

**INTERRELATIONSHIPS BETWEEN MITOCHONDRIAL FUNCTION,  
MAXIMAL OXYGEN CONSUMPTION, RUNNING ECONOMY, AND DIET IN  
ELITE MALE AND FEMALE RUNNERS**

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## ABSTRACT

The relationships between maximal oxygen consumption ( $VO_{2max}$ ), mitochondrial function and running economy were investigated in a population of twenty-one endurance trained males and females (range=18-54 yrs). The purposes of this study were: 1) to determine whether mitochondrial oxidative capacity, as defined by the maximal activities of citrate synthase (CS), cytochrome c oxidase (COX), and beta-hydroxyacyl-CoA dehydrogenase (BHAD), is a significant determinant of maximal oxygen consumption in endurance trained men and women, and 2) to observe the relationship between maximal oxygen uptake and running economy in the same population. Muscle biopsy samples were taken from the vastus lateralis prior to a 90-minute bout of submaximal running (65%  $VO_{2max}$ ) from which running economy was assessed. Running economy was observed to significantly negatively correlate with  $VO_{2max}$ , regardless if it was expressed in absolute or relative values: L/min ( $r = -.506$ ), ml/kg body mass/min ( $r = -.703$ ) and ml/kg FFM/min ( $r = -.700$ ). Data also show that maximal COX activity was significantly related to  $VO_{2max}$ , L/min ( $r = .789$ ). In addition, non-significant positive correlations were apparent between both CS and COX activity and all other expressions of  $VO_{2max}$  ( $r > .6$ ). BHAD activity was not related to any measured variable. These results confirm that an inverse relationship is present relating  $VO_{2max}$  and running economy. Also, data herein indicate that maximal activities of key marker enzymes of the citric acid cycle and oxidative phosphorylation are likely a significant factor determining whole-body maximal oxygen consumption in endurance trained males and females.

## CHAPTER 1: INTRODUCTION

Maximal oxygen consumption ( $VO_{2max}$ ) has long been considered the benchmark measurement of the cardiovascular systems' capacity to perform aerobic exercise. The term "maximal oxygen uptake" dates back to the 1920's when Archibald Vivian (A.V.) Hill began taking measurements of oxygen consumption while running around an 85-m grass track (1). Today it is universally regarded to be the premier indicator of the body's ability to take up and consume oxygen during strenuous exercise. Despite challenges to its physiological relevance (2),  $VO_{2max}$  testing has emerged as a proven and reliable method for demonstrating a training effect as well as characterizing the aerobic potential of the cardiopulmonary system. Maximal oxygen uptake cannot predict performance in sport; a myriad of factors such as skill level, motivation, and genetics contribute to performance in a given task. However, the knowledge of one's upper limit for aerobic capacity can accurately predict potential for vigorous muscular exercise (3). Thus, exercise physiologists have universally accepted and adopted this measure and its testing has been continually implemented as a tool in scientific interventions.

$VO_{2max}$  is the product of maximum cardiac output and maximum arteriovenous oxygen difference. Adaptations, which increase cardiac output, such as improvements in stroke volume with endurance exercise training, have yielded proportional changes in  $VO_{2max}$  (4). Additionally, the ability of active muscle to efficiently extract oxygen from increased blood flow is a crucial component to  $VO_{2max}$ .

Maintaining maximum arteriovenous oxygen difference relies upon several peripheral factors including, tissue capillarity, hemoglobin and myoglobin concentration and mitochondrial content and distribution. Many studies have demonstrated increases in

muscle capillarity following exercise training (5). Increases in capillary density enhance the ability to transport oxygen to active tissues. Increases in hemoglobin concentration improve the oxygen carrying capacity of blood. Ultimately, the aerobic capacity of skeletal muscle is reliant on the oxidative capacity of the mitochondria therein.

