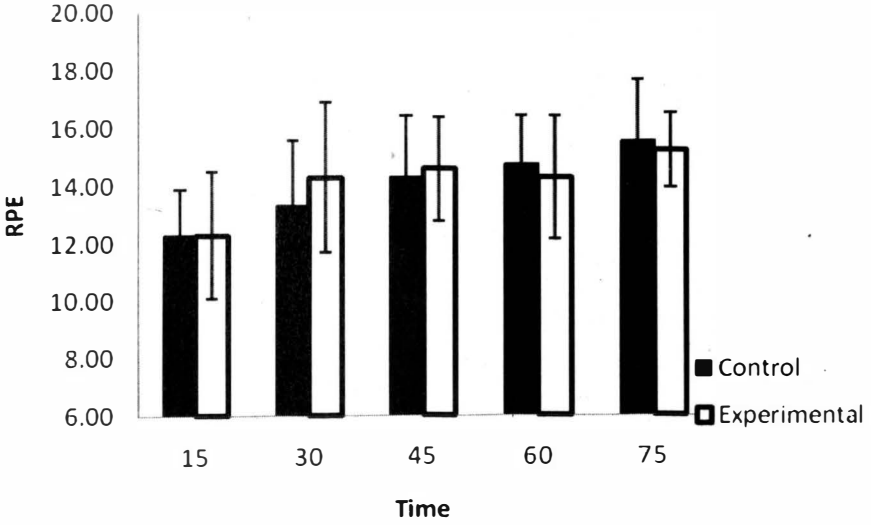


Figure 10



Tables

Table 1. Macronutrient and Caffeine Intake

Trial	CAF(mg)	CHO(g)	PRO(g)	FAT(g)	Kcal
EXP	79±135	376±91	84±24	108±34	2800±569
PLA	76±130	352±135	102±21	111±32	2940±576

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Appendix A

Participant Consent Form

Participation in a Research Project

South Dakota State University

Brookings, SD 57007

Department of HPER

Project Director Lee Weidauer Phone No. 507-829-1631

e-Mail laweidauer@jacks.sdstate.edu Date _____

Please read (listen to) the following information:

1. This is an invitation for you _____ to participate in a research project under the direction of Lee Weidauer _____.

2. The project is entitled The effect of consuming a commercial, herbal-based supplement prior to exercise on endurance performance.

3. *Purpose of the project*

The purpose of this investigation is to determine if consumption of a commercially available pre-exercise specific supplement designed to improve mental clarity, focus, and cardiac output, will improve cycling performance during a 40 km time trial.

4. *If you consent to participate, you will be involved in the following process*

You will make a total of four visits to the Human Performance Laboratory (HP 118) on the campus of South Dakota State Univeristy over a 3 week period. The first visit will include the initial screening, and will take ~30 min. Each subsequent visit (preliminary testing procedures and two performance trials) will take ~ 90 min each. Total time required for each subject will be approximately 5 hrs. Preliminary testing procedures and performance trials will each be separated by one week (see the time-table below).

Week 1	Week 2	Week 3
Initial screening, testing procedures	Performance Trial #1	Performance Trial #2

Body Composition

Body composition (% body fat) will be estimated by air-displacement plethysmography using the BodPod (LMi, Concord, CA), a fiberglass plethysmograph that determines body volume by measuring changes in pressure within a closed chamber. You will sit comfortably inside the BodPod while pressure sensors determine the amount of air displaced by your body, which is used to estimate the body density. Using this data, body composition can then be calculated. Values are based on the average of 2-3 trials, with a complete analysis in 5-7 minutes.

Maximal O_2 Consumption (VO_{2max})

VO_2 will be determined by means of indirect calorimetry (Parvo Medics TrueOne Metabolic Measurement System, Sandy, UT) during an incremental cycling test to exhaustion performed on a cycle ergometer. You will begin the test cycling at 95 watts (W), and increase 35 W every 3 min until volitional exhaustion (21-30 min depending on fitness level). VO_{2max} will be determined as the highest VO_2 value recorded prior to test termination. Heart rate will be monitored continuously by means of radio telemetry (Polar Electro, Lake Success, NY). Ratings of perceived exertion (RPE) will be based off of the Borg 6-20 RPE scale and will be assessed at the end of each stage.

Lactate Threshold

Samples for lactate determination will be collected by finger stick at the end of each 3 min stage during the incremental cycling test to exhaustion. The skin around the site will be cleansed with alcohol, followed by a small finger prick with a lancet device. A capillary tube will be filled, and the wound will be covered with gauze. Lactate values will be determined using an YSI model 1500 sport lactate analyzer (YSI, Yellow Springs, OH). The lactate threshold will be determined as a non-linear rise in blood lactate of at least 1 mmol/L with increasing cycling intensity (Coyle et al. *Med Sci Sports* 16: 120-121, 1984).

Performance Trials

On the day of the trial you will report to the lab following an overnight fast. You will arrive 2 hrs prior to the trial. Upon arrival, you will ingest an energy bar and a serving of sports beverage, then rest. 45 min prior to exercise you will consume either the supplement or placebo, then complete a 40 km simulated time trial on a cycle ergometer. VO_2, HR, RPE, and blood lactate measurements will be taken as described previously at 15 min intervals during each trial. The goal for each trial will be to complete the simulated distance as fast as possible.

