EFFECTS OF HIGH FAT LOADING ON SUBSTRATE UTILIZATION AND PERFORMANCE DURING INTERMITTENT EXERCISE IN TRAINED ATHLETES

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By

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The undersigned, appointed by the Dean of the Graduate School,
Have examined the Thesis entitled

EFFECTS OF HIGH FAT LOADING ON SUBSTRATE UTILIZATION AND PERFORMANCE DURING INTERMITTENT EXERCISE IN TRAINED ATHLETES

Presented by Jonathan G. Garlow

A candidate for the degree of Master of Arts

And hereby certify that in their opinion is worthy of acceptance.

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Professor John Thyfault

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ABSTRACT

High intensity intermittent exercise is the most common form of exercise in competitive sport yet dietary factors that may affect an athlete's performance in this capacity are rarely investigated. Recent evidence has shown that intramuscular triglyceride (IMTG) is a readily available source of fuel in type I muscle fibers, can easily be supercompensated with a diet high in monounsaturated fat, and is significantly depleted after exercise of varying intensity and modality. **Purpose:** The purpose of this investigation was to see if a 5 d high fat load alters substrate oxidation and performance during high intensity intermittent exercise in trained athletes. **Methods:** Nine wrestlers at the University of Missouri were given a 5 d supplementation of 2.2 g/Kg/d of olive oil. The subjects were tested on a 40 min intermittent bout of treadmill running at work:recovery ratios of 6:9 s and 24:36 s at speeds that produced an RER between 0.95 and 0.99 while gas was collected, then in elliptical locomotion in which distance traveled (Km) and power output (peak Km/hr) was measured during four separate 8 min maximum:active rest bouts of 15:15 s. **Results: S**ubjects had a greater fat oxidation (4.87% ± 0.07 vs. 11.36% ± 1.06% $P < 0.01$) after a 5 d high fat load. There was a significant improvement in 8 min high intensity intermittent performance in total distance, sprint distance, and power output ($P < 0.01$) after the 5 d high fat load. Following the 5 d high fat diet, plasma non-esterified fatty acid (NEFA) was unchanged after the 40 min bout, lower after the third 8 min performance bout ($P < 0.05$) and unchanged after the fourth 8 min performance bout. There was no change in plasma lactate or pH following the 40 min bout or performance bouts three and four. Plasma glucose was significantly higher

($P < 0.01$) following the 40 min sustained intermittent running bout after the high fat load suggesting a possible plasma glucose sparing effect. **Conclusion:** Five days of high fat loading increases fat oxidation without reducing glucose availability and improves performance in high intensity intermittent exercise designed to simulate a wrestling tournament in trained wrestlers.

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INTRODUCTION

During exercise it is well known that two types of fuel sources are used: carbohydrates and fats. One specific type of fat fuel under recent investigation is intramuscular triglycerides (IMTG). Found primarily in Type I fibers, IMTG's are situated closely to the mitochondria for quick access. Recent reviews on the subject of IMTG have concluded that IMTG are used during prolonged exercise, and percentages can depend on various factors such as exercise intensity, duration, dietary content, training status, gender, age, obesity, or Type Two Diabetes (28, 63, 59). VanLoon et. al. describes IMTG as readily oxidized during exercise conditions, and representing a pool of readily available fatty acids (60).

IMTG use during continuous exercise

Although it is well known that the percent of fat oxidation decreases with intensity in continuous exercise, there has been little study in fat utilization during intermittent exercise. A brief review of the literature in continuous exercise shows that in trained cyclists on a normal diet, at 75% maximal oxygen consumption (VO_2max), roughly 10% of total fuel comes from non plasma free fatty acids, presumably IMTG (60). This presumption comes from investigations that have demonstrated that IMTG represent up to 90% of non adipose derived fat oxidation during exercise and even more in trained individuals (38). Furthermore, endurance training may reduce the role of free fatty acids as an energy source during prolonged exercise due to a decrease in sympathodrenal response, while IMTG make up the difference with increased utilization with training due to β-adrenergic stimulation (39). Numerous other studies have

demonstrated marked decreases in IMTG after various exercise protocols. Johnson et. al. noted an 80% depletion in IMTG after a cycle time trial lasting around 3 h, while Krssak et. al. found a 67% drop in soleus IMTG just after, and an 83% drop during a recovery phase following exhaustive sub max jogging (27, 34). Additional investigations found a 62% drop in IMTG after a 2 h cycle at 60% VO_{2max}, with triglyceride (TG) derived oxidation representing around 15% of the total (60).

Fat use during intermittent exercise

Although a wealth of work on fat oxidation exists, little research has been conducted on intermittent exercise. Early studies showed an unexpected use of lipid and aerobic energy release during high intensity intermittent exercise when compared to continuous exercise matched for average power output and oxygen uptake (16). Essen et. al. had subjects perform an intermittent intense exercise session at 15:15 s work:rest ratio and a 60 min continuous trial that were nearly identical in average power output and oxygen uptake (VO_2). The study surprisingly revealed similar glycogen depletion as well as free fatty acid (FFA) and glucose uptake between trials, suggesting that lipid utilization during the intermittent exercise was similar to steady state continuous exercise. This novel finding meant that fat can be just as important of a fuel source as carbohydrate (CHO) during high intensity intermittent exercise.

Recent investigation has shown that the work:recovery ratio can further alter substrate utilization (7). One study observed the effects of a high fat load on fat oxidation in intermittent exercise. The subjects ran at a set speed corresponding to a work intensity of roughly 110% VO_2 peak with either a 6:9 s or 24:36 s work:recovery

ratio; therefore traveling the same distance over the set time period (7). The authors used a novel design to measure substrate oxidation during the high intensity intermittent bouts. By establishing a steady lactate (La) during the exercise (confirmed by blood draws), therefore confirming a stable bicarbonate (HCO_3) pool, the authors were able to use indirect calorimetric methods using the respiratory expiratory ratio (RER) to calculate fat and CHO oxidation. Because the rest sessions were 1.5 times the work sessions, the average RER was able to stay below the 1.0 even with an intensity of 110% VO_{2peak} during the work portions. An RER below 1.0 is desired for accurate indirect calorimetry calculations. The results found the 6:9 s group to oxidize three times more fat than the 24:36 s group. Furthermore, the 24:36 s group oxidized three times more CHO, had a significantly higher plasma La despite a lower calculated energy expenditure, and had a lower muscle oxygenated hemoglobin (HbO_2) count (7). The authors suggested that the lower HbO_2 in the 24:36 s group may have contributed to the observed decrease in fat oxidation, thus increased CHO use and higher plasma La accumulation. The knowledge that carbohydrate metabolism increases in skeletal muscle when oxygen (O_2) supply is reduced supports these findings (68)

The only known investigation directly assessing IMTG use during high intensity intermittent exercise found a 30% depletion in IMTG after a weight lifting session (17). The researchers used 30 s of exhaustive quadriceps extension (generally 6-12 reps) with 60 s of rest between for a total of 30 min. As expected, they found a decrease in both IMTG and muscular glycogen after the test. The most interesting finding of the study was that the individuals who had higher baseline levels of IMTG stores used a higher percentage during the test. They concluded that the starting metabolic profile of the

muscle influenced the substrate utilization during the high intensity intermittent exercise bout.

Loading IMTG

With the evidence pointing to IMTG being a vital addition to total energy during exercise, many researchers are attempting to increase resting levels in attempts to burn more IMTG thus sparing whole body glycogen stores that will be needed during a prolonged exercise bout. In a review on the subject, Johnson et. al. concluded that IMTG formation may be relatively rapid (28). One researcher noted a 36% increase in IMTG after only 2 d of fat loading on a 60% fat diet (66). Other findings included a replenished IMTG store after 24 h and a supercompensation after a 3 h exercise bout at 55% VO_2max in only 48 h on a 40% fat diet (61). Similarly, a 35% fat diet replenished IMTG in 22 h and supercompensated them at 70 h after a 25% IMTG depletion during a 2 h cycle at 67% in women, while a low fat diet of 10% never replaced the loss in IMTG (35). One investigation even revealed a single high fat load after 2 h of cycling significantly increased IMTG after a 12 h overnight fast (55). Although all these previous studies have been performed on trained individuals, one more study revealed an IMTG supercompensation after a 2 h 50% VO_2max cycle in 30 h in both trained and untrained subjects (12). It seems clear from the existing literature that IMTG can be rapidly loaded and even supercompensated, which was the main goal of the high fat diet that was used in the current investigation.

The type of dietary fat also plays a role in the extent of IMTG loading. One recent investigation revealed that monounsaturated fat is preferential to saturated fat following heavy exercise (62). The study found that labeled monounsaturated fats (1-^{13}C

oleate) was oxidized significantly more than labeled saturated fat (d_{31}-palmitate) following heavy exercise. This is substantial because IMTG may have a preference for dietary monounsaturated fats. IMTG is composed almost entirely of palmitate (16:0), oleate (18:1), and linoleate (18:2) (21). It has been demonstrated that safflower oil, high in monounsaturated and low in saturated fat raised IMTG, while fish oils, high in polyunsaturated fat but low in monounsaturated and saturated fat lowered IMTG stores (42). Therefore, by increasing monounsaturated fats IMTG can be loaded more efficiently than a standard fat intake of mixed saturated, monounsaturated, and polyunsaturated fats, and an increased fat oxidation following heavy exercise should occur.

Loaded IMTG and performance

Because IMTG are a vital source of fuel during and after exercise, and possibly more important during intermittent bouts than continuous, and it has repeatedly been demonstrated that it is possible to replace them and even supercompensate them in only a matter of a few days, the question arises as to whether a loaded IMTG supply can improve high intensity intermittent performance. In continuous intense bouts, it has been demonstrated that when running above lactate threshold, IMTG accounted for more fat oxidation than FFA's (30). The reviews on the subject suggest that a short term high fat diet can reduce RER, increase percent fat oxidation, and reduce percent CHO oxidation but may impair endurance performance, while a long term high fat diet can maintain or reduce endurance performance (28, 63, 61). However, it has yet to be assessed whether a short term high fat loading period can improve high intensity intermittent exercise.

One researcher found that the metabolic changes during exercise can occur with only a 5 d high fat diet (4,5). Furthermore, the changes were maintained after a carbohydrate load the day before exercise testing (4,5). Despite the lower RER and higher fat-ox during the 2 h bike protocol and 30 min time trial, performance was not enhanced with the 5 d high fat diet. However, the experimental group was not compared to a control group or to baseline measurements, so it is unknown if their individual performance was truly enhanced following a 5 d high fat diet and a 1 d CHO load.

It has recently been observed that power can be enhanced, lactic acid (LA) can be reduced, and pH and plasma HCO_3 can be better maintained in a glycogen reduced state due to increased fat use during high intensity exercise (44). Osborne and Schneider purposely reduced the type I muscle glycogen by having subjects perform a moderately paced cycle exhaustion protocol lasting roughly 143 min. Prior investigation has found that this protocol severely depletes glycogen in type I fibers while only an intermediate loss in type IIa fiber glycogen and a minimal loss in type IIb fiber glycogen (58). The following day, the subjects returned and performed an 8 min constant load heavy exercise bout. The power output was significantly higher at min 5-8, blood La and HCO_3 were significantly lower at min 6-8, and blood pH was elevated at min 3-8 in the glycogen reduced state. The authors attributed the enhanced performance to an increased use of type I fiber IMTG as they observed an 88% fat oxidation in the glycogen reduced vs. 42% fat oxidation in the control trial, and to altered motor recruitment pattern, via summoning more of the stronger type II fibers which retained most of their glycogen stores. The authors speculated that the former was more contributory to total energy production due to the reduced LA seen in the glycogen reduced trial. If type II fibers

(glycogen rich) contributed more that the type I fibers (IMTG rich and glycogen depleted) then LA would have increase during the heavy exercise bout; this was not the case. Therefore, the authors believed that the type I fiber IMTG pool was a primary fuel for this high intensity 8 min exercise bout.

Wrestlers as subjects

Wrestling is a unique sport because it consists of a two to three hour practice at moderate to high intensity for a single 7 min match, or in a tournament setting, 4-8 matches in one day at intense intermittent workloads: thus wrestling is a fine mix of aerobic and anaerobic oxidation. Additionally, wrestling is a weight class sport, resulting in considerable pressure to maintain a lean body composition and regularly lose weight for competition. A survey in 1990 revealed that 89% of collegiate wrestlers regularly lose weight for competition with an average loss of 4.4 kilogram (kg) over a 3 d period (56). A more recent national survey conducted in 2003 reported an average weekly loss of 2.9 kg in an attempt to reach a desired weight class (43). That survey found that wrestlers achieve their desired weight by additional exercise (75.2%), dieting (79.4%), or fasting (54.8%) (43). Exercise related energy expenditure to maintain a lean composition was reported to be 71 kilocalories (kcal)/kg/wk for high school wrestlers, equating to 691 kcals/day for a 150 pound (lb) male (50). Individual case reports on elite Olympic wrestlers have shown a 2100 kcal/day exercise related energy expenditure in preparation for competition (65). A recent laboratory investigation reported that the weight loss practices commonly performed by wrestlers resulted in a large decrease in muscle

glycogen (57). Furthermore, it has also been shown that a single wrestling match can reduce muscle glycogen by 21% (23).

Studies on refeeding diets attempting to replace the glycogen lost during weight cutting practices have had mixed results. Finn et. al (18) reported no positive effects of a 1.5g/kg CHO load taken 1 h before competition just after weigh ins compared to placebo. Rankin (48) found that a 5 h high carbohydrate refeeding vs. a medium carbohydrate refeeding returned the total work performed by subjects closer to baseline values (99.1% vs. 91.5%) but only reached a significance of $P = 0.10$ while the interventions had no significant effect on peak power or average power. Additionally, the lactate levels in the high carbohydrate group were significantly higher and the pH was significantly lower than the medium carbohydrate group. Another study (2) has shown no effect of bicarbonate taken 60-90 min before a simulated wrestling match.

It seems clear that the designed interventions aim to improve glycogen stores and reduce lactic acid; however, no research has been performed on wrestlers' performance in a simulated tournament setting using a 5 d high fat diet. It is apparent that a high fat diet can quickly increase the quantity and availability of IMTG. It is also apparent that IMTG are an important fuel source during and after high intensity exercise. It has been established that high fat diets for 5 d can alter muscle metabolism, burning a higher percent of fats than CHO at rest and during exercise as is evident by the lowered RER values, even if a CHO load is given the day before competition. The net result of a higher percentage of fat utilization during and after exercise is a reduced La production thus a lower pH, as well as a sparing effect of the precious glycogen stores that will be needed during portions of a high intensity intermittent workload.

Purpose and Hypothesis

Purpose:

The purpose of this study was to determine: (1) if a 5 d high monounsaturated fat load can alter substrate oxidation during sustained high intensity intermittent exercise bouts of 6:9 s and 24:36 s and (2) whether performance can be enhanced in a separate set of four high intensity intermittent exercise bouts.

Hypothesis:

In baseline tests:

Sustained intermittent workloads of 6:9 s will oxidize more fat than 24:36 s

In sustained intermittent workloads:

After 5 d high fat load:

(1) Both 6:9 s and 24:36 s sustained intermittent workloads will oxidize a higher percentage of fat when compared to baseline.

(2) As a result, RER and La will be lower during the exercise protocol

In performance based bouts:

After 5 d high fat load:

(1) La production will be lower after each performance based bout when compared to baseline.

(2) As a result, performance (a. total sprint distance , b. average sprint distance, c. total distance traveled, d. total peak power output, e. average peak power output, and f. rate of perceived exertion) will be improved when compared to baseline measurements.

METHODS

Subjects

As a member of the wrestling team at the University of Missouri, the subjects participated in 20 h of intense physical activity per week consisting of 3 weight lifting sessions and 6 wrestling practices. This study did not include the practice of weight loss in preparation for competition, as we did not want to compound calorie restriction, excessive exercise, and dehydration with the experimental protocol. Subjects (N=9) were required to provide hydrated urine specimens before each exercise protocol. None of the subjects had a history of any cardiovascular disease risk factor or any other disease symptom as determined by the American College of Sports Medicine (1). The subjects were required to discontinue the use of any health supplement 2 wk before, and caffeine 48 h prior to baseline testing and discontinued their use during the study. Subjects composed a 7 d physical activity and dietary record beginning 2 d before baseline testing and through the end of the dietary intervention. The subjects were familiar with both treadmill and elliptical locomotion as they are both commonly used for cardiovascular conditioning, high intensity interval training, and used when athletes are injured and cannot practice. The intensities used in the current trial are similar to intensities used in such training. Finally, subjects refrained from physical activity 36 h prior to baseline or post exercise tests. Before any testing portion of the study, subjects were informed of their responsibilities and obligations and supplied a signed informed consent form.

Table 1. Demographic Data

Subject Variable (N = 9)	
Age (yrs)	21 ± 1.6
Height (cm)	174 ± 6.5
Weight (Kg)	
Pre	81.7 ± 4.4
Post	81.0 ± 4.4
% Fat (skin folds)	
Pre	9.32 ± 2.6
Post	8.69 ± 2.8
VO2 max (ml/kg/min)	56.55 ± 5.6

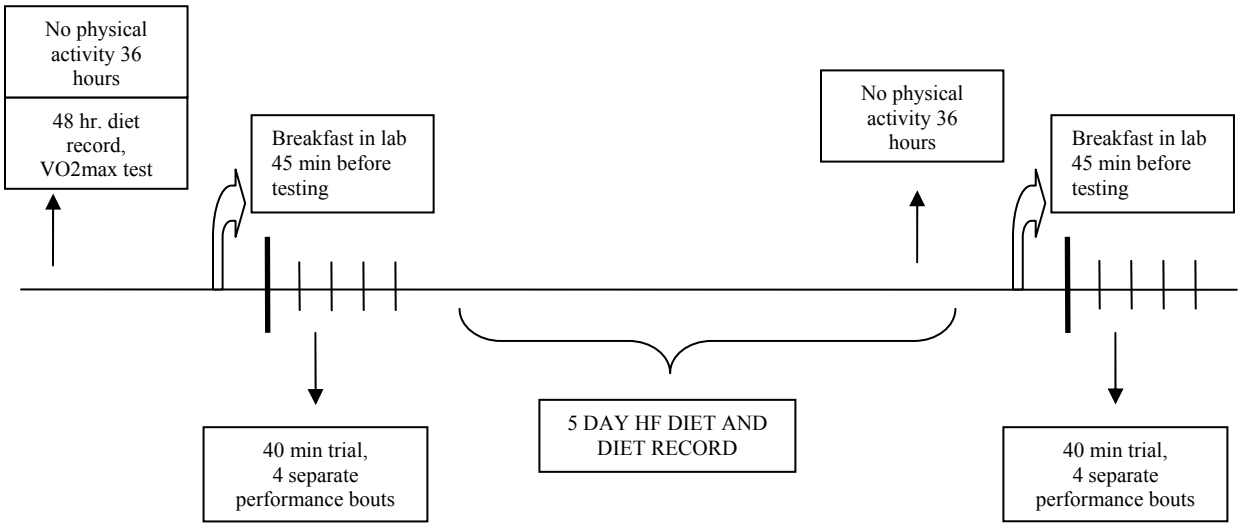
Values are reported as means ± SD. No significant difference was observed.