Since increases in oxidative enzyme capacity would be expected to increase arteriovenous oxygen difference (6), it seems logical to expect that there exists a relationship between the respiratory capacity of skeletal muscle mitochondria and  $VO_{2max}$ . However, the role of skeletal muscle aerobic capacity as an independent determinant of whole-body oxygen consumption has been the subject of widespread debate. There is literature to suggest that maximal oxygen uptake is limited strictly by central factors: pulmonary capacity, cardiac output and the oxygen carrying capacity of blood (7-16). Contrasting evidence suggests that peripheral factors such as skeletal muscle characteristics are responsible for limiting oxygen consumption (8, 17-22).

Evidence also suggests that there may be time-course differences in the response of central and peripheral adaptations to training. Certainly training would be expected to impart immediate responses in both categories of adaptations, however it is believed that changes in cardiac output, particularly stroke volume are more influential than local factors to improving  $VO_{2max}$  during the early stages of training in untrained populations (4,6,11). Longitudinal studies have revealed that acute increases in  $VO_{2max}$  result primarily from increases in maximal cardiac output rather than from a widening in arteriovenous oxygen difference (4,11). Following 20 days of training in sedentary, improvements in  $VO_{2max}$  resulted from an 8% increase in cardiac output and a corresponding 3.6% increase in arteriovenous oxygen difference (11). No changes were

noted in heart rate, thus it appears that changes in stroke volume resulting from increased blood volume is the predominate factor affecting  $VO_{2max}$  in short-term training.

Understanding what mechanisms are most responsible in determining  $VO_{2max}$  has far-reaching implications. This knowledge would prove invaluable to practitioners wishing to develop training programs that better enhance endurance performance.

Achieving a high  $VO_{2max}$ , although important, is not the sole factor influencing one's ability to excel in endurance events. Economy, or the energy cost at a given workload, has been shown to be strongly related to endurance performance and varies significantly among trained athletes (23). It would be expected then, that  $VO_{2max}$  and running economy are positively correlated. Extensive research has sought to understand this relationship however efforts to arrive at a consensus have been futile. There is research suggesting that  $VO_{2max}$  and economy are positively correlated (24, 25) while other investigations have shown negative correlations (23, 26, 27). The importance of running economy to successful endurance performance is well documented (28). Interventions to better grasp the inter-workings of economy and its relationship to physiological measures, i.e.  $VO_{2max}$ , is therefore warranted.

## PURPOSE

The purpose of this study is two-fold: 1) To determine whether mitochondrial function, as defined by maximal activities of citrate synthase (CS), cytochrome c oxidase (COX), and beta-hydroxyacyl-CoA dehydrogenase (BHAD), is a significant determinant of maximal oxygen consumption in endurance trained men and women, and 2) To observe the relationship between maximal oxygen uptake and running economy in the same trained population.

## RESEARCH HYPOTHESIS

It is hypothesized that muscle mitochondrial aerobic capacity is an important predictor of whole-body peak oxygen consumption. It is also proposed that  $VO_{2max}$  will correlate inversely and significantly with running economy.

## CHAPTER 2: REVIEW OF LITERATURE

### THE RELATIONSHIP BETWEEN MAXIMAL OXYGEN CONSUMPTION AND MITOCHONDRIAL FUNCTION

#### Pulmonary Limitations

Research has demonstrated the existence of pulmonary limitations on the body's ability to perform maximal aerobic exercise (12-16). Trained subjects have been shown to be more vulnerable to arterial oxygen desaturation during maximal work compared to normal, untrained subjects (12). Endurance trained subjects exhibiting higher cardiac output values experience decreased transit time of the red blood cell in the pulmonary capillary. Therefore, in this group, the ability of the pulmonary system to fully oxygenate the blood is compromised. When highly trained subjects obtain oxygen-enriched air, these limitations appear to be overcome. Powers et al. (13) compared  $VO_{2max}$  values in trained and untrained populations breathing either room air or air containing 26% oxygen. Under a condition of higher atmospheric oxygen concentration, the trained subjects improved their  $VO_{2max}$  from 70.1 to 74.7 ml/kg/min compared to normal room air. These changes were not apparent in the untrained group demonstrating that the oxygen saturation levels were limiting the trained group.