Additional requirements

You will be prohibited from intense physical activity within 24 hrs of testing procedures and performance trials. In addition, you will need to make a 24 hr diet and activity record during the day leading up to the initial performance trial, which will be followed for the subsequent performance trial.

5. *Participation in this project is voluntary*. You have the right to withdraw at any time without penalty. If you have any questions, you may contact Lee Weidauer at the number listed above.

6. Potential risks associated with participation in this investigation are as follows:

Risks associated with the incremental cycling test to exhaustion

Little risk is assumed during this test, but nonetheless, you will be closely monitored to prevent any possible abnormal events.

Risks associated with blood sampling procedures

There may be slight discomfort when using the lancet device to obtain blood samples from your finger. This could lead to bruises and possibly result in infection.

Risks associated with supplement consumption:

This product is currently in use, and to date, no adverse effects have been reported. The ingredients contained within this supplement (First Endurance PreRace; L-Taurine, Citrulline Malate, Quercetin, DiMethyl Amino Ethanol, Caffeine, Metabromine, Catechin, and Malic acid) are not determined to cause adverse events according to the Food and Drug Administration database. Because this product contains caffeine (200 mg), potential side effects may include,

dizziness, tremors, nervousness, headaches, elevated heart rate, and tingling sensations; however, previous findings from our laboratory reported no adverse effects in subjects who consumed a single 200 mg dose of caffeine (Ballard et al. *Clin Exp Pharmacol Physiol* 33:310-314, 2006). Any sign of adverse events adverse effects during either trial, will result in immediate test termination.

To minimize potential risks, blood sampling procedures will be performed by qualified personnel utilizing sterile techniques. The stresses associated with these procedures are minimal. The risk of hematoma or sepsis is minimized by using appropriate precautionary measures. These procedures will be performed utilizing the universal precautions for the handling of blood and potentially infectious material. Blood samples will be properly disposed of following lactate analysis.

To minimize risks associated with exercise, all exercise sessions will be monitored by laboratory personnel. Heart rate will also be monitored during the exercise sessions.

In the event of an injury, you will be taken to Student Health (West Hall, 688-4032) and if the injury is too severe or occurs after normal Student Health hours, then you will be taken to Brookings Medical Clinic or Brookings Hospital.

7. Benefits of participation include valuable information about your maximal aerobic capacity, lactate threshold during exercise, maximal sustainable power output during a simulated 40 km effort, and body composition.

8. Promotional items, including water bottles, visors, and supplements will be provided by the sponsoring agency if you successfully complete all testing and performance trials.

9. Your responses are strictly confidential. When the data and analysis are presented, you will not be linked to the data by your name, title or any other identifying item.

10. The University is unable to offer financial compensation nor to absorb the costs of medical treatment should you be injured as a result of participating in this project. In the event of an injury, you will be taken to Student Health (West Hall, 688-4032). If the injury is too severe or occurs after normal operating hours, you will be taken to Brookings Medical Clinic (697-9599) or Brookings Hospital (696-9000). You are responsible for the cost of the treatment.

As a research participant, I have read the above, have had any questions answered, and agree to participate in the research project. I will receive a copy of this form for my information.

Participant's Signature _____ Date _____

Project Director's Signature _____ Date _____

If you have any questions regarding this study you may contact Lee Weidauer. If you have questions regarding your rights as a participant, you can contact the SDSU Research Compliance Coordinator at (605) 688-6975 or SDSU.IRB@sdstate.edu.

Appendix B

Subject Characteristics

Subject:_____Date:_____

Height:_____Weight:_____BodyComposition:____Age:_____

Years Cycling Experience:_____

AbsoluteVO_{2max}:_____RelativeVO_{2max}:_____

Absolute Max W:_____Relative Max W:_____

Lactate Threshold W:_____

Lactate Threshold % Max:_____

Max Heart Rate:_____

Appendix C

VO_{2max} Test Information

Subject:_____ Date:_____

Height:_____ Weight:_____ Age_____

Time	Watts	VO_2	Heart Rate	Lactate	Pulse Power	RPE
0-3	95					
4-6	130					
7-9	165					
10-12	200					
13-15	235					
16-18	270					
19-21	305					
22-24	340					
25-27	375					
28-30	410					
31-33	445					
34-36	480					

Stop Time:_____

Appendix D

Time Trial Data

Subject_____Date:_____

Height:_____Weight:_____Age_____

Trial:_____Tire Pressure:_____

Calibration Setting:_____

Max HR:_____VO_{2max}:_____Max W:_____

Lactate Threshold W:_____

Time	VO_2	$\%VO_{2max}$	HR	$\%HR_{max}$	RPE	PP	La
15							
30							
45							
60							
75							

Stop Time:_____

Average RPM:_____Peak RPM:_____

Average Watts:_____Peak Watts:_____

Average Velocity:_____Peak Velocity:_____

Appendix E

Food Log

Subject:_____

Food	Amount	Time