Experimental Design

A motorized treadmill was used for the VO_2max test as well as the sustained intermittent test to measure substrate oxidation. Previous studies have validated the specific intermittent exercise protocol that was used (7). A whole body elliptical cross trainer machine was used for the performance based bouts as it utilizes both upper and lower body exercise and the precise resistance can be increased to more closely simulate intermittent athletic competition better that a treadmill or cycle ergometer. A treadmill does not offer the resistance that an elliptical cross trainer can, and a bicycle only utilizes lower body activity. Therefore, performance in power output cannot be assessed using a treadmill, and a cycle performance test is specific only for cyclists. Using a whole body elliptical machine provides a natural movement of both the upper and lower body so it is not sport specific, and it can easily measure performance by applying distance traveled to the set resistance.

All exercise testing were performed in the exercise physiology lab at the University of Missouri. Baseline testing consisted of a VO_2max test on a treadmill. One week later, the subjects completed the baseline testing with a 40 min sustained

intermittent exercise trial followed by four separate 8 min performance based bouts one hour apart. Blood was drawn 5 min before and 5 min after the 40 min sustained intermittent exercise trial (B40 and A40) and 5 min prior to and 5 min after performance bout 3 (B3 and A3) and performance bout 4 (B4 and A4) for a total of 6 blood draws during the day. The subjects then consumed a 5 d high fat diet consisting of three standardized high fat shakes each day. Following the 5 d intervention, the subjects returned to the lab for a second round of exercise testing under the same format as baseline testing. The timeline of the study is illustrated in fig 1.



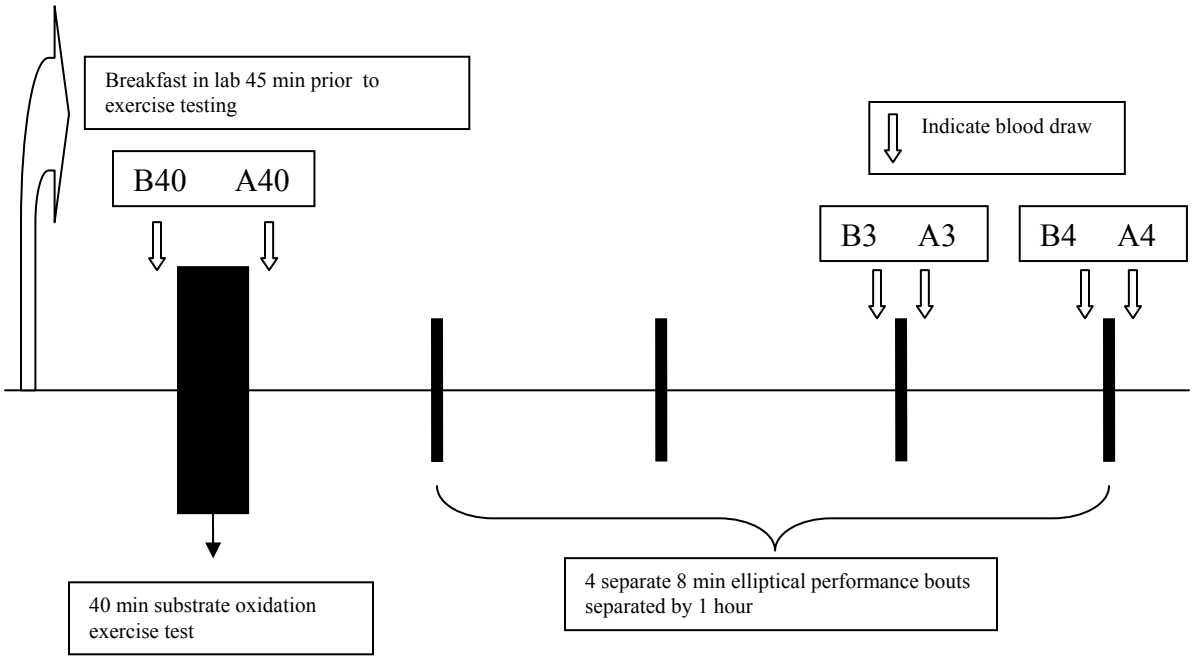


Figure 1: Study Timetable

Diet

A 2 d dietary record was taken before the experiment began to examine dietary practices of the subjects. Diets were recorded during the 5 d intervention for a total of 7 d of dietary record. Subjects were instructed to maintain a scaled down but normal diet during the 5 d intervention. Subjects were void of any nutritional supplements for 2 wk and caffeine for 48 h before baseline testing and throughout the experiment. The 24 h diet consumed before baseline testing was repeated in a scaled down version prior to post exercise testing.

High fat diet

Subject received 3 dietary shakes per day in the morning, afternoon, and evening, supplemented with a total of 2.2 $g \cdot kg^{-}1 \cdot day^{-1}$ of olive oil. The subjects were met and given their morning shakes after their morning practice (around 9:00 AM) on Monday,

Wednesday, and Friday. On Tuesday and Thursday morning, the subjects were responsible for consuming their morning shake that was provided the night before. Before every afternoon practice (around 3:00 PM), subjects were given their afternoon shake, and after each afternoon practice (around 6:00 PM), subjects were given their evening shake to be consumed with the rest of their dinner later that evening.

Olive oil was chosen as the preferential fat due to the high monounsaturated and low saturated and polyunsaturated fatty acid content. As discussed earlier, IMTG are composed almost entirely of oleate, palmitate, and linoleate. These happen to be the three fatty acids found in olive oil with a majority of the monounsaturated fatty acid being comprised of oleate. Additionally, olive oil has a mild taste, is easily digestible, and can be added to a liquid dietary shake with ease. Shakes did not comprise all of the nutrition; subject consumed smaller portions of their normal diet such that the combination of the two results in a 50-60% fat diet. Special care was taken to instruct the subjects on how much they should eat to achieve similar caloric intake that was observed in their 48 h dietary log.

Example: 70 kg subject consuming roughly 3000 kcal/d

This subject would receive 2.2 $g \cdot kg^{-1} \cdot d^{-1}$ of olive oil for a total of 154 fat grams from olive oil per day. This results in 1386 kcals and approximately 11.5 tablespoons of olive oil per day (olive oil contains 120 fat kcals/ Tbs). With a 3000 kcal diet this would result in 46% of energy coming from olive oil in the shakes. Because the remainder of the diet will be composed of some fat, the total fat intake will be between 50 and 60%.

Breakfast in Lab

Subjects consumed a breakfast meal in the lab 45 min before baseline and post exercise testing to avoid hunger and hypoglycemia during testing. The meal consisted of commercially available nutrition bars consisting of 40:30:30 CHO:fat:protein. One bar contained 200 kcals and portions were matched per kg body weight (BW) such that a 70 kg subject received 2 bars, an 80 kg subject received 2.25 bars, and a 90 kg subject received 2.5 bars. Subjects also received a sports drink at the rate of 2 oz per 10 kg BW. Water was provided after the 40 minute sustained intermittent bout and throughout the rest of testing *ad libitum*.

Exercise Protocols

A. VO_{2max} test. The VO_{2max} test was performed on a treadmill (Quinton Model 18-60) in order to best determine workloads that will be applied during the sustained intermittent trial. The protocol began with a warm-up period at 3.0 mph to allow subjects a familiarization period with the breathing apparatus and review of the protocol. The first workload began at 3.5 mph and was sustained for 2 min. Treadmill speed was then increased 0.5 mph at the end of each minute until a speed of 6.0 mph was reached. This pace was held for the duration of the test. After successfully completing 1 min at 6.0 mph, the grade of the treadmill was increased at the rate of 2º / min until exhaustion. The physiological criteria for a successful VO_2max test was a maximal RER $\geq$ 1.1, a maximal heart rate within 10% of age predicted max, and a leveling of O_2 consumption ($\leq$ 2 ml/kg/min difference between two successive workloads).

B. Sustained intermittent test. The sustained intermittent exercise trial to evaluate substrate oxidation began with a 5 min continuous warm up and familiarization period at 4.0 mph. After familiarization, the treadmill speed was increased to 6mph and maintained for the duration of the test while the grade was increased represent 110%$V0_{2peak}$, and was adjusted throughout the test. Intermittent periods of 6 s of exercise to 9 s of rest were achieved by the subject stepping on or off the treadmill when instructed. This pace was designed to elicit an average RER of 0.95 to 0.99 during the test. The average RER stayed under 1.0 for accurate indirect calorimetry. If the incline needed to be adjusted to achieve this desired RER, it was and the new incline was recorded. This 6:9 s intermittent procedure was repeated for a total of 20 min. At the 20 min mark, the subject switched to 24 s of 110% VO_2peak followed by 36 s off the treadmill. Again, the incline was adjusted as needed to achieve an average RER of 0.96 to 0.99. This also repeated for a total of 20 min. Half of the subjects begin with 6:9 s and half begin with 24:36 s.

After the 40 min of high intensity intermittent exercise, the subject were allowed a 5 min cool down period at their own pace. Blood was taken prior to and 5 min after completion of the exercise test. Because it was believed that a 5 d high fat diet would lower resting and possibly exercising RER, the speed used for post tests was the same as was used for baseline test and the RER was again recorded.

Calculations used to determine substrate oxidation followed the stoichiometric equations and assumptions outlined by Frayn (19) and Romijn (52). According to values calculated by Frayn, total O_2 consumption was represented as

$$VO_2 \text{ (l/min)} = 0.746\ c + 2.03\ f + 6.04\ n \quad (1)$$

and total CO_2 was assumed

$$VCO_2 \text{ (l/min)} = 0.746\ c + 1.43\ f + 4.89\ n \quad (2)$$

whereby (c) and (f) represented grams of carbohydrate and fat oxidized per minute and (n) represented grams of urinary nitrogen excreted per minute. These equations were then solved for c grams of carbohydrate (as glucose) and f grams of fat per minute as

$$c = 4.55\ VCO_2 - 3.21\ VO_2 - 2.87\ n \quad (3)$$

$$f = 1.67\ VO_2 - 1.67\ VCO_2 - 1.92\ n \quad (4)$$

where VO_2 and VCO_2 represented O_2 consumption and CO_2 production (L/min).

Romijn outlined principles to determine fat oxidation with the use of expired O_2 as opposed to CO_2 which may provide more accurate assessment of substrate oxidation using indirect calorimetry and is represented as

$$\text{fat oxidation (g/min)} = 0.493\ VO_2 - 0.367\ c - 2.975\ n \quad (5)$$

Nitrogen excretion rate was assumed in accordance to Romijin to be 135 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Even with a 30% error rate in this value, there would be no significant effects on calculated values of CHO and fat oxidation.

C. Performance Bouts. One hour after the sustained intermittent exercise trial, a series of four 8 min performance bouts was carried out on an elliptical machine. Each bout was separated by 1 h. The performance based bouts used a whole body elliptical machine. Gas collection was not required. The resistance was set at 16 out of a possible 20. There was a warm up period of 2 min at 8 out of possible 20 resistance and 80-90 rpm cadence. The subject sprinted as hard as possible for 15 s with verbal encouragement followed by 15 s of rest at a pace of 30 revolutions per minute (rpm).

Because total distance was used to determine total work, each subject maintained this slow cadence during the rest portion and was aided by a metronome to achieve this pace as quickly as possible. Distance traveled and watts produced during selected sprint portions were also recorded as well as the subjects' rate of perceived exertion (RPE). Blood was taken 5 min prior to and 5 min after for the final two bouts. The same protocol was repeated after the 5 d high fat diet.

Blood Collection

Blood was collected in standard fashion via the anetcubital vein. For each blood draw (before 40 min (B30), after 40 min (A30), before 3rd performance bout (B3), after 3rd performance bout (A3), before 4th performance bout (B4), and after 4th performance (A4) bout) 10 milliliters (ml) was drawn and transferred to a vacutainer containing EDTA. The samples were separated by centrifugation in a Marathon 22100R centrifuge (Fisher Scientific, Pittsburg, PA). The plasma was then drawn off, alloquated and stored in cryogenic vials at -70ºC for later analysis.

Plasma Volume.

Hematocrit (Hct) values was used to determine plasma volume (PV) shifts. Hct was measured immediately after each blood draw in 4 randomly selected subjects to determine if corrections are needed for calculations involving PV. The procedure for measuring Hct was to fill a micro hematocrit capillary tube 2/3 full with whole blood and to seal them with capillary tube sealant. The capillary tubes were then spun in a micro hematocrit centrifuge for 3 min (Model MB, International Equipment Company, Needham Heights, MA). The spun capillary tubes were then read using a hematocrit tube reader according to manufacture instructions (Model CR, International Equipment

Company, Needham Heights, MA). The Hct. percent was adjusted (0.96) for trapped plasma located within the Hct portion, as well as an additional adjustment (0.93) for venous-to-total body Hct ratio (9).

In addition to Hct, hemoglobin (Hb) was also measured to more accurately assess plasma volume shifts. A Nova stat analyzer (Model pHOx plus L , Waltham, MA) was used for Hb determination. The stat analyzer used direct ISE, amperometric, conductivity, and optical reflectance to determine blood levels of several blood electrolytes and gasses. A 125µL previously alloquated plasma sample was taken from each pre-exercise and post-exercise trial and inserted into the Nova stat analyzer for Hb measurement.

Calculations used to determine PV changes were in according to Dill and Costill and Costill and Fink (13,9). Baseline PV% (PV_B) was determined by baseline Hct (Hct_B) measurement and adjustments previously mentioned.

$$PV_B = \text{Total blood} - Hct_B / 100 \quad (6)$$

Post exercise PV (PV_A) was determined via the Hb adjusted methods described by Costill and Fink whereas the subscript B refers to before exercise and the subscript A refers to after exercise.

$$PV_A = (100 \times Hb_B/Hb_A) - [(100 \times Hb_B/Hb_A) \times Hct_A] \quad (7)$$

Percent change in PV (%ΔPV) was then be calculated as

$$\%\Delta PV = 100\ (PV_A - PV_B)/PV_B \quad (8)$$

Blood Glucose and Lactate Analysis

The Nova stat analyzer previously mentioned was also used for determination of blood glucose and lactate. Previously alloquated plasma samples were used. All blood trials were examined for glucose and lactate. The measurement of glucose is determined by measuring the H_2O_2 produced during an enzymatic reaction between glucose and oxygen in the presence of the glucose oxidase enzyme (Glucose + O_2 + Glucose Oxidase → Gluconic acid + H_2O_2). A constant potential of 0.70 volts is applied and the electroactive H_2O_2 is then oxidized at the surface of the platinum anode, producing a measurable current proportional to the glucose concentration of the sample ($H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$). Lactate is measured in a similar manner with a lactate oxidase enzyme (Lactate + O_2 + Lactate Oxidase → Pyruvate Acid + H_2O_2) then applying a constant potential of 0.70 volts yielding a measurable current proportional to the lactate concentration of the sample ($H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$). A coefficient of variation (CV) was calculated to be 3.0% for glucose and 3.3% for lactate.

Gas Analysis

Gas collection and analysis during the VO_2max and sustained intermittent exercisee protocols was obtained using the Parvo Medics TrueOne 2400 metabolic cart (Parvo Medics Inc, Sandy, UT). The headgear used for gas collection was a Hans Rudolph head support (Hans Rudolph model # 2726, Kansas City, MO) which holds a bi-directional valve attached to a rubber mouthpiece. The valve connects to 4 ft of lightweight tubing which was directly connected to the pneumotach of the TrueOne 2400 metabolic cart. Subjects were allowed time to become familiar with the apparatus before

any testing began. During the VO_2max test, gas was collected to determine that an RER of at least 1.1 was reached, helping to validate the test. During the 40 min sustained intermittent exercise trial, RER was constantly be monitored and speed was altered in order to obtain an average RER between 0.95 and 0.96 during the test. Because it was believed that a 5 d high fat diet may lower RER during the sustained intermittent trial, RER was recorded every minute throughout the trial for comparison to baseline.

Plasma Free Fatty Acid Analysis

Plasma free fatty acid, or non esterified fatty acid (NEFA), were analyzed by a commercially available assay kit using the enzymatic colorimetric method. The HR series NEFA-HR (2) (Code No. 999-34691) manufactured by Wako Pure Chemical Industries, Ltd. Distributed by Wako Diagnostics, Richmond VA was used. Nine runs were performed, one for each subject. The CV ranged from 0.4% to 2.9% with an average of 1.9%.

Work Analysis

During the performance bouts of 15:15 s work:recovery, several evaluations of work were assessed. Distance traveled was used to determine the total work performed as well as work performed during the second sprint of each minute. That sprint distance was examined as a sum total as well as an average over the test. Peak power was measured by observing the highest cadence or Km/hr achieved during the second sprint of every minute. The peak power was also examined in a sum total and average. Finally,

RPE was taken at min 2, 4, 6, and 8 of each performance bout. Taken together, the total work performed, the work performed during selected sprint portions, the peak power, and RPE provided sufficient evidence for performance on the 4 separate 8 min bouts.

Statistical Analysis

Two way ANOVA with Repeated Measures was performed using SPSS version 11.0.

(1) Comparing the means of the pre test and post test values for body composition, body weight, food intake, fat oxidation percent in the 6:9 sec and 24:36 sec subsections of the sustained intermittent bout, total sprint distance, average sprint distance, total distance, total peak power, average peak power, RPE, lactate, glucose, pH, and NEFA.