Exercising at high altitudes (3,000-5,000 m) has also shown to exert pulmonary limitations (14,15) due to a decreased oxygen concentration in air at higher altitudes. Individuals with asthma and other chronic pulmonary diseases experience similar limitations to aerobic exercise (16). For these individuals it is impaired pulmonary transport facilitating the condition. In these situations, exercise capacity can be augmented following exposure to oxygen-enriched air, providing further evidence of the presence of pulmonary limitations.

## Maximal Cardiac Output and Mitochondrial Oxidative Capacity

During whole-body exercise there is evidence to suggest that maximal oxygen uptake is predominantly limited by cardiac output and the capacity for skeletal muscle to extract and utilize oxygen is of little significance in limiting oxygen uptake. Experimental evidence has shown that the enzymatic capacity to utilize oxygen is well above the measured  $VO_{2max}$ . Blomstrand (7) found that maximal activities of succinate dehydrogenase (SDH) and citrate synthase (CS) were 55% and 940% higher than the estimated Krebs cycle flux (4.6  $\mu\text{mol}/\text{min}/\text{g}$ ). Gollnick and Saltin (8) argue that the ability to increase extraction of oxygen is much smaller than the increase that is typically seen in enzyme capacity following training. Thus, if the enzymatic capacity within muscle exceeds the maximal oxygen flux, then, muscle mitochondrial capacity becomes relatively insignificant in limiting peak oxygen consumption. Instead, peak oxygen consumption should be solely dependent on the *transport* of oxygen.

In agreement with this theory, Henriksson and Reitman (9) illustrate effects of training and detraining on  $VO_{2max}$  and oxidative enzyme activity. They reported a significantly more rapid post-training decline in muscle oxidative enzyme activities compared to  $VO_{2max}$ . Eight weeks of endurance training yielded a 19% increase in  $VO_{2max}$  and a 32 and 35% increase in the activity of SDH and cytochrome c oxidase (COX) above pre-training levels, respectively. Following a six-week detraining period,  $VO_{2max}$  was still 16% above pre-training levels, however SDH activity returned to pre-training levels. COX activity declined to pre-training levels only 2 weeks post-training, and was reduced to levels below pre-training within the following month. The results of this investigation suggest that enhanced oxidative potential is not a necessity for an increase

in whole-body  $VO_{2max}$ , and thus is not a limiting factor. Instead, it appears that an adaptation in  $VO_{2max}$  resulting from training was associated with, but not dependent on skeletal muscle oxidative potential. They propose that muscle oxidative capacity may be more important during submaximal work, contributing to a shift in substrate utilization from carbohydrate to fat metabolism. Providing further evidence that oxidative enzyme capacity is not a significant and independent determinant of  $VO_{2max}$ , Bebout (10) demonstrated that a 68% reduction in CS activity following limb immobilization in dogs did not have an effect on  $VO_{2max}$ .

Despite literature discrediting the role that oxidative enzymes play in the determination of whole-body  $VO_{2max}$ , there is also evidence to suggest the counter. Significant correlations between maximal oxidative enzyme activities and maximal oxygen consumption in humans have been widely reported (8, 17, 18, 20). A significant correlation ( $r = 0.75$ ) was found between vastus lateralis cytochrome c oxidase activity and  $VO_{2max}$  corrected for body weight (17). Findings from Blomstrand (7) indicated a significant correlation between leg oxygen uptake and maximal activity of Krebs cycle enzymes:  $r = 0.79$ ,  $0.72$ , and  $0.73$  for CS, oxogluterate dehydrogenase and SDH respectively.

There are numerous studies that show strong correlations between training induced adaptations in muscle aerobic capacity and improvements in  $VO_{2max}$ . There is evidence to indicate that endurance training results in peripheral adaptations (i.e. increases in muscle oxidative enzyme activity) that increase peak oxygen consumption independent of cardiac output. In comparing sedentary and endurance-trained rats, McAllister and Terjung (19), found the peak oxygen consumption of the trained group

( $3.93 \pm 0.27 \mu\text{mol/g/min}$ ) to be 20% greater than that of the sedentary group ( $3.28 \pm 0.28 \mu\text{mol/g/min}$ ). Blood flow was not different between groups indicating that the increase in  $\text{VO}_{2\text{max}}$  was achieved by greater oxygen extraction/utilization and independent of changes in cardiac output.

Although it is understood that whole-body oxygen consumption can be no greater than the rate of which it is supplied via cardiac output, it is apparent that, despite inconsistencies in the literature, peripheral factors appear to play a large part in determining the extent to which  $\text{VO}_{2\text{max}}$  may be realized. It is highly conceivable that local muscle factors specifically, maximal mitochondrial aerobic capacity, is an important independent determinate of  $\text{VO}_{2\text{max}}$ .

#### THE RELATIONSHIP BETWEEN MAXIMAL OXYGEN CONSUMPTION AND RUNNING ECONOMY

Running economy is traditionally defined as the energy cost for a given submaximal running pace. Economy is typically measured on a treadmill, and, although this can be different from over ground running, its measurement provides researchers with a convenient approach to characterize the efficiency at which various athletes operate. The origins of interventions into running economy date back to the early 1920's when A.V. Hill and colleagues began measuring oxygen consumption at different running speeds (29). Since these early studies, decades of investigations have sought to understand what factors influence running economy, to identify various methods of training to improve it, and to assess its affect on performance (23-28, 30-41, 43, 45). It would be expected that runners who are more efficient would be able to better endure prolonged periods of aerobic exercise. Multiple studies suggest this relationship indeed exists. Investigating well-trained male distance runners, running economy has been

shown to account for variations in 10 km running time (30). Up to 65% of variations in race performance could be attributed to differences in running economy. Conley and Krahenbuhl (31) showed that in similarly endurance trained males ( $\text{VO}_{2\text{max}} \sim 72 \text{ ml/min}$ ), 10 km time was significantly related to efficiency ( $r = 0.83$ ). In addition, it has been shown that in elite or near-elite runners, economy is a better predictor of endurance performance than  $\text{VO}_{2\text{max}}$  (32, 27).

The extensive literature relating the importance of economy in predicting performance has spawned numerous investigations aimed at identifying key factors that predominately regulate economy in trained populations. Several biomechanical and physiological variables have been revealed to significantly influence running economy (24). Biomechanical characteristics such as flexibility, ground reaction forces, and elastic properties of skeletal muscle have been correlated positively with economy. Since running involves harmonious conversions of muscular forces, similar to a spring, the ability of major muscle groups to effectively store and release elastic energy is important determinant of high performance running. Efficient exploitation of this model (i.e., effective storage and release of elastic energy, and lower ground reaction forces) has been shown to be a major feature differentiating those runners with good economy from those without (33). Muscle stiffness is significantly related to running economy ( $r = 0.80$ ) with stiffer muscles exhibiting lower resonant frequencies and better economy.

Several studies have examined the effect of biomechanical variables on running economy following exhaustive exercise and reported little differences in running kinematics (31-35). Examining the effects of running a marathon on efficiency, both submaximal  $\text{VO}_2$  and respiratory exchange ratio (RER) increased following the run (34).

Kyrolainen et al. found that after the marathon, significant increases were observed in oxygen consumption during submaximal running, ventilation, heart rate, RER, and serum creatine kinase activity. In comparison, only small changes in running kinematics (slight increase in stride frequency/decrease in stride length) were noted. Thus, it was concluded that the impaired economy could not be completely explained by mechanical factors; rather, physiological stressors appear to be the predominate mechanism reducing economy. Another study observed the effects of a 5km race on running economy and biomechanics in trained female athletes (35). Running economy steadily decreased throughout the race while no alterations in mechanical variables were evident, indicating that physiological processes were playing a larger part in reducing economy. The literature taken collectively suggests that although intra-individual differences in running mechanics both exist and are important, impairments in running economy that are associated with fatiguing exercise (i.e. marathon running, or competitive middle-distance running) are most likely due to physiological factors.