RESULTS

Blood draws were successful at most time points. At time points where a sample was unachievable due to difficulty in venipuncture or prolonged time in achieving adequate sample quantity, averages were created without missing sample.

Body Composition

Subjects' weight and body composition was taken before the 40 min intermittent bout each day in the lab. There were no significant differences in weight or fat percentage after 5 d of a high fat load. Data is summarized in Table 1.

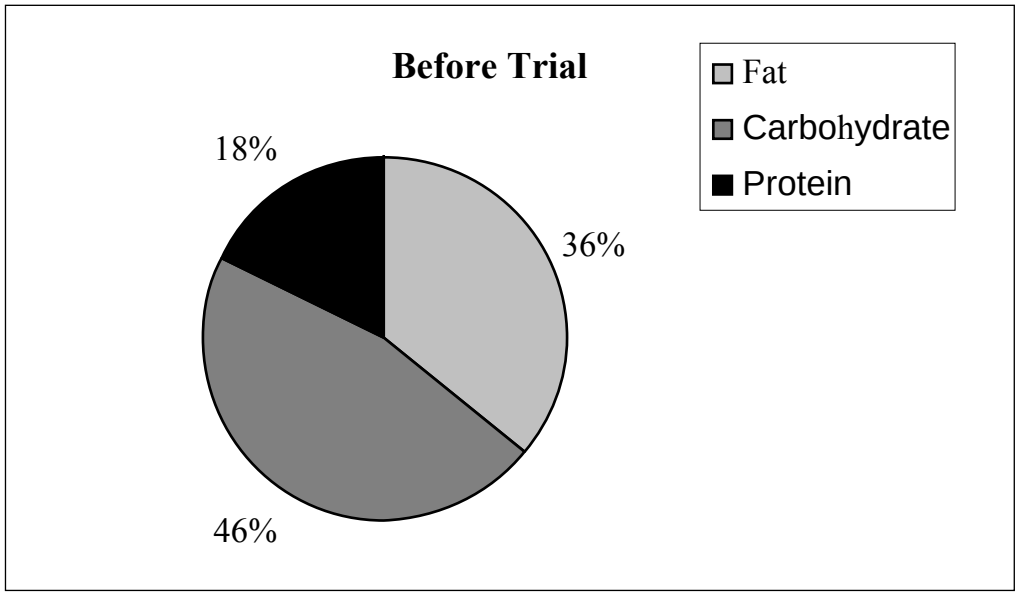
Intake

Food diaries were analyzed for total calories, fat, carbohydrate, and protein for two days before the first exercise trial and for the five days of high fat loading. The subjects reported consuming significantly more calories and fat during the 5 d high fat load. Subjects also reported consuming significantly less carbohydrates and no statistical difference in protein during the 5 d load. Data is summarized in Table 2. Before the trial, the subjects reported consuming 34% fat, 44% carbohydrate, and 17% protein. During the 5 d high fat load, the subjects reported consuming 59% fat, 28% carbohydrate, and 14% protein. Data is summarized Figure 1.

Table 2. Dietary Intake

	Calories (kcal)	Fat (g)	Carb (g)	Protein (g)
Pre	2848 ± 178	106 ± 17	313 ± 23	123 ± 11
Post	3492 ± 75 *	230 ± 4 *	248 ± 9 *	124 ± 7

Values reported as Means ± SE. * Significant difference between pre and post treatment. $P<0.01$.



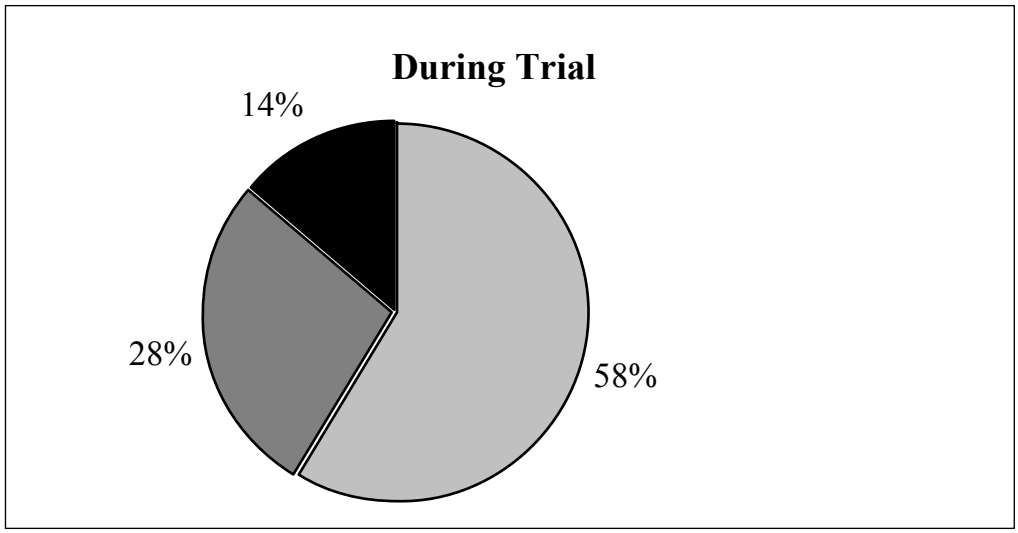


Figure 2. Percent of calories from Fat, Carbohydrate, and Protein before and during trial.

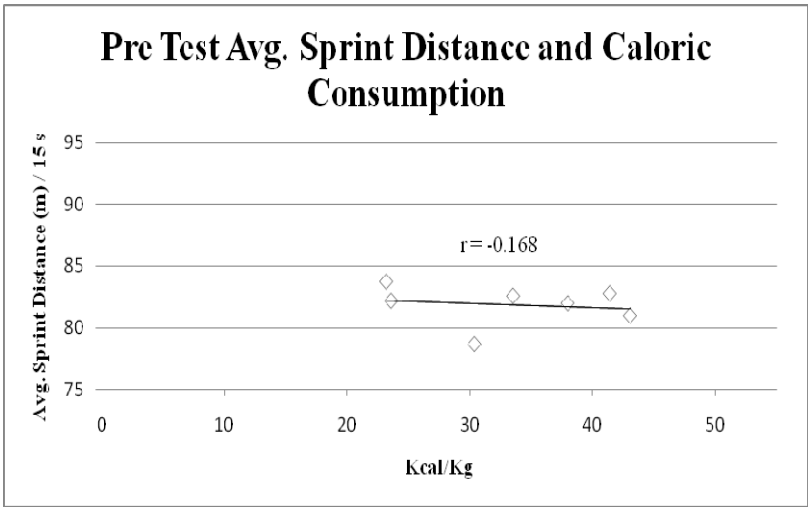
Dietary Intake

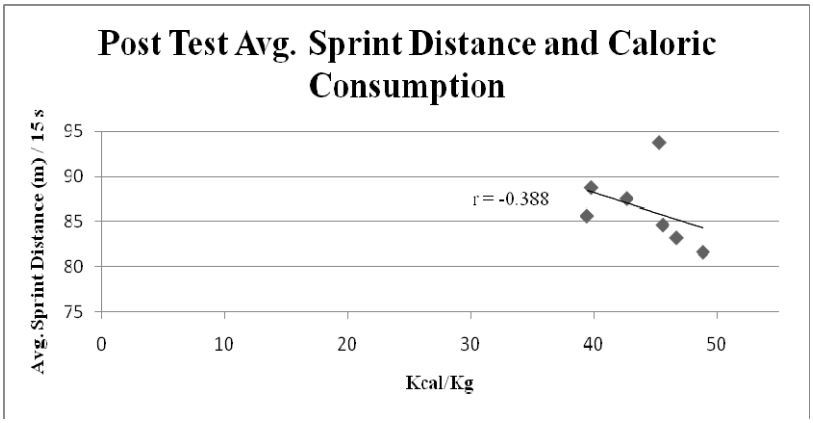
	df	F	P value
Calories	1	15.245	0.000
Fat	1	105.15	0.000
Carbohydrate	1	9.522	0.003
Protein	1	0.007	0.931

Because total caloric intake was statistically higher, correlations were run between total caloric intake and performance as well as fat intake and performance. The data is described following statistic table and in figures 3 and 4.

Performance Pre vs. Post

	df	r	R^2	F	P Value
Kcals/Kg & Avg. Sprint (pre)	1	-0.168	0.028	0.145	0.719
Kcals/Kg & Avg. Sprint (post)	1	-0.388	0.150	0.889	0.39
Kcals/Kg & Avg. Sprint (comb)	1	0.311	0.096	1.281	0.28
Fat & Avg. Sprint (pre)	1	0.172	0.090	0.152	0.713
Fat & Avg. Sprint (post)	1	-0.188	0.350	0.183	0.687
Fat & Avg. Sprint (comb)	1	0.558	0.311	5.412	0.038





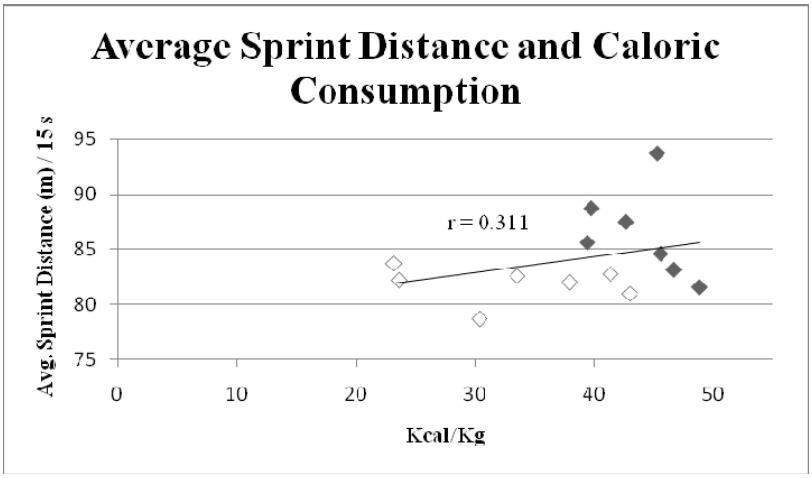
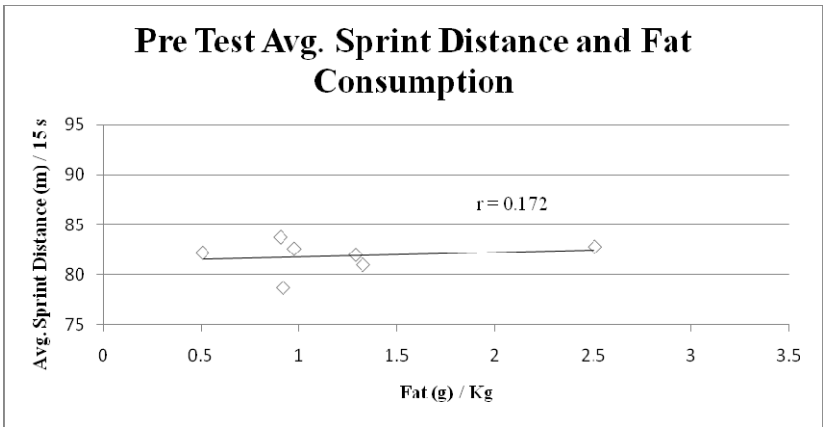
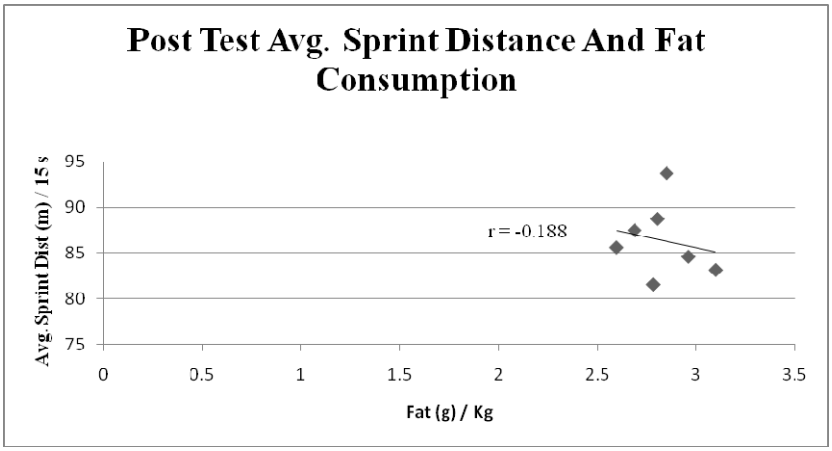


Figure 3. Sprint Distances and Total Caloric Consumption for Pre, Post and Combined. Correlation coefficients (r) values were: -0.168, -0.388, 0.311 respectively.





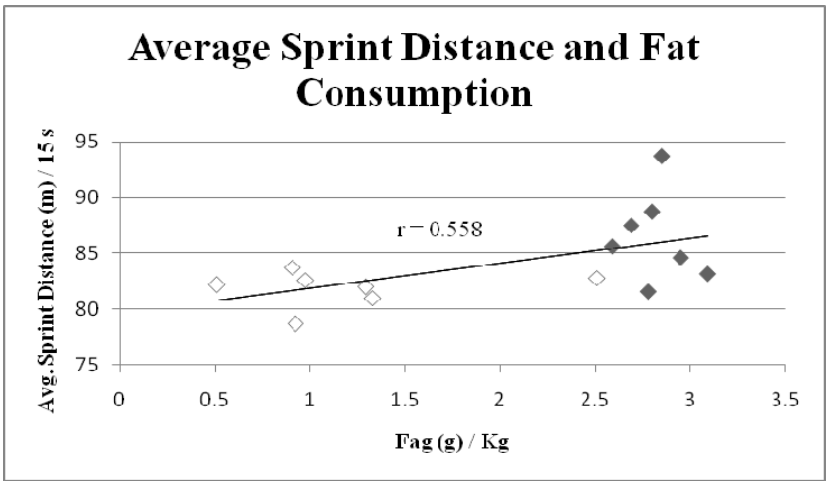


Figure 4. Sprint Distance and Fat Consumption for Pre, Post and Combined. Correlation coefficients (r) values were: 0.172, -0.188, and 0.558 respectively. Combined Pre and Post Fat Consumption only significant correlation $P < 0.05$.

Fat Oxidation

Fat oxidation during the 40 min intermittent bouts are shown in Table 3 and Figure 5. There were significant differences in pre and post fat oxidation. Subjects burned significantly more fat after the high fat intervention (11.4% vs. 4.8%) in the 6:9 s intervals and the 24:36 s interval (10.5% vs. 3.2%). There was no significant difference when comparing 6:9 s and 24:36 s before treatment (4.87% vs. 3.21%) or 6:9 s and 24:36 s post treatment (11.36% vs. 10.54%). The average RER's during the pre test at 6:9 s and 24:36 s were 0.96 and 0.97, and 0.91 and 0.95 during post tests at 6:9 s and 24:36s.

Table 3. Fat Oxidation During 40 min Trial

	6:9s	**24:36s**
Pre		
g/min	0.129 ±0.066	0.082 ± 0.015
Percent	4.8 ± .07	3.2 ± 1.7
Post		
g/min	0.303 ± 0.09*	0.249 ± 0.11*
Percent	11.4 ± 1.1*	10.5 ± 1.2*

Values are reported as Means ± SE. *Significant change from pre to post.
$P < 0.001$

Fat Oxidation pre vs. post

	df	**F**	**P value**
6 : 9 sec	1	23.95	0.001
24 :36 sec	1	10.95	0.011

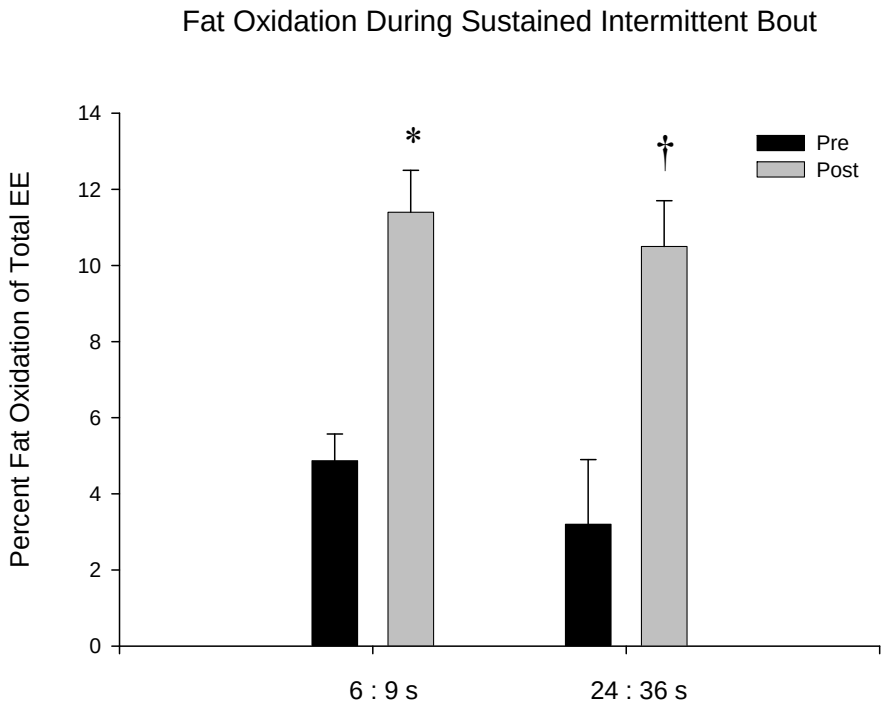


Figure 5. Percent fat oxidation during 40 minute high intensity intermittent bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, P = 0.001 † Significant difference from pre to post testing P = 0.011.

Total Sprint Distance

Sprint distance was measured during the second sprint of each minute in the 8 min bout. A significant difference was observed in total sprint distance measured before and after the 5 d high fat load. Subjects traveled farther during the sprint portion of the 15:15 s intervals after a 5 d high fat load (683 m vs. 648 m). Data is summarized in Table 4 and Figure 6.