Changes in physiological variables such as body temperature, heart rate, ventilation, and skeletal muscle metabolic and structural characteristics have been proposed to significantly affect running economy in athletes (25, 31, 35-40). Thomas et al. found that various physiological parameters were associated with changes in running economy (35). During a 5 km race involving trained males and females operating between 80-85%  $VO_{2max}$ , a decline in running economy was correlated with minute ventilation and higher core temperature (41). The study concluded that the effect of increased circulation, and higher ventilatory rates and body temperatures are responsible

for impairments in running economy. Thus it follows that training to enhance economy should concentrate on improving the regulation these physiological characteristics.

Training to improve these factors would certainly be expected to result in training-enhanced  $VO_{2max}$  improvements. Thus, one would expect that better running economy would be associated with higher  $VO_{2max}$  values. The relationship between  $VO_{2max}$  and running economy is not clearly understood. A classic study comparing elite male distance runners ( $VO_{2max} \sim 79$  ml/kg/min) with good male distance runners ( $VO_{2max} \sim 69.2$  ml/kg/min) found that the elite runners had a better running economy (24). The elite runners with a greater capacity for oxygen uptake were running the same pace at a lower percentage of their  $VO_{2max}$  compared to the other runners, an indicator of a better running economy. In support of the notion that economy and  $VO_{2max}$  are positively related is a case study that followed American mile record holder Steve Scott over a 6-month training period. Improvements in  $VO_{2max}$  (3.8% or 74.4 to 77.2 ml/kg/min) paralleled improvements in his running economy at 16 km/hr (6.6% or 48.5 to 45.3 ml/kg/min). These adaptations enabled Scott to reduce the intensity required of him to run 16km/hr by 10% (65.1% to 58.6% of  $VO_{2max}$ ). As expected he was able to parlay these improvements into improved endurance performance (25, 43).

These findings support the existence of a positive correlation between running economy and maximal oxygen uptake, however there is recent work to suggest the opposite. Hunter (26) examined the relationship between  $VO_{2max}$  and walking economy at 3 mph in a large group of sedentary women. The results indicated that economy was negatively correlated with  $VO_{2max}$  ( $r = -0.28$ ,  $p < 0.05$ ). When adjusted for sleeping energy expenditure the relationship strengthened ( $-0.37$ ,  $p < 0.01$ ). The role of oxidative

metabolism as it relates to economy was also investigated in this same group. Citrate synthase correlated inversely with muscle metabolic economy (-0.56,  $p < 0.05$ ) and also appeared to correlate inversely with walking economy (-0.25,  $p = 0.33$ ). Altogether these results show that, in walking, a significant negative correlation exists between economy and  $VO_{2max}$ . Lucia et al. also examined this same relationship in elite trained male cyclists, (23) and found a similar negative correlation. Cycling economy and  $VO_{2max}$  correlated inversely among 11 professional male cyclists (-0.65,  $p < 0.05$ ). It could be speculated that the high economy exhibited in these world-class cyclists compensates for their relatively low  $VO_{2max}$ , allowing them to separate themselves from other competitors of similar capacity for oxygen uptake. There also exist bodies of literature showing no relationship between exercise economy and  $VO_{2max}$ . When 55 active male and female runners were classified as exhibiting low, medium, or high economy, no significant differences or trends were evident in  $VO_{2max}$  (28). Another study found no significant correlations between oxidative enzyme activities (CS, LDH) and running economy (45). Clearly inconsistencies exist in the literature and further research is warranted to better understand the relationship between running economy and  $VO_{2max}$ .