Table 4. 8 min performance data

	Pre	Post	% Change
Total Sprint Distance (m)	648.8 ± 4.75	683.2 ± 6.0 †	5.0%
Avg. Sprint Distance (m)	81.1 ± 0.59	85.4 ± 0.75 †	5.0%
Total Distance (Km)	1.71 ± 0.01	1.74 ± 0.01 *	1.8%
Total Peak Power (Km/h)	186.6 ± 1.14	192.1 ± 0.94 †	3.0%
Avg. Peak Power (Km/h)	23.3 ± 0.14	24.0 ± 0.12 †	3.0%
RPE (1-20)	17.1 ± 0.2	16.9 ± 0.2	1.2%

Values are reported as Means ± SE. *† Significant difference between pre and post. * P=0.014, † P<0.001

Performance Pre vs. Post

	df	F	P value
Total Sprint Distance	1	21.91	0.000
Avg Sprint Distance	1	21.91	0.000
Total Distance	1	11.85	0.002
Total Peak Power	1	16.95	0.000
Avg Peak Power	1	16.95	0.000
RPE	1	0.887	0.353

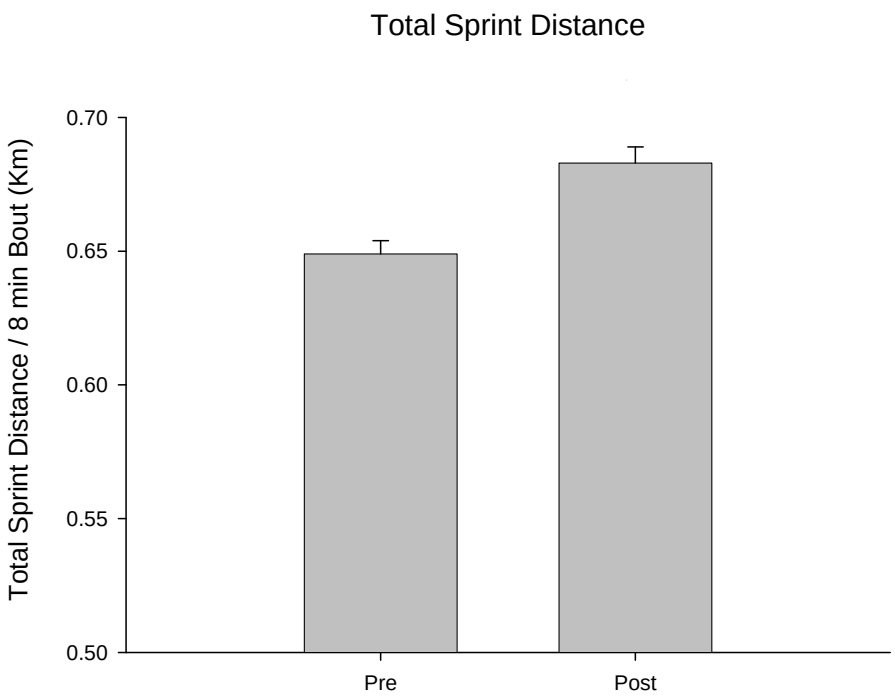


Figure 6. Total sprint distance during 8 min performance bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, $P < 0.001$.

Average Sprint Distance

The average distance traveled during the 8 measured 15 s sprint intervals was significantly greater after the 5 d high fat load. The subjects overall average was 81.1 m in 15 s before treatment and 85.4 m in 15 s after treatment. Data is summarized in Table 4 and Figure 7.

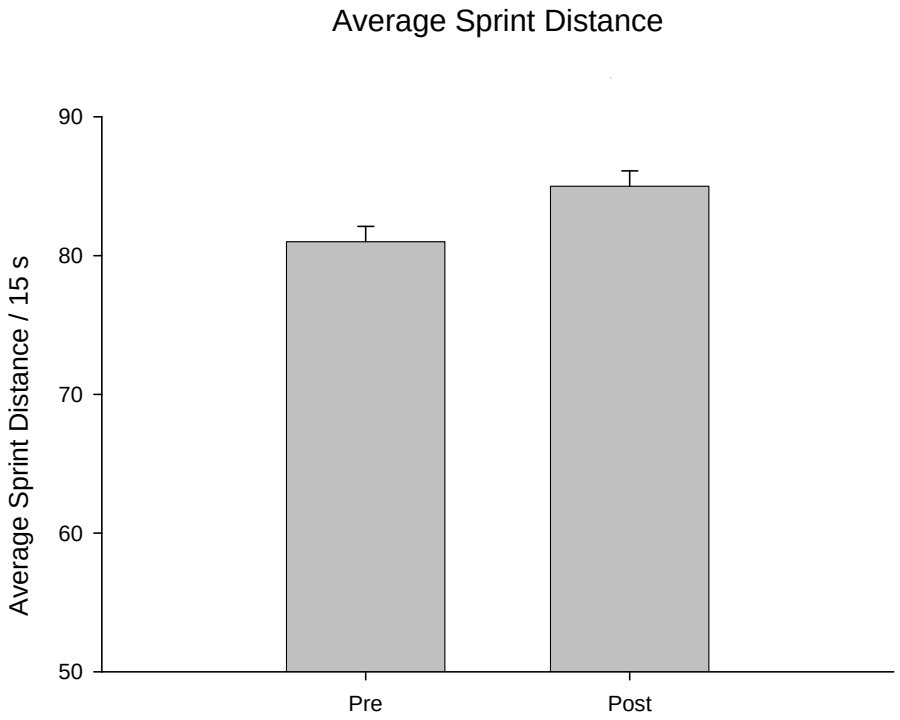


Figure 7. Average sprint distance during 8 min performance bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, $P < 0.001$.

Total Distance

Total distance was derived by subtracting the finishing distance by the distance traveled during the warm up period. This distance included the portion of the bout where subjects were required to follow the cadence 30 rpm guided by a metronome during the non-sprint 15 s interval. This distance was also significantly farther after the 5 d high fat load. Subjects traveled 1.70 Km before the intervention and 1.74 Km after. Data is summarized in Table 4 and Figure 8.

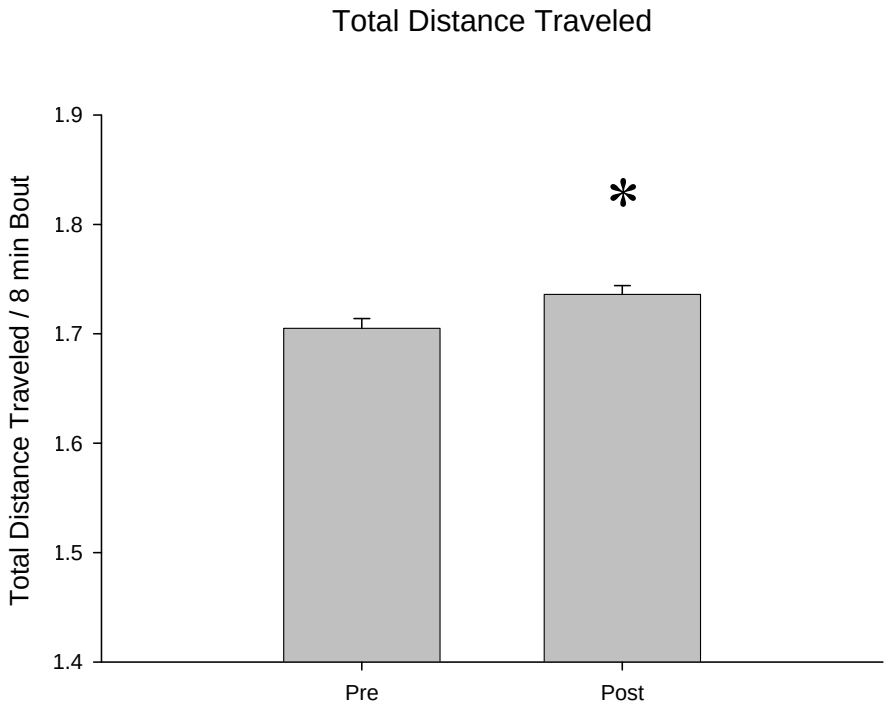


Figure 8. Total distance traveled during 8 min performance bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, P = 0.01

Total Peak Power Output (cadence)

Total peak power output was determined by observing and adding the highest cadence or Km/hour achieved during the second sprint bout of every minute. The total output observed was significantly greater following the 5 d high fat load. Data is summarized in Table 4 and Figure 9.

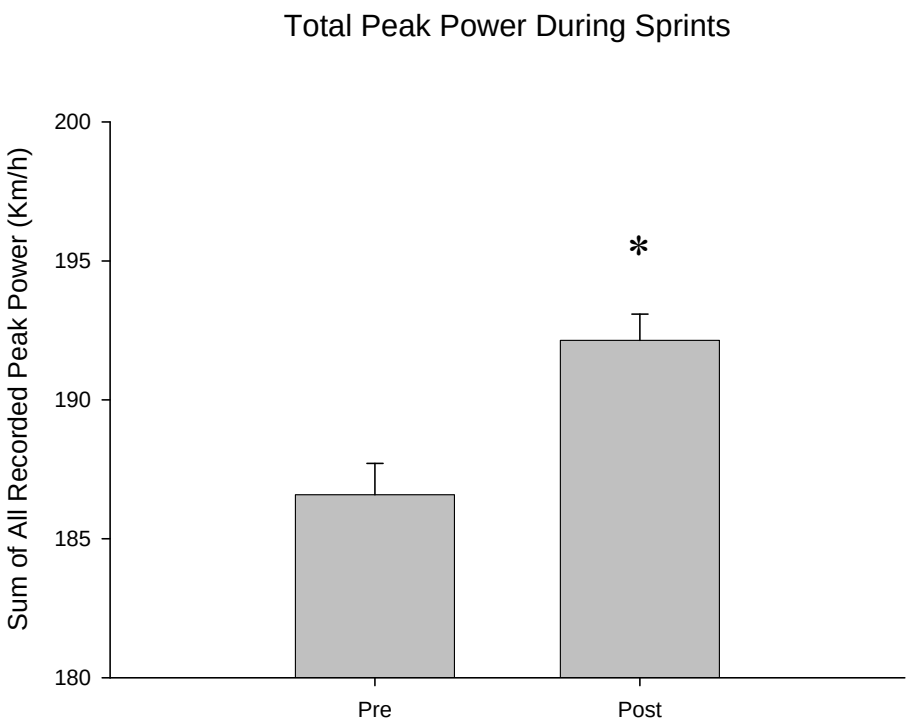


Figure 9. Total distance traveled during 8 min performance bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, $P < 0.01$

Average Peak Power Output (cadence)

Average peak power output was determined by observing and averaging the highest cadence or Km/hour achieved during the second sprint bout of every minute in the 8 min bout. The average peak power output was significantly greater following the intervention (24.017 vs. 23.323) than at baseline. Data is summarized in Table 4 and Figure 10.

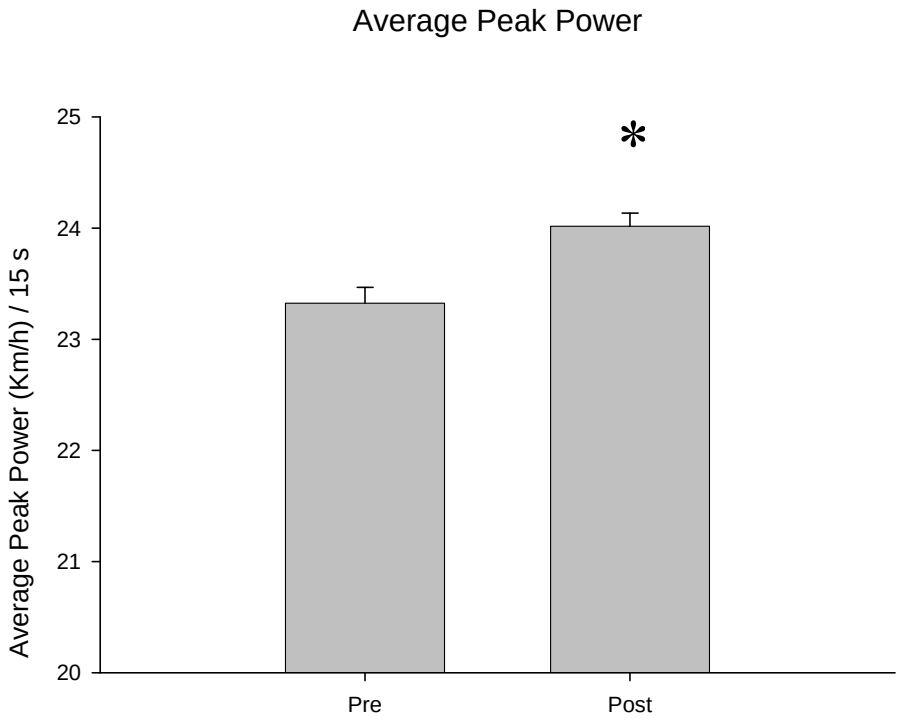


Figure 9. Average peak power measurements during 8 min performance bouts. Values reported as Means ± SE. * Significant difference from pre to post testing, $P < 0.001$

RPE

Rate of perceived exertion was obtained at 4 time points during each 8 min sprint bout. The average RPE was not statistically different when comparing before and after treatment. Data is summarized in Table 4 and Figure 11.

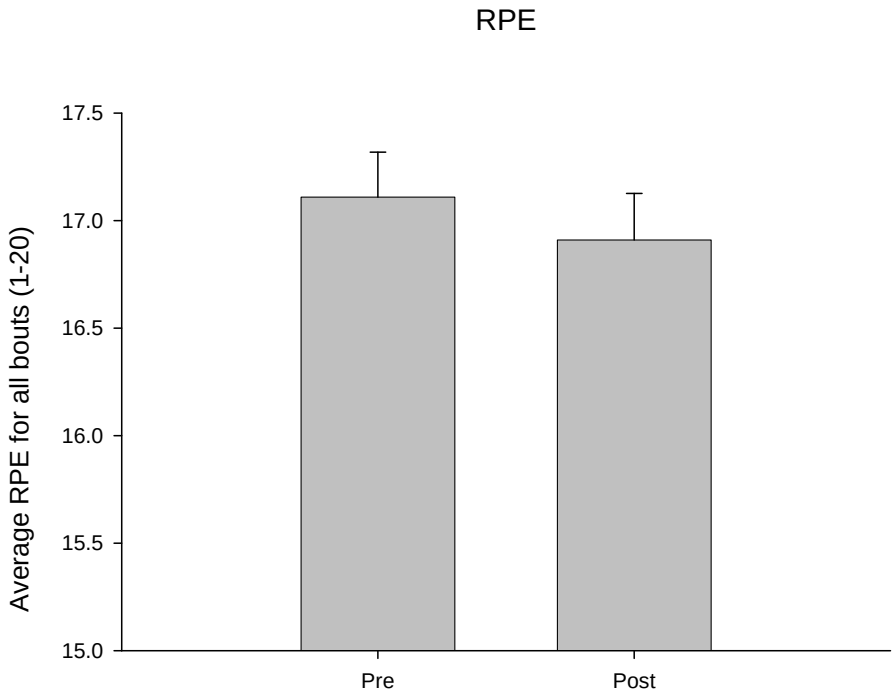


Figure 11. Average RPE. Values reported as Means ± SE. No significant difference was observed.

Lactate

Lactate was analyzed for all 6 blood draws pre and post. No time point was significantly different after a 5 d high fat load. Data is summarized in Table 5 and Figure 12.

Table 5. Glucose, Lactate, and pH

	B40	A40	B3	A3	B4	A4
Glucose						
Pre	99.67 ± 13.07	122.29 ± 12.08	101.63 ± 4.57	124.29 ± 7.88	97.40 ± 4.91	116.83 ± 10.71
Post	103.00 ± 9.46	128.78 ± 3.83 †	103.33 ± 4.00	128.56 ± 3.41	97.57 ± 4.03	131.13 ± 5.49
Lactate						
Pre	3.83 ± 0.92	2.47 ± 0.25	4.48 ± 0.38	15.44 ± 1.04	5.54 ± 0.93	14.33 ± 1.52
Post	3.82 ± 0.75	2.71 ± 0.29	6.62 ± 1.16	15.98 ± 1.40	5.49 ± 0.73	16.59 ± 0.83
pH						
Pre	7.59 ± 0.02	7.62 ± 0.01	7.60 ± 0.01	7.45 ± 0.02	7.59 ± 0.01	7.47 ± 0.03
Post	7.60 ± 0.01	7.63 ± 0.01	7.61 ± 0.01	7.46 ± 0.02	7.59 ± 0.01	7.46 ± 0.02

Values reported as Means ± SE. † Significant difference from pre to post, $P < 0.05$.

Lactate by time point pre vs. post

	df	F	P value
1	1	0.001	0.975
2	1	2.002	0.207
3	1	2.314	0.172
4	1	0.067	0.804
5	1	0.016	0.905
6	1	3.264	0.145

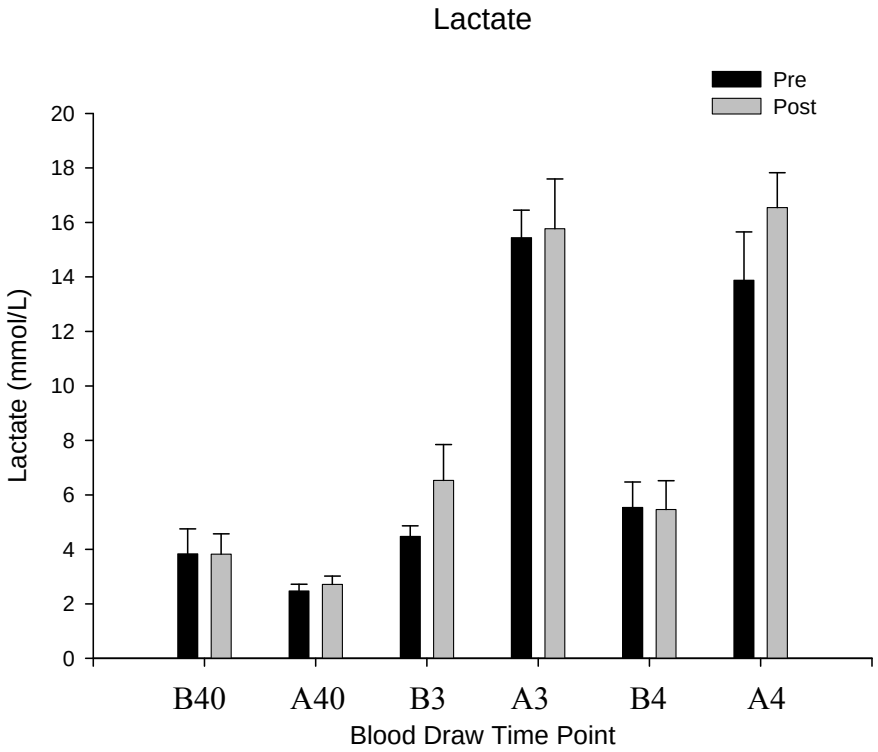


Figure 12. Average Lactate at each time point. Values reported as Means ± SE. No significant difference was observed from Pre to Post at any Draw.