## CHAPTER 3: METHODOLOGY

### STUDY PARTICIPANTS

Twenty-one healthy endurance-trained runners (11 males and 10 females) ranging from the ages of 18-44 for males and 18-54 for females were recruited to participate in this study. All volunteers were regularly performing endurance running ( $\geq 20$  miles/week) and had maximal oxygen uptake values  $\geq 50$  ml/kg/min for women and  $\geq 55$  ml/kg/min for men. Subjects were excluded if they smoke, demonstrated signs of renal, endocrine, hepatic, hematological, gastrointestinal, pulmonary or cardiac disease, alcoholism or other substance abuse. All participants were given informed consent and were made aware of all potential risks and benefits associated with participation in the experimental protocol.

### EXPERIMENTAL DESIGN

Following the consumption of a baseline diet (25% fat, 60% carbohydrate, 15% protein) for 3 days, the athletes performed a 2-hr bout of endurance exercise at 65%  $VO_{2max}$  designed to deplete muscle glycogen and intramuscular lipid (IML) stores. Subjects were then randomly assigned, in crossover design, to a very low fat (10% fat, 75% carbohydrate, 15% protein) or a moderate fat (35% fat, 50% carbohydrate, 15% protein) recovery diet for 3 days. The diets were designed to provide at least moderate amounts of carbohydrate allowing them to be practical and real world applicable to athletes undergoing regular exercise training. Following 3-day adherence to the experimental diet, to normalize glycogen stores, participants in both groups were fed a 24-hr glycogen-loading diet immediately upon completion of a 20-minute run at 70% maximal oxygen consumption. An endurance performance test, designed to test the

effects of the experimental diet (low fat vs. moderate fat), was performed on the morning following a day of glycogen loading. This test consisted of two phases, a 90-min bout of submaximal endurance running (65%  $VO_{2max}$  “preload” run) followed by 10-kilometer time trial. The participants were encouraged to complete the time trial as fast as possible. The same design and protocol was implemented 3-4 weeks later as each subject completed the crossover diet treatment. The experimental design is summarized below.

Day 1: Begin baseline diet (25%fat)

Day 2: Baseline diet

Day 3: Baseline diet

Day 4: 2hr endurance run, Begin experimental diet (10 vs. 35% fat)

Day 5: Experimental diet

Day 6: Experimental diet

Day 7: 20-min run, begin 24 hr glycogen loading phase

Day 8: Pre-exercise muscle biopsy, 90-min run (65%  $VO_{2max}$ ), 10-K time trial

#### MAXIMAL OXYGEN CONSUMPTION

Prior to initiation of the experimental protocol, all subjects were tested for aerobic fitness. Maximal aerobic power ( $VO_{2max}$ ) was determined using a treadmill test designed to exercise participants to exhaustion. Subjects began at a warm-up pace for about 5 minutes and then increases in speed (0.5 mph) or grade (2.5%) incurred every minute until the subjects reached exhaustion. The subject, using hand gestures, determined the increase in workload, speed vs. grade. This protocol has been previously implemented and was developed specifically for well-trained athletes (46). Heart rate was monitored continuously and blood pressure was measured pre-exercise, during the third minute of

warm-up, during the second minute of the protocol, and during recovery. A Sensormedics Vmax 2900 Series metabolic cart analyzed the volume of oxygen consumed and carbon dioxide produced continuously. Ratings of perceived exertion were obtained at specific intervals, scaling from 6 (very, very light) to 20 (very, very hard). For the test to qualify as a valid  $\text{VO}_{2\text{max}}$ , 2 out of 3 measurement criteria must have been met: 1) a plateau in  $\text{VO}_{2\text{max}}$ , as defined by an increase of no more than 2 ml/kg/min with increased workload, 2) maximum heart rate within 10 beats of age-predicted max ( $208 - 0.7 \cdot \text{age}$ ), and 3) respiratory exchange ratio  $\geq 1.10$  (47).