Glucose

Glucose was significantly different after the 40 min sustained bout (A40) in post tests when compared to pre tests $P < 0.01$. No other time point was statistically significant. Data is summarized in Table 5 and Figure 13.

Glucose by time point pre vs. post

	df	F	P value
1	1	0.046	0.836
2	1	32.32	0.001
3	1	0.328	0.585
4	1	0.116	0.745
5	1	0.107	0.760
6	1	0.973	0.380

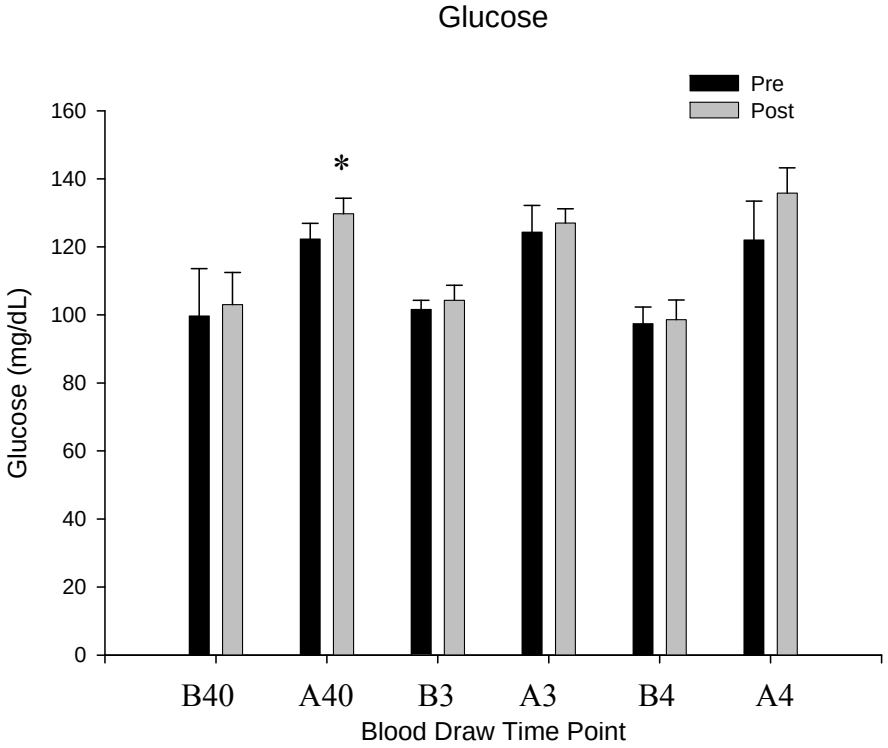


Figure 13. Average Glucose at each time point. Values reported as Means ± SE. * Significant difference from Pre to Post $P < 0.01$.

pH

There was no statistical difference in pH before or after the 5 d high fat load during any of the 6 time points. Data is summarized in Table 5 and Figure 14.

pH by time point pre vs. post

	df	F	P value
1	1	0.214	0.656
2	1	1.357	0.288
3	1	0.44	0.529
4	1	1.437	0.276
5	1	0.143	0.724
6	1	1.750	2.56

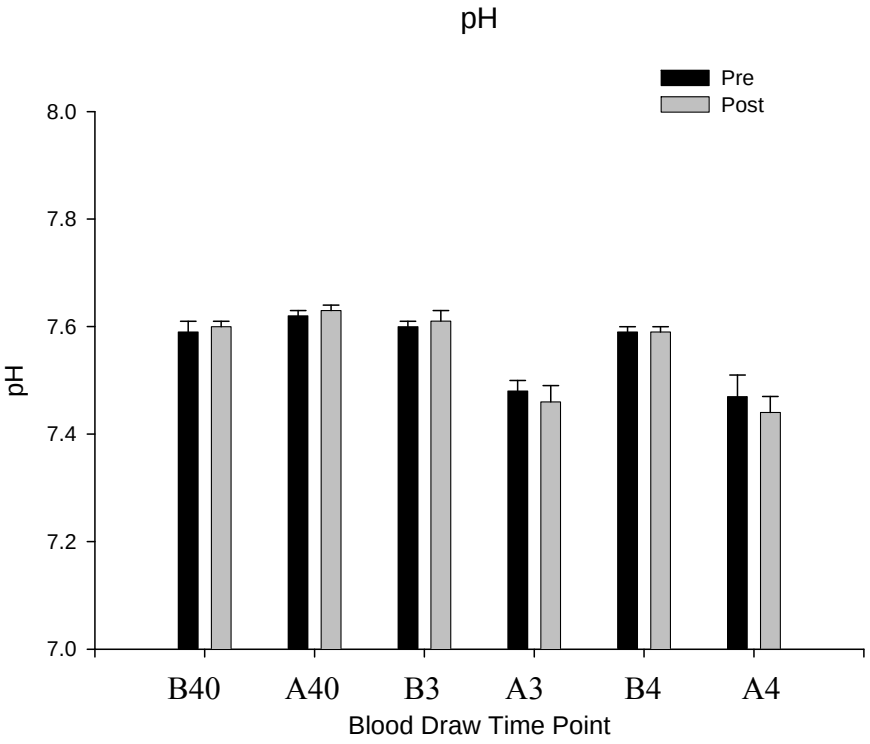


Figure 14. Average pH at each time point. Values reported as Means ± SE. No significant difference was observed from Pre to Post at any Draw.

NEFA

When comparing the same time points before and after intervention, plasma NEFA was significantly lower after performance bout 3 (A3) when compared to pre tests. Before the 40 min sustained bout (B40) and after performance bout 4 (A4) did not show a significant difference after intervention. Data is summarized in Table 6 and Figure 15. B40, A3, and A4 were also examined against each other in pre test intervention and

against each other in post tests. In pre tests the 5 d high fat load, plasma NEFA at A3 and A4 was significantly higher than B40. There was no difference between time point A3 and A4 in pre tests. In post tests however, a significant effect was seen between B40 and A4, but not B40 and A3, or A3 and A4. In other words, A4 was significantly higher than B40, but B3 showed no statistical difference from B40 or A4. Only pre to post data is summarized in Table 6 and Figure 15.

Table 6. NEFA (mEq/L)

	1	**4**	**6**
Pre	0.297 ± 0.015	0.854 ± 0.079	0.839 ± 0.286
Post	0.374 ± 0.065	0.535 ± 0.047 *	0.651 ± 0.095

Values reported as Means ± SE. * Significant difference from Pre to Post, $P < 0.05$.

NEFA by time point pre vs. post

	df	**F**	**P value**
1	1	1.593	0.247
4	1	9.259	0.023
6	1	1.038	0.383

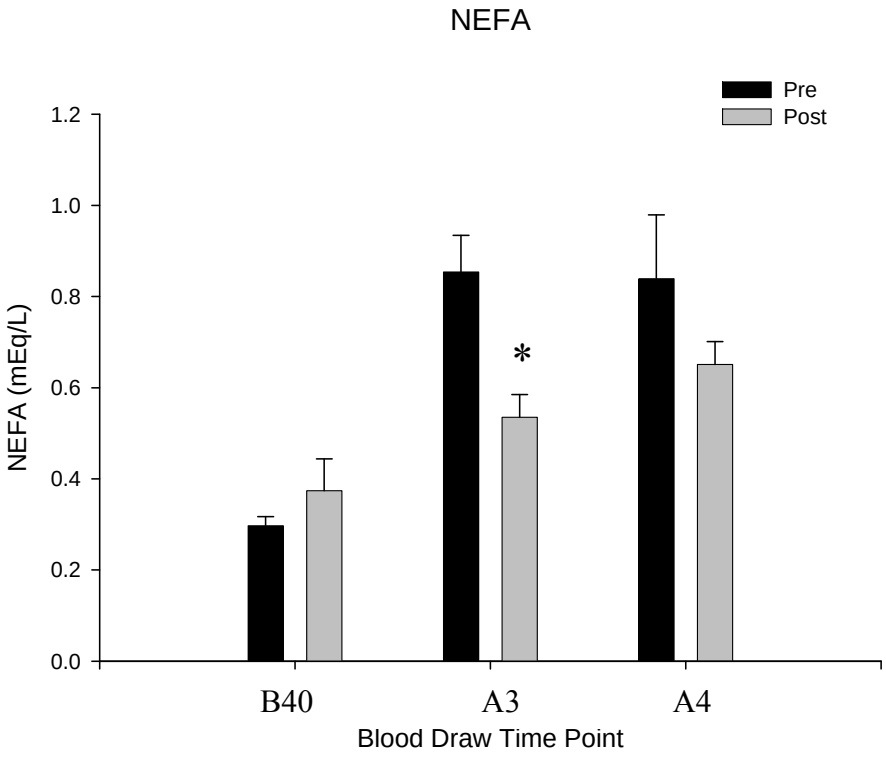


Figure 15. NEFA at selected time points. Values reported as Means ± SE.
* Significant difference from pre to post testing, $P < 0.05$.

DISCUSSION

Subjects and Intake

As reported in the results, the weight and body composition of the subjects after 5 d of high fat loading did not change. Subjects documented all food and activity for two days before and during the 5 d trial. During that time, subjects achieved a 59% total fat intake which was in the range desired. According to their food journals, the subjects consumed the shakes during regular meal times while consuming a scaled down version of a normal diet. The subjects also participated in normal wrestling and weight lifting

practices except for the 36 h before baseline and post testing in which they refrained from heavy physical activity.

The fact that the subjects consumed more total calories during the 5 d high fat load raises some suspicion that increased intake in general, not just monounsaturated fat, could have led to the improved performance. An argument could be made that the subjects were in a negative energy balance before the 5 d high and the increase in fat simply led to a neutral or positive energy balance providing them with more energy to complete the rigorous exercise protocols before fatigue set in. The negative correlations in pre trail diet analysis and during trial analysis suggested that total calories per Kg did not play a role in improved performance, however, the positive correlation when looking at combined data suggested that it could. The only significant finding was in combined fat intake and average sprint performance ($P < 0.05$). It was therefore confirmed that the high fat intake had the larger role in increasing performance in the current trial than total caloric consumption.

Fat Oxidation

Fat oxidation was markedly increased during both 6:9 s and 24:36 s sustained intermittent exercise after the 5 d high fat load. In regards to the hypothesis that 6:9 s would oxidize more fat than 24:36 s at baseline, there were no significant differences observed. The intervals of 6:9 s and 24:36 s were used in order to compare our data with Christmass et al. who observed a three fold greater fat oxidation at 6:9 s than at 24:36 (7). The values of percent fat oxidation found in both 6:9 s and 24:36 s in post tests were similar to those seen at 6:9 s by Christmass (7). The percent oxidation observed in the

current study in the pre test at both 6:9 s and 24:36 s were similar to the lower values observed in 24:36 s intervals in Christmass' study.

Christmass et al. concluded that his subjects burned significantly more fat at 6:9 s due to a higher observed value of plasma lactate and pyruvate accumulation in the 24:36 s intermittent portion of the study. This increase in plasma lactate and pyruvate correlated with reduced O_2 availability validated by near infrared spectroscopy. The reduced intramuscular O_2 in turn led to a reduced utilization of Type I muscle fiber and thus a reduced fat oxidation in the 24:36 s bout portions of the bout.

An explanation for the discrepancy is that our subjects as trained wrestlers likely contain a lower percentage of type I muscle fiber than the distance runners used in the Christmass et. al. (6,7) studies. The pre test values of 4.87% and 3.21% fat oxidation for 6:9 s and 24:36 s respectively suggest little use of Type I fiber during the sustained high intensity intermittent bout. However, once the Type I fibers were loaded with IMTG after the 5 d high fat load the wrestlers burned 11.36% and 10.54% fat, similar to values observed by Christmass et. al. (7), suggesting a greater utilization of the fat loaded Type I fibers they did have.

To explain one possible metabolic regulatory factor within the muscle fiber that could be responsible we can turn to O_2 (VO_2) kinetics. The preferred method to determine oxidative fuel utilization during exercise is steady state indirect calorimetry. The limitation to this method is the steady state requirement. Whipp et al. (64) introduced VO_2 kinetics during non steady state exercise providing the subject remains below lactate threshold intensities (64). Three phases were isolated. Phase I involves a rapid rise in pulmonary VO_2 during the first 15-20 s of exercise. In phase II VO_2 rises

exponentially over the next period of a few seconds to a few minutes to a steady state (phase III). Phase II and III VO_2 kinetics can be used to represent the sum of fuel usage in the working muscle.

Mole and Hoffmann described and quantified the dynamics of fat and CHO oxidation over the first 5-10 m of mild exercise (41). They were able to calculate a biexponential model accurately describing phase II VO_2 kinetics over a wide range of sub 1.0 RER's. In their investigation Mole and Hoffmann isolated a fast and slow component in their model representing fat and CHO oxidation. The fast (fat-ox) component was inversely related to RER and the slow (CHO-ox) component was positively related to RER. As intensity increases, so too does RER supporting the fact that fat oxidation decreases with high intensity steady state exercise. However, in addition to their biexponential fat-ox and CHO-ox mode, they found that IMTG oxidation during phase II could respond more quickly than CHO when a step increase in work rate is encountered. Intermittent exercise is essentially reoccurring step increases and decreases in work rate. In the current study, the subjects were asked to go from a slow steady cadence on the elliptical machine to maximal effort for 15 s. If the theories of Mole are applied to the current investigation, we can suggest a higher IMTG oxidation with a step increase in work rate, or at the onset of every single sprint bout in the performance test protocol.

Mole and Hoffmann (41) also observed metabolic adaptations to a high fat diet which increased the duration or length of theVO_2 fast component meaning their subjects spent longer time in phase II but not because of an increase in the slow component or CHO oxidation but rather a more abundant supply of fuel for the type I fibers before reaching a steady state. This can be because the step increase in work rate at the initial

onset of exercise was managed more by the super compensated IMTG oxidation in Type I muscle fibers than those reporting a normal fat diet. All this suggests that the dynamics of intramuscular fat oxidation can be immediate at the onset of high intensity exercise or at every step increase in work rate thereafter.

Several researchers have expanded on the findings of Christmass looking at exercise intensity and phase II kinetics. Kappo et al. (31) examined phase II VO_2 kinetics at varying outputs. The findings revealed progressively slower kinetics (subjects took longer to achieve a steady state) at higher outputs in trained and untrained subjects meaning they utilized more type II motor units and not as many type I motor units as intensity increased. This is supported by Pringle et. al. (47) who found a negative correlation between the primary time constant or time spent in phase II kinetics and percentage of type I fibers. However, Pringle also demonstrated a higher primary gain (Δoxygen uptake/Δwork rate) with a higher percentage of type I fibers at moderate, heavy, and severe exercise suggesting that those with a higher percentage type I fibers achieved more work per mL O_2 per min even at severe intensities. It has even been proposed by Whipp that the recruitment of low-efficiency type II muscle fibers during heavy exercise is a likely cause of reduced mechanical efficiency, again suggesting type I fibers may be beneficial to use at high intensities (64). Finally, it has been found that slower phase II kinetics or a larger VO_2 slow component than fast component are predicted to have a detrimental effect on performance during heavy exercise (46). When combining the findings of Christmass, Mole and Hoffman, Kappo, Pringle, Whipp and Pool a theory arises and suggests that the IMTG rich type I fibers can and do supply more power output than previously thought at the initial onset of heavy exercise especially if it

is short in duration and intermittent helping to explain the mechanism behind the current investigation.

Performance

Following a 5 d high fat load, this study found an increased performance in every parameter measured. Total sprint distance, average sprint distance, total distance traveled, total peak power output, and average peak power output were all enhanced after the 5 d high fat load. The current results suggest that the underlying mechanism that is able to shift the widely accepted theory of the muscles' preference for CHO at high intensity to fat-ox may also be capable of improving high intensity intermittent performance.

Few researchers have examined performance after a high fat load. Burke et al. (5) found metabolic adaptations toward fat oxidation (RER values dropping from 0.90 ± 0.01 to 0.87 ± 0.01 ($P < 0.05$) and fat oxidation from 61 ± 5 g/2h cycle to 94 ± 6 g/2h cycle ($P < 0.05$)) during a 2 h sustained cycling bout at 70% VO2 max after a 5 d high fat load. The time trial performance following showed a tendency toward a faster time after a 5 d high fat load (30.73 ± 1.12 vs. 34.17 ± 2.48 min) but the statistics failed to reveal a significant difference ($P = 0.21$). The time trial to determine performance however was performed immediately after 2 h of cycling at 70% VO_2 max. These results are then specific only to cyclist performance after a 2 h bout at 70% VO_2 max.

Osborne et al. took a different approach to the question by deliberately reducing the glycogen content of type I fibers by using a previously proven method of sustained low intensity exercise (44). Osborne et al. then looked at blood lactate, pH and power output by the vastus lateralis and vastus medialis the following day over an eight minute

performance test at 50% maximum output and 75 rpm. The results showed a significant drop in blood lactate in minutes six and eight as well as a significant increase in pH in minutes three through eight. The muscle power output proved to be higher at minutes five through eight in the performance bout as well. Osborne attributed the differences to increased fat metabolism in and greater phase II amplitude in type I muscle fibers after being glycogen reduced.

Although Osborne et al. showed a higher fat metabolism, lower lacate, higher pH, and increase muscle force production after reducing type I muscle glycogen, no mention was made to distance traveled during the eight minute performance bout. It seems that the true parameter of performance in a standard cycle or running test should be distance traveled in a given time point, not just power output, VO_2 and blood lactate or pyruvate levels. In the present study, it was displayed that power output as well as distance traveled can be increased by a 5 d high fat load.