#### RUNNING ECONOMY

Since subjects inevitably had varying maximal oxygen uptake values and were thus running at a different speed during their 65%  $\text{VO}_{2\text{max}}$  90-minute preload run, this presented a non-ideal situation for measuring running economy. To circumvent this, the American College of Sports Medicine (ACSM)  $\text{VO}_2$  prediction equation was used to estimate expected running speed at a given oxygen cost (48). The equation,  $\text{VO}_2 = (0.2 \cdot \text{speed}) + (0.9 \cdot \text{speed} \cdot \text{grade}) + 3.5$ , was applied to treadmill running and used to predict the oxygen cost the athlete would be expected to consume at his submaximal running pace. Deviations from that predicted speed were expressed as a percent and used to represent subjects' relative economy. Zero percent deviation was representative of "average" economy while negative and positive values were indicative of better than average and poorer economy, respectively.

#### TISSUE ANALYSIS

Muscle biopsy samples were taken from the vastus lateralis just prior to the performance test and immediately frozen in liquid nitrogen. Approximately 50mg of

tissue was designated to assay gene expression and another 50mg to determine maximum activities of key metabolic enzymes. Tissue was weighed and homogenized in a buffer containing 0.250M Sucrose, 1mM EDTA, 0.01M Tris-HCL, and 2mM ATP at pH 7.4. Samples were then sonicated for a total of 30 seconds and stored at  $-80^{\circ}\text{C}$  until ready for analysis.

### Citrate Synthase

Citrate synthase catalyzes the formation of citrate and CoASH from acetyl-CoA and oxaloacetate. CoASH reduces DTNB and CS activity was determined from the reduction of DTNB over time. Ten microliters of a 5:1 diluted muscle homogenate was added, in duplicate, to 170 $\mu\text{l}$  of a solution containing Tris buffer (0.1M, pH 8.3), DNTB (1mM, in 0.1M in Tris buffer) and oxaloacetate (0.01M, in 0.1M Tris buffer). The spectrophotometer (SPECTRAMax PLUS 384, Molecular Devices Corporation, Sunnyvale California) was calibrated and 30 $\mu\text{l}$  acetyl CoA (3mM in ddH<sub>2</sub>O) was added to initiate the reaction. Absorbance was measured at 405nm at 37C every 12 seconds for 7 minutes. Maximum CS activity was calculated and reported as  $\mu\text{mol}/\text{min}/\text{mg}$ .

### Cytochrome C Oxidase

Cytochrome c oxidase activity was ascertained by measuring the rate of cytochrome c oxidation over time. The reaction media was prepared using the following protocol. 130 mg of reduced horse heart cytochrome c (2mg/ml) was dissolved in 6.5 ml 0.01M KPO<sub>4</sub> (in ddH<sub>2</sub>O). 260 $\mu\text{l}$  of 10mg/ml sodium dithionite (prepared in 0.01M KPO<sub>4</sub>) was added along with 6.5 ml 0.01M KPO<sub>4</sub> and 53 ml ddH<sub>2</sub>O to generate a total volume of 65ml reaction media. Following calibration of the spectrophotometer (SPECTRAMax PLUS 384, Molecular Devices Corporation, Sunnyvale California),

245 $\mu$ l of reaction media was added to 5 $\mu$ l of muscle homogenate, in triplicate, and absorbance was recorded at 550 nm every 10 seconds for 7 minutes at 37C. Maximum COX activity was calculated and reported as  $\mu$ mol/min/mg.

#### Beta-hydroxyacyl-CoA Dehydrogenase

For the determination of  $\beta$ -hydroxyacyl-CoA dehydrogenase, oxidation of NADH to NAD was measured. In triplicate, 20 $\mu$ l of muscle homogenate was added to 190 $\mu$ l of a buffer containing 0.1M liquid triethanolamine, 5mM EDTA tetrasodium salt dihydrate, and 0.45mM NADH. The spectrophotometer (SPECTRAmax PLUS 384, Molecular Devices Corporation, Sunnyvale California) was calibrated and 15 $\mu$ l of 2mM acetoacetyl CoA was added to initiate the reaction. Absorbance was measured at 340 nm every 12 seconds for 6 minutes at 37C. Maximum BHAD activity was calculated and reported as  $\mu$ mol/min/mg.