The mechanism behind the improved performance can also be attributed to VO_2 kinetics. The intermittent nature of this trial lent itself to a continuous step increase and decrease in work rate. Research by Mole et al. in phase II VO_2 kinetics suggests that it was the IMTG in type I muscle fiber that was responsible for the fast component when a step increase in work rate was applied (41). Mole showed that even as RER values approached 1.0 the fractional contribution of fast kinetics or fat-ox in phase II were as high as 35% (41). In their findings Mole et al. also found a slower rate in achieving phase III or steady state and a reduction in the slow component or CHO-ox when the subjects were fat loaded. As mentioned above, Kappo et al. provided evidence that heavy intensity and increased training slowed phase II VO_2 kinetics even further (31) . The

extent of training in the subject and the high intensity and intermittent nature of the present trail did not allow the subjects to achieve phase III kinetics or a steady state in their 8 min bout. Therefore they were repeatedly if not constantly in phase II kinetics; a state type I fiber percent increases the primary gain (47) and where IMTG have been show to provide a quicker and nearly exclusive percent of fatty acid oxidation in those type I fibers (41).

When examining the current results the multiple bouts performed by the subjects must be taken into account. Two researchers have examined VO_2 kinetics in heavy exercise following prior heavy exercise (15, 45). Both studies set out to examine if the change in VO_2 kinetics was due to an increased blood flow to the working muscle after the first bout. Endo et al. found consecutive six minute bouts of heavy bilateral knee extension at a rate halfway between the lactate threshold and VO_2 peak separated by six minutes of 10 watt cycling elicited a reduced slow component or reduced CHO oxidation in phase II despite no enhancement in femoral blood flow (15). Because O_2 delivery exceeded demand, the team suggested a mechanism other than O_2 delivery more proximal to the muscle tissue. Paterson et al. found similar results in a similar study examining two six minute bouts of heavy intensity one legged knee extension separated by six minutes of loadless exercise. Phase II VO_2 kinetics were increased, suggesting higher percent of type I fiber use, again despite no difference in limb blood flow. Paterson et al. also ruled out increased limb blood flow as the underlying mechanism behind increased VO_2 response after prior heavy intensity exercise.

Combining all the evidence mentioned, it can be surmised that the increased performance observed in the present study may be due to increased type I muscle fiber utilization and IMTG oxidation in the working muscle.

Lactate and pH

The present study found no difference between pre and post tests in lactate or pH after performance bouts three and four. Lactate levels were significantly higher and pH levels were significantly lower immediately following performance bouts 3 and 4 (comparing B3 to A3 and B4 to A4) as expected. It was hypothesized that lactate levels would be lower in post tests due to increased utilization of IMTG as a fuel source in type I fibers. This effect was not observed. The explanation for this effect is that the subject showed an increase in performance, thus had a higher work output in post tests than pre tests. As explained above, the increased work can be attributed to increased type I fiber use. As Endo et al. and Paterson et al. displayed O_2 delivery was not limiting in phase II kinetics, therefore type I fibers would be able to dominate over type II when called upon in short duration high intensity intermittent exercise (15, 45). We can then speculate that type II fibers worked at the same rate and intensity as in pre tests, so the unchanged lactate levels should be expected. It would be interesting to see if lactate levels would be reduced and pH would be increased if work volume was matched not increased, but that was not the aim of this study. The primary objective was to examine if performance could be increased following a 5 d high fat load, not to see if lactate would be reduced.

Glucose

Although the subjects reported consuming a diet consisting of 59% fat 28% carbohydrate and 14% protein for 5 d, plasma glucose was unchanged from pre to post at

B40, B3, A3, B4, A4 and significantly higher at A40 (after 40 min sustained bout). As expected, plasma glucose did rise statistically higher in both pre and post tests after each exercise bout (comparing A40 to B40, A3 to B3 and A4 to B4), but again, there was no observed difference in that rise between pre and post intervention.

Subjects were asked to consume half the amount of the same meal the night before post testing as they did the night before pre testing along with their high fat shake. They were fed identical amounts of commercially available nutrition bars consisting of 40:30:30 CHO:fat:protein and sports drink during before post testing as they were before pre testing.

To explain the plasma glucose results, we look at the normal response to high intensity exercise, release of glycogen and increased gluconeogenesis from hepatic cells to replace stores in the muscle. The 40 min sustained intermittent bout was matched to pre test outputs and the subjects burned significantly more fat during that test, thus setting up the potential to spare muscle glycogen. Because muscle stores were not measured via biopsy before and after this or any other test, it is hard to quantify the glycogen sparing effect seen in these results. If taken at face value however the higher blood glucose after the 5 d high fat load following the 40 min sustained intermittent bout would suggest a slight glycogen sparing effect of the intervention.

In the performance bouts it is theorized that phase II VO_2 kinetics increased IMTG loaded type I muscle fiber use to increase power output. This increase in IMTG use did not slow the release or cellular absorption of glycogen into the blood stream. The rise and fall in plasma glucose is the expected normal response to such high intensity exercise. It is well known that glucose transport and glycogen synthase activity are

elevated in response to exercise (49). It is also known that insulin sensitivity is enhanced after exercise (40). Therefore glucose is rapidly released, circulated and then absorbed with great efficiency after intense exercise. The importance of this response, the observation that those involved in regular physical activity develop an enhanced ability to store glycogen, and the fact that glycogen depletion is highly correlated with the onset of fatigue is evidence to the significant contribution of glycogenolysis in exercise metabolism, especially at high intensity (25, 3).

Although the primary objective of this study was to examine fat oxidation and performance, one hope was that an increased fat oxidation would spare hepatic, plasma or muscle glycogen for later use. Although plasma glucose was significantly higher after the 40 min bout after the 5 d high fat load, because no biopsies were taken, we cannot speculate on this question. It can however be said that a 5 d diet consisting of 58% fat and only 28% CHO had no observed lowering effect on plasma glucose either at rest or after one 40 min bout and four 8 min bouts of high intensity intermittent exercise.

NEFA

Comparison of NEFA by draw after the 5 d high fat intervention revealed similar results in B40, significantly lower values in A3 (after performance bout 3), and similar results again at A4 (after performance bout 4). Early work on the topic of fat utilization in intermittent exercise showed substantial lipid usage at intensity levels that would be CHO dominated if continuous (16). Similar NEFA availability during high intensity intermittent exercise at 6:9 s and 24:36 s (which were reproduced in the current study) have also been observed (7). The authors found a decline in fat oxidation during 24:36 s

intervals compared to 6:9 s despite similar NEFA availability. Combined with research that the NEFA levels they did observe in the 24:36 s portion of the trial were above values suggested to impair fat oxidation, Christmass et al. determined again that a metabolic factor within the oxidative muscle fiber to be responsible for the difference seen in substrate utilization (52,7).

Because it has been shown that IMTG oxidation appears to be influenced by baseline profile or storage level, the current investigation aimed to discover whether NEFA would be similar post high intensity intermittent after a 5 d high fat load (16). The current investigation was based on the belief that muscle metabolism would place a preference on the most proximal source, IMTG. Kanaley et al. found that especially at high intensities above LT, IMTG accounted for more fat oxidation than FFA's (30). The results we observed with NEFA staying statistically similar to baseline values after the 40 min bout and two 8 min bouts at A3 would support this notion. In pre tests, NEFA displayed a large stair step increase from B40 to time points A3 and A4 whereas in post tests, the increase in NEFA appeared gradual or linear from B40 to A3 then to A4. The interesting finding that NEFA was similar at A4 reveals the thought that by the end of all exercise protocols, (40 min sustained intermittent test and four separate 8 min performance bouts) the IMTG stores had been used up.

Through muscle biopsy and subsequent Western blotting, Roepstorff et al. observed HSL activity at rest, after 30 min and after 60 min of bicycle exercise at 65% VO_2 peak (51). The main objective of the investigation was to determine which Ser phosphorylation site on HSL was most active. One interesting finding was that at 30 min of exercise, mean HSL activity was 117% that of baseline, but at 60 min of exercise

levels had returned to those observed at baseline. The authors suggested that the IMTG hydrolysis occurred primarily in the first 30 min of continuous exercise at 65% VO_2 peak. The theories of VO_2 kinetics mentioned previously would support this suggestion. Once the subject achieves a steady state or phase III, the fast component which is positively correlated with IMTG-Ox, type I fiber usage, and RER (41) would slowly reduce as the slow component and steady state RER values associated with 65% VO_2 peak took over.

The difference with the present study is again a lack of achieving a steady state during the intermittent performance bouts. Assuming the 40 min sustained intermittent bout and the first three 8 min performance bouts began depletion of IMTG stores due to the constant step increases in work rate and fast component activation, it would appear that in pre tests, NEFA was higher at A3 (after third performance bout) to attempt to replace IMTG whereas in post test at A3 there was enough IMTG left over or regenerated from FFA during the hour long breaks between bouts to not need a significant increase in NEFA. Because the NEFA values are statistically similar after performance bout 4 (time A4) the idea arises as to whether the IMTG stores were finally used up in post testing as it would have been in pre testing.

Limitation to the Study

There were a few limitations to the present study. Concerning the subjects, there was no control group available to perform the tests. It is always a concern of a learned behavior or protocol adaptation when using a testing method unfamiliar to the subjects. The placebo effect must also not be overlooked. The subjects were expecting to do better on the post testing therefore their competitive nature could have affected their efforts. The elliptical machine was chosen because all subjects participate in high intensity

workouts on the elliptical machine on a regular basis in their training as well as the fact that total body activation during testing was desired. The learned adaptation to the equipment or protocol then hopefully was kept to a minimum by using the make and model of elliptical machine the subjects regularly train on. Secondly concerning the subjects, having them self monitor and report daily intake of food was a risk that had to be taken. It is understood that self monitoring and reporting of dietary intake is not always performed accurately, but limited funding made this a necessity. It is believed that daily delivery and collection of high fat shakes and inquiries to the subjects regarding their intake and quantity was sufficient to produce accurate estimates of intake.

Regarding the design of the study, it would have been highly beneficial to obtain muscle biopsy before and after treatment and exercise. The risk to the subjects was one the department was not willing to take considering the requirement that the subjects continue to practice in direct physical contact with other athletes as is required in their sport. Future research on this topic would provide greater evidence of IMTG loading and usage if biopsies were obtained.

Summary of Results

The results suggest that wrestlers have a greater fat oxidation in a 40 min sustained high intensity intermittent exercise after a 5 d high fat load. There was a significant improvement in 4 separate 8 min high intensity intermittent performance bouts in total sprint distance, average sprint distance, total distance traveled, total peak power, and average peak power in the current investigation as well. These results demonstrate no difference after a 5 d high fat intervention in plasma lactate, glucose, or pH except for glucose after the 40 min intermittent bout (A40). Finally, the results

suggest that IMTG was used because significantly more fat was oxidized during the 40 min sustained intermittent trial however plasma NEFA was lower after three of the four 8 min performance bouts (A3). The results would also suggest that after the fourth and final performance bout, IMTG could have been used up as plasma NEFA levels were elevated to levels seen before the 5 d high fat diet.

Conclusion

Five days of high fat loading consisting predominantly of monounsaturated fat increases fat oxidation without reducing glucose availability and improves performance in high intensity intermittent exercise designed to simulate a wrestling tournament in trained wrestlers.

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APPENDIX A

EXTENDED LITERATURE REVIEW

VO_2 Kinetics and Performance

Working muscle requires oxygen, that O_2 uptake has been deemed VO_2 kinetics. Three phases of VO_2 kinetics have been separated in exercise below lactate threshold (64). Phase I represents the rapid rise in pulmonary VO_2 in the first 15-20 s of exercise. This phase was termed the cardiodynamic phase due to the rapid delivery of O_2 poor blood to the lungs for exchange. Phase II displays an exponential rise in VO_2 until a steady state is observed and can last anywhere from 30 s to several minutes depending on the mode and intensity of exercise. The steady state has been termed phase III. One remains in phase III until a step increase or decrease in intensity is encountered. Most research has focused on phase II and III kinetics because they represent the sum of metabolically active tissue oxidation.

There are two portions to the phase II whole, the fast component and the slow component. The fast portion has been found to be negatively correlated with RER and the slow portion positively associated with RER. Mole provided a reliable biexponential model to represent fast or fat and slow or CHO sub portions in phase II kinetics (41). The proportion of the fast component and slow component in VO_2 on-kinetics appear to be dependent on several factors.

In vitro studies have demonstrated that O_2 delivery does not appear to influence rest to exercise transition at a moderate intensity of 60% VO2peak (20) and several human trials have demonstrated no difference in VO_2 kinetics with an increase in capillarity at moderate, heavy, or severe intensities (47) or O_2 availability up to 80% VO_2 peak (31). It has however been reported that VO_2 on-kinetics slow during heavy intensities when inspiratory hyperoxia was administered (37) and that enhancing

convective O_2 delivery in dog gastrocnemius preparations increased VO_2 on-kinetics at VO_2 peak (20). It is therefore possible that O_2 availability may affect VO_2 kinetics during near maximal exercise.

Prior exercise may also affect VO_2 kinetics. Paterson et al. had subjects performing heavy knee extension exercise for 6 min followed by 6 min of rest then another 6 min bout of heavy knee extension in efforts to observe changes in VO_2 kinetics (45). The results demonstrated accelerated overall VO_2 kinetics (5 s or 18% faster) by increasing the fast component involvement with no change in the slow component. Paterson et al. also found O_2 delivery to exceed O_2 consumption in both 6 min trials meaning that the change was not due to O_2 availability. Other research by Endo et al. (15) with nearly identical methods also demonstrated a speeding effect on the overall phase II kinetics, but observed a reduction in the slow component. It therefore appeared that prior heavy exercise sped VO_2 kinetics either by increasing immediate fat oxidation or reducing immediate CHO oxidation.

The type of muscle fiber present appears to play a larger role in primary VO_2 kinetics. Pringle et al. found a significant negative correlation with type I muscle fiber percent and a significant positive correlation with type IIX fiber percent for heavy and severe exercise. This meant that the more type I fibers present, the faster the VO_2 kinetics were. Pringle postulates this relationship with either potential difference in O_2 delivery due to capilarization differences or oxidative enzyme activity between the fiber types. His studies as well as others (31, 45, 15) however confirmed capillarity or O_2 availability not be correlated with the primary VO_2 component in high intensity exercises over ventelatory threshold (VT) in human subjects.

This then leaves the potential for the enzymatic differences between type I and type II fibers to be the mechanism behind on-kinetics differences observed in high intensity exercise in humans. Although several attempts have been made to determine the exact mechanism of faster VO_2 on-kinetics in type I fibers in exercise over VT, it has yet to be determined. Pringle and others suggest the answer lies in the differences in pyruvate dehydrogenase complex, tricarboxylic acid cycle, or electron transport chain between type I and II fibers.

Available fuel may also be an area to investigate. Mole's biexponential model of VO_2 kinetics demonstrated that fat oxidation is correlated with the fast component and CHO oxidation is correlated with the slow component in phase II kinetics (41). It has also been displayed that trained individuals have a greater fast component gain and therefore a faster phase II on-kinetic response than untrained (31). This idea that fat not carbohydrate may be the preferred fuel source at the immediate onset of exercise in trained individuals has been displayed even higher intensities. Pringle et al. (47) demonstrated a shorter phase II thus increased fat oxidation with increased type I fiber cross section percentage. Additionally, Pringle found a higher primary gain (O_2 ml/min per unit work) in individuals with a higher distribution of type I fibers at moderate, heavy, and severe exercise intensities. This may at first seem counterintuitive, requiring more O_2 per unit of work or power, but trained individuals have a larger capacity to process that O_2 in the working muscle than untrained. The increase in absolute power seen in trained individuals by Kappo when normalized relative to the change in power output demonstrated the trained subjects had higher fast component gains. Kappo et al. suggests that even during heavy exercise in which type I and type II fibers are likely to be

recruited, trained subjects will recruit proportionately more type I fibers than untrained subjects and the on-kinetics will be faster, suggesting higher fat oxidation.

VO_2 kinetics and dietary intake

One interesting finding by Mole et al. (41) was that RER at mild exercise was not related to cycling power output, but to dietary CHO intake ($P < 0.01$). This was also supported by preliminary work to the study where subjects consumed a high fat or carbohydrate diet and VO_2 kinetics were changed. There was an increase in the fast component with a fat diet and an increase in the slow component in the high CHO diet. This evidence further supported Moles' phase II substrate usage model. Osborne et al. (44) looked at this from a different angle. Instead of loading fat or CHO before testing, he reduced the glycogen content of type I fibers by having his subjects perform a long slow cycle bout previously shown to severely deplete type I fiber glycogen. He then had his subjects perform an 8 min high intensity cycle bout and observed the differences to previously recorded controls with the same subjects. His studies revealed several interesting findings: a lower lactate at min 6-8, a higher mean power frequency developed from EMG on Vastus Lateralis and Vastus Medialis at min 5-8, a higher VO_2 amplitude or fast component, but no change in the total phase II VO_2 time.

This suggested a two part explanation. One, that the reduced CHO availability in the type I fibers led to increased fat oxidation in type I fibers which Mole and Hoffman (41) have shown to be IMTG, thus adding a larger fast component which would explain the higher VO_2 amplitude. The larger fast component also explained the lower RER value 0.74 (88% fat oxidation) vs. 0.87 (42% fat oxidation) in the glycogen reduced trial

at the same workload. Second, due to the total VO_2 time constant being similar in the glycogen reduced trial compared to control, there must also have been an increase in CHO oxidation in type II fibers which could have increased the slow component. Osborne explained under the opposing conditions, phase II time constant would be unchanged despite increased power output (44). Related to the current investigation, Mole and Hoffman and Osborne provided evidence that altering the starting energy profile of type I fibers could have an effect on substrate oxidation and performance in high intensity workloads.

VO_2 kinetics and modality

VO_2 kinetics may also be altered by modality. Differing modalities require varying levels of concentric, eccentric, isometric, and elastic sub portions to contribute to total work. A.V. Hill's primitive force velocity curve developed in the late 20's and early 30's described the varying amount of force that can be produced at specific velocities is still used today (22). As velocity increases, the force production drops. Maximum power is generated at approximately 30% maximum velocity; however maximum force or greatest economy production occurs in eccentric contraction. This greater economy of eccentric contraction was a suggested mechanism for a more current investigation of VO_2 kinetics in running vs. cycling. Nearly every VO_2 kinetics study has been performed with cycle ergometry. Jones et al. examined kinetics at matched VO_2peak values in running and cycling (29). The results revealed a higher slower component in cycling which was attributed to running having a more economical pattern due to the decreased metabolic cost of eccentric and elastic additions not present in cycling as well as cycling having

greater isometric contraction which increases metabolic cost with no generation of external power.

In relation to the current investigation, an in depth look at the biomechanics of elliptical cycle locomotion was researched. Hovais et al. investigated the biomechanical and physiological aspects of elliptical machine movement (24). The study revealed the upstroke phase of one lower limb was achieved by the downstroke of the opposite limb in addition to upper limb activation at absolute top and absolute bottom center of the cyclic pathway. The authors concluded that elliptical is different from cycling and running in that the entire body contributes to power output as well as having a lack of eccentric phases during footstrike present in running. With elliptical locomotion being predominantly concentric with minor isometric portions, less economy of motion and a larger slow component than running can be expected. What might happen if the amount of slow component addition to phase II VO_2 kinetics in cycling or elliptical motion through dietary or other means could be reduced? Would RER's be changed, would subjects burn more fat, would performance be increased? Although this was not the primary objective of the current investigation, it would be an interesting addition to the current literature on VO_2 kinetics.

Given all the literature suggesting that a faster phase II component improved performance, that the fast component represented fat oxidation in type I fibers and that that fat oxidation was predominantly IMTG even at high intensities, that several factors affected VO_2 kinetics, the question remains, can a high fat loading improve high intensity performance? More specifically for traditional thinking, can type I fibers provide enough power to improve performance during high intensity activity?

Muscle Fiber Type and Force/Velocity Production

Looking back at the force velocity curve, it is known that maximum power is not maximum strength; it represents velocity and force. When comparing red and white dogfish fibers (type I and type II respectively), it was shown that red fibers contained only 23% the power that white fibers contained (36). The lower capacity to produce force and the slower intrinsic velocity of filament sliding contributed to their lower power production. However, when compared to typical mammalian (mouse) fibers, the dogfish red fibers were more effective relative to maximum isometric output. The dogfish red fibers which are known to have a larger fat oxidation potential than normative mammalian fibers, are relatively identical to their white fibers relative to isometric or maximum strength potential whereas mammalian red fibers express only 86% of relative maximum strength compared to their white counterparts.

The authors also found a much greater velocity in the red fibers when placed on stretch, concluding the velocities of red and white dogfish fibers are similar when stretch reflex was implemented. Finally, the authors attempted to replicate sinusoidal movement patterns that resembled swimming and found higher power outputs when intermittent stimulation and stretch was applied. The relaxation phase of movement allowed the red fibers to recover metabolically and then utilize the stretch reflex to generate a stronger faster contraction than isometric loading alone.

Muscle fiber type and fatigue

It is well documented that type II muscle fibers fatigue more quickly in a top down manner from type IIX, to IIXa, to IIXx, to IIA, to type I (54). One recent investigation observed the effects of reduction in power during a 25 s maximal sprint effort in different muscle fiber types following a fatiguing 6 min 90% VO_2max bout (54). Prior to the primary test, the authors determined maximum power output cadence of 120 RPM on an isokinetic cycle by developing force/velocity curves with their subjects. They confirmed the top down effect in maximum effort of 20 s following fatiguing exercise. More specifically, they found no change in ATP in type I fibers after 10 s and only a modest decreases of 5% after 25 s of maximum power cycling whereas type IIA fibers only retained 60% starting values after 10 s and 40% after 25 s and type IIX fibers only retained 30% after 10 s and was unchanged after 25 s. The authors suggest that there is limited contribution from type IIX fibers in the second half of the 25 s maximal effort and underlies the whole-muscle fatigue often observed in maximal dynamic exercise. They conclude that the profound loss of power may be attributed to selective fatigue of a relatively small population of fast fatigue sensitive fibers.

Again in relation to the current investigation, if energy stores in type II fibers were used up after one 10-20 s bout, and the exercise bout required more than that, type I fibers would be called upon. Those type I fibers would therefore need a larger immediate energy source to draw upon as they would be the main force producers in maximal efforts despite their known lower power production start values. If those type I fibers were loaded with IMTG, the bouts offered normal stretch reflex action, and short periods of rest to reload contraction and metabolic contributors were present, a mechanism becomes

ever clear that may provide the opportunity to use those fatigue resistant fibers to generate more power than previously thought.

APPENDIX B

INFORMED CONSENT

FOR HS IRB USE ONLY

APPROVED

Dan Vieira MD 3/14/07

HS Authorized Representative Date

EXPIRATION DATE: _____

CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Jonathan G. Garlow
Tom R. Thomas, PhD
Project #: 1081657
Approval date: February 28, 2007

STUDY TITLE: EFFECTS OF HIGH FAT LOADING ON SUBSTRATE UTILIZATION AND PERFORMANCE DURING INTERMITTENT EXERCISE IN TRAINED ATHLETES

Introduction

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.

This is a research study. Research studies include only people who choose to participate. As a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends. You are being asked to take part in this study because you are a highly trained athlete in high intensity intermittent exercise.

This study is being sponsored by the University of Missouri Athletic Department and the Exercise Physiology Laboratory.

In order to participate in this study, it will be necessary to give your written consent.

Why is this study being done?

The aim of this study is to determine if a 5 day high fat diet can alter the type of fuel that our body burns for energy, and consequently improve performance during high intensity intermittent exercise.

How many people will take part in this study?

We intend to have 10 subjects from the University of Missouri Wrestling team participate in this study.

What is involved in the study?

You will be participating in a series of exercise tests followed by a 5 day dietary intervention. You will then return for a second round of exercise tests.

Two Days Before the Exercise Tests

You will record your food intake. You will undergo a maximum oxygen consumption exercise test.

Day 1:
You will be asked to complete the following tests (see the description of each test below):

- body fat percent using the standard 3 site skin fold measurement (caliper measurements)
- sustained intermittent test
- performance bouts
- blood test
- urine test

You will record your food intake for the next 5 days. You will discontinue the use of any supplements including protein shakes during the study. You will refrain from heavy exercise for 36 hour prior to the exercise tests. Finally, please do not eat or drink anything with caffeine in it during the 48 hour time period before the exercise tests.

Day 6:
You will be asked to complete the following tests (see the description of each test below):

- body fat percent using the standard 3 site skin fold measurement (caliper measurements)
- sustained intermittent test
- performance bouts
- blood test
- urine test

Exercise tests:
Maximum oxygen consumption test: The VO_{2max} test will be performed on a treadmill in order to best determine workloads that will be applied during the sustained intermittent trial. The protocol will begin with a warm-up period at 3.0 mph to allow you a familiarization period with the breathing apparatus and review of the protocol. The first workload will begin at 3.5 mph and will be sustained for 2 min. Treadmill speed will then be increased 0.5 mph at the end of each minute until a speed of 6.0 mph is reached. This pace will be held for the duration of the test. After successfully completing 1 min at 6.0 mph, the grade of the treadmill will be increased at the rate of 2º/min until exhaustion. The physiological criteria for a successful VO_{2max} test are a maximal RER $\geq$ 1.1, a maximal heart rate within 10% of age predicted max, and a leveling of O_2 consumption ($\leq$ 2 ml/kg/min difference between two successive workloads).

Sustained intermittent test: This is a 40 minute test. You will be given a 2 minutes warm up. You will then be asked to run on the treadmill at a speed calculated from the VO2 Max test you completed earlier. You will be asked to complete 20 minutes at an exercise ratio of 6:9 seconds work to rest. This means you will exercise for 6 seconds and rest for 9 seconds for the 20 minutes. Rest is accomplished by stepping off the treadmill when instructed. After you have completed the 20 minutes we will switch the ratio of work to

rest to 24 seconds of work to 36 seconds of rest. You will then be given 5 minutes to cool down. We will be measuring your oxygen use during this test.

Performance Bouts: One hour after the completion of the sustained intermittent test you will begin a series of four 8 minute exercise bouts separated by one hour each. During your time off, you may watch movies in the lab, complete homework, browse the internet, but we ask you to stay in the building. These 8 minute bouts will consist of 15 seconds maximum effort on an elliptical machine followed by 15 seconds at a walking pace.

Dietary Treatment: You will ingest a high fat diet for 5 days consisting of olive oil blended with a chocolate milk shake supplement three times a day. The shakes will be made by me by combining Gatorade supplement shakes with olive oil in a blender and pre packaging them for you to consume. The shakes will be dropped off to you after you lift weights in the morning and before practice in the afternoon. On the days where you do not lift weights in the morning, your morning shake will be given to you the night before. The amount of olive oil will be determined by your body weight; such that each subject consumes 2.2g/kg/day. That equates to about 3.8 tablespoons added to each shake for a 150 lb subject. This amount of supplementation will be sufficient to sustain about half your daily intake; therefore, you will consume roughly half the food you normally do. On the test days, you will be given between 1.5 and 2.5 balance bars and some Gatorade based on your weight 45 minutes before exercise begins.

Other tests: Because of the nature of wrestling as a weight class sport, and dehydration methods are often used to lower body weight, you will not intentionally reduce your body weight during the study and must provide a hydrated urine specimen on the two days of exercise trials. When you arrive to the lab on exercise testing days, your urine will be tested to make sure you are well hydrated. The method used is the same that you are familiar with when certifying for your weight class. You will provide a small urine sample and a swab will be placed on a hand held refractometer. The specific gravity must be under 1.02 to be considered properly hydrated. If you are not properly hydrated, you will consume fluids and we will try again 1 hour later. This process will continue until you are properly hydrated.

We will draw 10 ml (about one teaspoon) of blood 5 minutes before and 5 minutes after the Intermittent Exercise and again 5 minutes before and 5 minutes after the 3rd and 4th Performance Bouts for a total of 6 times for each day of exercise testing.

You will not change your exercising habits during the study. That is, you will participate in all practice related activities and abstain from additional exercise.

How long will I be in the study?
After the consent form has been signed, you will be scheduled for a maximum oxygen consumption test which will take about an hour. Following that, only one week of testing and treatment will be required from you. The testing days in the lab will be on Saturday

and Sunday and will require a total of about 6 hours of your time starting at either 7:00 or 8:00 am.

What are the risks of the study?
Because you are an active wrestling team athlete, we expect no risk of the exercise tests on your cardiovascular system. However, there is always a small risk with any intense exercise activity. Additionally, treadmill speeds will be set at a fast pace and there is always a risk of falling, or having an unforeseen injury.

Are there benefits to taking part in the study?
You will gain information on your maximum oxygen uptake and maximal heart rate from the VO2max test. Because oxygen uptake is the gold standard test for cardio respiratory fitness, you may be able to determine your personal exercise levels. Additionally, knowing a maximum heart rate is vital information to any level of athlete in training. Finally, if a benefit is shown as a result of this dietary intervention, you will have obtained sports nutrition information. That you may find helpful in your athletic training program

What other options are there?
The only other option is to not participate in this study.

What about confidentiality?
Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. If the investigator conducting this study is not your primary, or regular doctor, he must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in this trial.

Results of this research may be published and reports may be made to government agencies, funding agencies, manufacturers or scientific bodies, but you will not be identified in any such publication or report.

Although your coaches are aware of the study, they are not aware of who will participate in it and will receive no personal information or data gained from it. Again, participation is completely voluntary.

What are the costs?
There is no cost to you. Examinations and tests for this research will be paid for by the Exercise Physiology Laboratory.

Will I be paid for participating in the study?
There will be no monetary compensation for participation in this study.

What if I am injured?
It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

What are my rights as a participant?
Participation in this study is voluntary. If you do not volunteer or if your participation is ended for any reason, this will not affect any care or consideration to which you are entitled.

In addition, the investigator of this study may decide to end your participation in this study at any time after he has explained the reasons for doing so.

Who do I call if I have questions or concerns?
Please ask any questions you have about this research or how it will affect you, and I will answer them. In addition, if you have any questions during your participation, I or one of my associates will be glad to discuss them with you. You may call me at **(573) 999-9228**
If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181
A copy of this consent form will be given to you to keep.

Signature
I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

_____ _____
Subject/Patient* Date

_____ _____

Legal Guardian/Advocate/Witness (if required)** Date

_____ _____
Additional Signature (if required) (identify relationship to subject)*** Date

*A minor's signature on this line indicates his/her assent to participate in this study. A minor's signature is not required if he/she is under 7 years old. Use the "Legal Guardian/Advocate/Witness" line for the parent's signature, and you may use the "Additional Signature" line for the second parent's signature, if required.

**The presence and signature of an impartial witness is required during the entire informed consent discussion if the patient or patient's legally authorized representative is unable to read.

Signature of study representative
I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

_____ _____
Study Representative** **Date**

****Study Representative is a person authorized to obtain consent. Per the policies of the University of Missouri Health Care, for any 'significant risk/treatment' study, the Study Representative must be a physician who is either the Principal or Co-Investigator. If the study is deemed either 'significant risk/non-treatment' or 'minimal risk,' the Study Representative may be a non-physician study investigator.

APPENDIX C

SUBJECT FORMS

40 Minute Intermittent Exercise Trial

Subject #: _____ Age: _____ Height (cm): _____ Weight (kg): ___

Time	Speed	Grade	HR (end)	RER	RPE	Comments
1	0	0				Resting
2	0	0				"
3	4	0				Warm up
4	4	0				"
5	4	0				"
6	6					6' : 9'
7	"					
8	"					
9	"					
10	"					
11	"					
12	"					
13	"					
14	"					
15	"					
16	"					
17	"					
18	"					
19	"					
20	"					
21	"					
22	"					
23	"					
24	"					
25	"					
26	"					24' : 36'
27	"					
28	"					
29	"					
30	"					
31	"					
32	"					
33	"					
34	"					
35	"					
36	"					
37	"					
38	"					
39	"					
40	"					
41	"					
42	"					
43	"					
44	"					
45	"					

RER		
High	**Low**	
	0.95	**0.99**
1	0.9	
1.01	0.89	0.97
1.02	0.88	0.96
1.03	0.87	0.95
1.04	0.86	0.94
1.05	**0.85**	**0.93**
1.06	0.84	0.92
1.07	0.83	0.91
1.08	0.82	0.9
1.09	0.81	0.89
1.1	**0.8**	**0.88**
1.11	0.79	0.87
1.12	0.78	0.86
1.13	0.77	0.85
1.14	0.76	0.84
1.15	**0.75**	**0.83**
1.16	0.74	0.82
1.17	0.73	0.81
1.18	0.72	0.8
1.19	0.71	0.79
1.2	**0.7**	**0.78**

6 sec : 9 sec				24 sec : 36 sec		
0:00	**on**	**0:06** **off**		**0:00**	**on**	**0:24** **off**
0:15	**on**	**0:21** **off**				
0:30	**on**	**0:36** **off**				
0:45	**on**	**0:51** **off**				

8 Minute Performance Bout

Subject#: _____ Age: _____ Height (cm): ___ Weight (kg): _____

Time		Distance	Cadence	Resistance	RPE
0:00	1:59		80-95	8	
2:00	2:14		30	16	
2:15	2:29			"	
2:30	2:44		30	"	
2:45	2:59			"	
3:00	3:14		30	"	
3:15	3:29			"	
3:30	3:44		30	"	
3:45	3:59			"	
4:00	4:14		30	"	
4:15	4:29			"	
4:30	4:44		30	"	
4:45	4:59			"	
5:00	5:14		30	"	
5:15	5:29			"	
5:30	5:44		30	"	
5:45	5:59			"	
6:00	6:14		30	"	
6:15	6:29			"	
6:30	6:44		30	"	
6:45	6:59			"	
7:00	7:14		30	"	
7:15	7:29			"	
7:30	7:44		30	"	
7:45	7:59			"	
8:00	8:15		30	"	
8:15	8:29			"	
8:30	8:44		30	"	
8:45	8:59			"	
9:00	9:14		30	"	
9:15	9:29			"	
9:30	9:44		30	"	
9:45	9:59			"	
10:00	11:59		80	8	

Total Distance Max Speed

University of Missouri-Columbia
Exercise Physiology Lab-Dietary Intake Log

SUBJECT #:_____ Date: Day of week:

Time	Food / Drink	Brand	Amount (tsp, cup, oz)	Condiments Food Prep	Location / Place Restaurant
BREAKFAST					
MORNING SNACK					
LUNCH					
AFTERNOON SNACK					
DINNER					
EVENING SNACK					

University of Missouri-Columbia Exercise Physiology Lab

Weekly Exercise Log

Date	Duration	Activity
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		
Sunday		

APPENDIX D

STATISTICAL RESULTS

One-way repeated measures ANOVA

Characteristics of subjects pre vs. post

	df	F	P value
Body Fat %	1	0.571	0.471
Weight Kg	1	1.05	0.336

Dietary Intake

	df	F	P value
Calories	1	15.245	0.000
Fat	1	105.15	0.000
Carbohydrate	1	9.522	0.003
Protein	1	0.007	0.931

Fat Oxidation pre vs. post

	df	F	P value
6 : 9 sec	1	23.95	0.001
24 :36 sec	1	10.95	0.011

Performance pre vs. post

	df	F	P value
Total Sprint Distance	1	21.91	0.000
Avg Sprint Distance	1	21.91	0.000
Total Distance	1	11.85	0.002
Total Peak Power	1	16.95	0.000
Avg Peak Power	1	16.95	0.000
RPE	1	0.887	0.353

Lactate by time point pre vs. post

	df	F	P value
1	1	0.001	0.975
2	1	2.002	0.207
3	1	2.314	0.172
4	1	0.067	0.804
5	1	0.016	0.905
6	1	3.264	0.145

Glucose by time point pre vs. post

	df	F	P value
1	1	0.046	0.836
2	1	32.32	0.001
3	1	0.328	0.585
4	1	0.116	0.745
5	1	0.107	0.760
6	1	0.973	0.380

pH by time point pre vs. post

	df	F	P value
1	1	0.214	0.656
2	1	1.357	0.288
3	1	0.44	0.529
4	1	1.437	0.276
5	1	0.143	0.724
6	1	1.750	2.56

NEFA by time point pre vs. post

	df	F	P value
1	1	1.593	0.247
4	1	9.259	0.023
6	1	1.038	0.383

APPENDIX E

RAW DATA

Characteristics of Subjects Before Treatment

Subject	Weight (kg)	Chest	Tricep	Subscap	BF%
1	84.1	6	7	10	6.2
2	84.1	4	8.5	7.5	5.5
3	80.2	7	15	17	11.7
4	76.8	5.5	10	5.5	6.5
5	84.5	7	9.5	12.5	9.3
6	75.5	13.5	12	13.5	12.6
7	84.1	9.5	8.5	12	10.2
8	88.6	6.5	9.5	13.5	10.3
9	77.7	9	12.5	10.5	11.4

Characteristics of Subjects After Treatment

Subject	Weight (kg)	Chest	Tricep	Subscap	BF%
1	85.5	7	10	12.5	7.9
2	78.2	4	7.5	10.5	5.5
3	79.8	14.5	20.5	19.5	15.3
4	77.3	9.5	10.5	10	8.1
5	84.1	6.5	8.5	10.5	6.7
6	74.1	10	11	12	8.9
7	84.1	11.5	9	11.5	8.7
8	87.3	6	9	14.5	7.9
9	78.6	9.5	13.5	10	8.9

Percent Fat Oxidation

Subject	6:9 pre	24:36 pre	6:9 post	24:36 post
1	7.4	2.1	15.2	16.2
2	0.9	2.3	13.8	12.5
3	7.4	0.0	8.1	9.9
4	5.3	4.8	7.6	3.0
5	4.4	5.6	7.1	8.1
6	3.9	6.9	10.7	8.9
7	5.6	3.6	14.8	12.7
8	3.7	4.5	13.6	12.8
9	5.2	8.2	11.7	10.5

NEFA (mEq/L) by Time Point Before Treatment

Subject	1	4	6
1	0.34	0.81	*
2	0.29	0.84	*
3	0.34	0.62	0.55
4	0.24	1.07	0.45
5	0.33	0.94	1.12
6	0.01	*	0.52
7	0.28	0.56	0.64
8	0.23	*	1.04
9	*	1.13	*

NEFA (mEq/L) by Time Point After Treatment

Subject	1	4	6
1	0.32	0.53	*
2	0.35	0.76	*
3	0.37	0.5	0.7
4	0.11	0.36	*
5	0.55	0.43	0.52
6	0.34	*	*
7	0.71	0.59	0.74
8	0.25	0.49	0.65
9	0.3	0.56	0.97

Glucose by Time Point Before Treatment

Subject	1	2	3	4	5	6
1	64	115	103	107	*	*
2	116	112	113	106	*	*
3	49	117	98	101	103	96
4	159	125	96	120	*	*
5	79	146	113	152	107	153
6	59	113	96	*	*	91
7	132	*	100	139	106	146
8	139	*	*	*	85	106
9	100	128	94	145	86	109

Glucose by Time Point After Treatment

Subject	1	2	3	4	5	6
1	116	126	130	111	*	125
2	79	122	92	133	*	135
3	85	123	100	132	102	144
4	75	127	98	116	95	110
5	90	156	116	130	118	124
6	104	122	102	132	95	*
7	169	134	99	144	97	151
8	99	117	96	136	93	147
9	110	132	97	123	83	113

Lactate by Time Point Before Treatment

Subject	1	2	3	4	5	6
1	2.2	1.7	4.3	9.9	*	*
2	2.2	2.6	3.7	14.7	*	*
3	3	3.7	4.8	15	3.9	11.2
4	3.7	2.8	5.9	16.5	*	*
5	2.3	2	6.2	17.6	6	17.7
6	10.9	2.1	3.3	*	*	16.6
7	4.5	*	4.2	18.1	6.1	17.8
8	3.3	*	*	*	8.5	13.9
9	2.4	2.4	3.5	16.3	3.2	8.8

Lactate by Time Point After Treatment

Subject	1	2	3	4	5	6
1	3.3	2.3	15.5	5.4	*	15.3
2	3	2.5	5.9	19.8	*	18.6
3	3.4	4.4	6.1	19.3	5.4	18.6
4	3.7	2.9	5.7	16.9	5.7	16.1
5	2.4	2.2	6	16.2	5.5	18.2
6	9.5	2.8	4.4	16.3	5.4	*
7	2.8	1.7	4.6	17.2	3.9	16.6
8	4.4	3.7	7.4	17.1	9.3	17.7
9	1.9	1.9	4	15.6	3.2	11.6

pH by Time Point Before Treatment

Subject	1	2	3	4	5	6
1	7.69	7.64	7.61	7.58	*	*
2	7.61	7.64	7.60	7.52	*	*
3	7.64	7.63	7.58	7.47	7.62	7.47
4	7.61	7.59	7.60	7.44	*	*
5	7.59	7.59	7.56	7.45	7.56	7.38
6	7.50	7.59	7.63	*	*	7.46
7	7.58	*	7.62	7.42	7.61	7.42
8	7.59	*	*	*	7.55	7.51
9	7.58	7.63	7.61	7.48	7.63	7.58

pH by Time Point Before Treatment

Subject	1	2	3	4	5	6
1	7.59	7.64	7.52	7.60	*	7.54
2	7.65	7.69	7.65	7.40	*	7.42
3	7.64	7.62	7.65	7.42	7.58	7.43
4	7.62	7.64	7.65	7.44	7.62	7.49
5	7.60	7.60	7.59	7.45	7.59	7.41
6	7.57	7.66	7.60	7.46	7.62	*
7	7.57	7.64	7.61	7.43	7.57	7.42
8	7.60	7.59	7.61	7.48	7.59	7.40
9	7.62	7.58	7.63	7.46	7.61	7.54

Distance Traveled (Km) During Each Sprint Bout Before High Fat Diet

Subject	Bout	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	1	0.08	0.09	0.08	0.09	0.08	0.07	0.08	0.09
	2	0.09	0.08	0.09	0.08	0.09	0.07	0.08	0.09
	3	0.07	0.06	0.08	0.09	0.08	0.09	0.08	0.09
2	1	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07
	2	0.08	0.08	0.08	0.08	0.09	0.07	0.08	0.09
	3	0.08	0.08	0.08	0.07	0.09	0.07	0.08	0.08
3	1	0.09	0.09	0.09	0.07	0.08	0.09	0.07	0.09
	2	0.09	0.09	0.09	0.08	0.07	0.08	0.08	0.09
	3	0.09	0.08	0.08	0.08	0.07	0.07	0.08	0.09
	4	0.09	0.07	0.08	0.08	0.08	0.08	0.08	0.09
4	1	0.08	0.08	0.08	0.07	0.09	0.08	0.07	0.09
	2	0.08	0.09	0.09	0.06	0.09	0.08	0.08	0.09
	3	0.09	0.08	0.08	0.09	0.08	0.08	0.08	0.1
	4	0.09	0.08	0.08	0.08	0.09	0.09	0.08	0.1
5	1	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.07
	2	0.1	0.08	0.08	0.07	0.07	0.08	0.08	0.1
	3	0.09	0.08	0.08	0.07	0.08	0.08	0.08	0.09
	4	0.09	0.08	0.09	0.09	0.08	0.07	0.07	0.09
6	1	0.09	0.08	0.08	0.07	0.07	0.06	0.06	0.08
	2	0.09	0.07	0.08	0.08	0.07	0.07	0.07	0.06
	3	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08
	4	0.08	0.08	0.08	0.09	0.07	0.07	0.07	0.08
7	1	0.1	0.09	0.08	0.07	0.07	0.08	0.08	0.09
	2	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08
	3	0.08	0.08	0.08	0.08	0.07	0.08	0.09	0.08
	4	0.09	0.09	0.08	0.09	0.08	0.07	0.08	0.09
8	1	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.09
	2	0.09	0.09	0.09	0.09	0.08	0.08	0.09	0.09
	3	0.09	0.09	0.08	0.09	0.08	0.09	0.09	0.09
	4	0.09	0.08	0.08	0.08	0.07	0.07	0.08	0.08
9	1	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	2	0.08	0.09	0.08	0.08	0.07	0.08	0.07	0.09
	3	0.09	0.08	0.08	0.07	0.08	0.1	0.06	0.1
	4	0.09	0.08	0.07	0.07	0.08	0.07	0.07	0.1

Distance Traveled (Km) During Each Sprint Bout After High Fat Diet

Subject	Bout	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	1	0.09	0.09	0.07	0.09	0.08	0.08	0.08	0.09
	2	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.1
	3	0.1	0.09	0.1	0.09	0.08	0.08	0.09	0.09
2	1	0.09	0.1	0.09	0.1	0.09	0.09	0.08	0.09
	2	0.09	0.09	0.09	0.09	0.1	0.1	0.09	0.09
	3	0.1	0.11	0.1	0.09	0.09	0.09	0.1	0.1
3	1	0.09	0.09	0.08	0.08	0.09	0.08	0.08	0.09
	2	0.08	0.09	0.08	0.08	0.09	0.08	0.08	0.1
	3	0.09	0.08	0.09	0.08	0.08	0.08	0.09	0.09
	4	0.1	0.1	0.09	0.08	0.08	0.08	0.08	0.09
4	1	0.08	0.07	0.07	0.07	0.08	0.08	0.09	0.09
	2	0.08	0.08	0.09	0.08	0.09	0.08	0.09	0.1
	3	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.09
	4	0.09	0.09	0.08	0.09	0.09	0.08	0.09	0.1
5	1	0.1	0.09	0.09	0.08	0.08	0.08	0.09	0.1
	2	0.08	0.1	0.08	0.09	0.09	0.08	0.09	0.09
	3	0.09	0.08	0.08	0.07	0.09	0.08	0.07	0.09
	4	0.08	0.08	0.08	0.09	0.09	0.08	0.08	0.09
6	1	0.09	0.08	0.07	0.07	0.08	0.07	0.08	0.08
	2	0.09	0.09	0.09	0.08	0.08	0.08	0.09	0.08
	3	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.09
	4	0.09	0.08	0.09	0.08	0.09	0.08	0.08	0.08
7	1	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08
	2	0.09	0.09	0.1	0.09	0.08	0.08	0.07	0.08
	3	0.09	0.08	0.08	0.08	0.09	0.08	0.08	0.09
	4	0.1	0.07	0.08	0.08	0.08	0.07	0.08	0.09
8	1	0.09	0.09	0.08	0.09	0.09	0.08	0.08	0.09
	2	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
	3	0.09	0.09	0.09	0.08	0.08	0.08	0.09	0.09
	4	0.1	0.1	0.09	0.09	0.09	0.09	0.09	0.09
9	1	0.08	0.09	0.09	0.09	0.08	0.07	0.07	0.08
	2	0.09	0.08	0.09	0.09	0.08	0.07	0.08	0.08
	3	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.09
	4	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

Maximum Cadence Recorded (Km/hr) During Each Sprint Bout
Before High Fat Diet

Subject	Bout	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	1	24	21	21	20	22	20	20	25
	2	23.2	24.9	23.8	23.2	24.5	22.4	23.2	24.5
	3	20.9	22.4	20.4	22.5	23.9	22.5	22.7	22.2
2	1	24.4	24	22.7	22.9	23.8	23.7	22.8	23
	2	23.2	23.6	24.9	21.6	24.5	23.8	22.3	23.5
	3	22.7	22.3	22.3	22.3	22.1	22.7	22.2	22.4
3	1	25.5	23.7	23	22.3	23	22.9	20.3	24.3
	2	23.4	23	22.6	22.6	21.7	21.5	23	22.3
	3	24.2	23.9	22.6	22.3	22.4	20.9	22.1	23.3
	4	21.7	21.6	22	21.5	21	23	22.6	23
4	1	22.3	22.2	22.1	20.9	21.4	22.5	24.5	23.4
	2	22.8	21.9	23.2	20.5	23.5	23.5	23	23.8
	3	24.3	21.2	24.3	25.2	22.4	22.6	23	25
	4	22	21.6	22.6	20.9	22	22.2	23	22
5	1	26.5	25.6	24	25.2	23.9	20.6	24.5	25
	2	26.5	23.4	22.8	23.2	24.1	24.4	24.2	27
	3	25.3	25	24.3	25.2	24.8	23.8	24.5	25.3
	4	25.7	24.6	24.9	24.6	24	22.9	22.3	25.2
6	1	24.8	24.4	22.6	22.3	23	21.8	22.8	24.1
	2	25	24.4	24.2	22.5	20.5	21.4	22.3	21.6
	3	22.3	22.4	22.8	22.8	22.5	22.4	21.9	24.6
	4	23.8	24.5	22.3	22.2	21.6	22.5	21.8	24.7
7	1	25.5	25.3	25	23	23.2	23.2	22.4	23.3
	2	23.5	24.8	25	24.8	24.1	24.5	23	24.3
	3	22.5	23.8	24.4	24.2	24.2	24	25	24.7
	4	25.5	24.7	24.7	24.1	24.2	22.3	22.8	25
8	1	25.2	25.5	24	23	24	21.6	22.3	25.2
	2	25	22	23.2	25.1	21.8	21.8	25	25.3
	3	24.9	24.1	25	25.2	25.1	25	24.3	25.2
	4	24.7	24.6	23.4	24.1	24	21	23.9	25.1
9	1	25.1	24	22.4	23.2	22.1	21.5	22.4	25
	2	25.1	24	22.4	23.2	22.1	21.5	22.4	25
	3	22.9	23.8	23.8	22.7	21.6	26	21.8	26
	4	23	22	22.2	21.7	23.1	22.4	22.5	27.5

Maximum Cadence Recorded (Km/hr) During Each Sprint Bout After High Fat Diet

Subject	Bout	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	1	25.4	25.5	25.1	22.9	25.2	23.2	22.5	23.4
	2	22.1	23.3	23	22.5	22.7	22.5	24	23.4
	3	24.4	25.3	24	24.4	23.9	23.9	24	23.4
2	1	25.1	24.6	22.7	24.1	25.2	25.1	23.6	25
	2	25.2	24.9	25.2	25.5	25.1	25.5	25.2	25
	3	26	25	25.2	24.2	25	25.5	25.2	23.9
3	1	24.5	24.2	23	23.1	23.1	22.1	23.4	25.1
	2	22.9	22	23.8	23.4	24.6	23.2	24.7	24.2
	3	23.3	24.6	23.4	24.4	23.4	24.5	24.1	25.4
	4	24.4	25.5	23.8	22.8	23.7	21.7	22.5	24.1
4	1	23.4	22	21.7	21.8	22.4	22.1	24.5	24.7
	2	23.4	21.8	24.5	24	23	24	24	25
	3	23.4	23.1	24.2	23.9	24.2	22	23	25.2
	4	24	24	23	24.5	21.6	20.5	24.4	26.5
5	1	25.3	24.8	24.6	24.5	21	24.5	24.6	24.5
	2	24.6	24.6	25.2	24.2	25.2	24.5	24.6	24.5
	3	24.7	24	23	23	24	22.4	24	25
	4	24.8	25	24.5	25.5	24.5	24.6	25.2	26.7
6	1	23	23	22.8	22.5	23.6	21.9	21.5	22
	2	25.5	23.5	24	23.9	23.3	21.9	23.1	23.9
	3	24	24.2	23.8	23.7	22.7	23.4	23.6	23.7
	4	24.6	23.7	23.2	23.4	25.1	22.5	23	24.1
7	1	25	23	23	22.7	23.6	23	23	23.4
	2	25	25	25.1	24	24	24	22	23.1
	3	25.4	24.2	25.1	25.1	25	25.4	24.9	25
	4	26.2	23	23.2	24	22	22	22	25.2
8	1	25.4	25.5	23.7	25.3	24.1	23	23.3	22.3
	2	25.1	24	25.2	23.5	23.5	24.7	23.5	24.1
	3	24.5	23	24.5	24	22.4	24.5	23	23.5
	4	25.5	25.5	25.7	25.4	25.1	24.9	25	25
9	1	25.5	25.5	25.2	25.5	24.4	24	24.2	25
	2	25.2	25.4	25.2	25	23	25	23	24
	3	23	25	25.2	24	24	22	22	24.5
	4	24.2	25.3	24.1	24.8	23.4	25.4	25.2	25.3

RPE Before High Fat Diet

Subject	Bout	RPE 1	RPE 2	RPE 3	RPE 4
1	1	25.4	25.5	25.1	22.9
	2	22.1	23.3	23	22.5
	3	24.4	25.3	24	24.4
2	1	25.1	24.6	22.7	24.1
	2	25.2	24.9	25.2	25.5
	3	26	25	25.2	24.2
3	1	24.5	24.2	23	23.1
	2	22.9	22	23.8	23.4
	3	23.3	24.6	23.4	24.4
	4	24.4	25.5	23.8	22.8
4	1	23.4	22	21.7	21.8
	2	23.4	21.8	24.5	24
	3	23.4	23.1	24.2	23.9
	4	24	24	23	24.5
5	1	25.3	24.8	24.6	24.5
	2	24.6	24.6	25.2	24.2
	3	24.7	24	23	23
	4	24.8	25	24.5	25.5
6	1	23	23	22.8	22.5
	2	25.5	23.5	24	23.9
	3	24	24.2	23.8	23.7
	4	24.6	23.7	23.2	23.4
7	1	25	23	23	22.7
	2	25	25	25.1	24
	3	25.4	24.2	25.1	25.1
	4	26.2	23	23.2	24
8	1	25.4	25.5	23.7	25.3
	2	25.1	24	25.2	23.5
	3	24.5	23	24.5	24
	4	25.5	25.5	25.7	25.4
9	1	25.5	25.5	25.2	25.5
	2	25.2	25.4	25.2	25
	3	23	25	25.2	24
	4	24.2	25.3	24.1	24.8

RPE After High Fat Diet

Subject	Bout	RPE 1	RPE 2	RPE 3	RPE 4
1	1	15	15	17	18
	2	15	15	16	17
	3	14	15	17	17
2	1	13	15	16	17
	2	13	14	16	17
	3	14	15	17	18
3	1	13	14	15	19
	2	13	14	15	19
	3	13	15	16	19
	4	14	15	16	19
4	1	15	15	15	16
	2	15	15	15	16
	3	15	16	16	16
	4	16	17	18	17
5	1	15	17	18	18
	2	16	17	18	18
	3	16	17	17	18
	4	15	16	17	19
6	1	15	17	17	18
	2	16	17	18	19
	3	16	17	18	18
	4	16	17	18	19
7	1	14	16	17	19
	2	15	17	19	20
	3	15	17	18	20
	4	16	18	19	20
8	1	15	17	19	20
	2	16	18	19	20
	3	16	17	19	20
	4	17	19	20	20
9	1	16	18	20	20
	2	16	20	20	20
	3	16	19	20	20
	4	17	20	20	20