#### Real Time Quantitative RT-PCR

Total RNA was extracted using the acid phenol method of Chomczynski and Sacchi (49), with an additional DNase digestion step with concomitant acid-phenol extraction and ethanol precipitation. Primer and probe sequences were designed using Primer Express software package version 1.0 (Perkin Elmer, Norwalk, CT). Gene expression was measured using quantitative RT-PCR on an ABI Prism 7700-sequence detector. Briefly, 20 ng of diluted RNA was added to 2x TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems). PCR conditions consisted of a 2-step PCR. Step 1 included a 30 min reverse transcription stage at 48°C and another at 95°C for 10 min to activate AmpliTaq- Gold DNA Polymerase. Step 2 consisted of 40 cycles of a denaturing step at 95°C for 15 seconds and an extension stage at 60°C for 1 min. All samples for

each gene were run in duplicate simultaneously to control for amplification efficiency. To compensate for RNA input variation and the efficiency of reverse transcription, cyclophilin-B mRNA was quantitated and results were normalized to these values. To ascertain whether cyclophilin-B gene expression was modified by exercise and/ or intervention (moderate fat diet), absolute gene expression levels were quantitated and analysed as previously described (50). No exercise-dietary effect was detected on the absolute expression of cyclophilin-B, implying similar initial mRNA concentrations between samples.

#### STATISTICAL ANALYSIS

Pearson correlations comparing maximal oxygen consumption, mitochondrial enzyme activities and running economy were established using bivariate regression analysis with SPSS 14.0 software. The level of significance was set at  $p < 0.05$ .

## CHAPTER 4: RESULTS

### SUBJECT CHARACTERISTICS

Means and standard deviations of the subject's descriptive statistics are shown in Table 4.1. Twenty-one volunteers participated in the study of which 11 were male and 10 were female, ranging in ages from 18 to 54 years. One subject was an African American male and the remaining were all Caucasian. The average height was  $170.6 \pm 9.1$  cm ranging from 157 to 186 cm and average body mass was  $62 \pm 7.8$  kg and ranged from 46.6 to 74.2 kg. Mean percent body fat was  $16.8 \pm 4.8\%$  with values ranging from 9.97 to 25.2%. As would be expected in a similarly trained population, body mass index did not vary greatly across the participants. Average BMI was  $21.4 \pm 1.36$  kg/m<sup>2</sup> and ranged from 20.3 to 24.3 kg/m<sup>2</sup>.

Table 4.1 Subject Characteristics

	Mean	Standard Deviation	Range
Age (years)	27.81	8.09	19 - 45
Height (cm)	170.59	9.11	157 - 186
Weight (kg)	62.01	7.80	46.60 - 74.20
Body Fat %	16.80	4.83	9.97 - 25.20
BMI (kg/m <sup>2</sup> )	21.45	1.36	20.30 - 24.30

BMI=Body Mass Index

### MAXIMAL OXYGEN CONSUMPTION

All study participants met at least 2 of the 3 criteria used to validate the test: 1) a plateau in  $VO_{2max}$ , 2) RER greater than or equal to 1.10 and 3) achieve within 10 beats of maximal predicted heart rate. Results from the tests are summarized in Table 4.2. Resting heart rate averaged  $58.6 \pm 7.95$  bpm and ranged from 42 to 74 bpm. Mean resting systolic blood pressure was  $109 \pm 12.8$  mmHg while mean resting diastolic blood pressure was  $67.9 \pm 9.91$  mmHg. Average maximal heart rate was  $194 \pm 10.2$  bpm and ranged from a

minimum of 178 to a maximum of 217 bpm. Maximal RER averaged  $1.19 \pm 0.06$  and ranged from 1.09 to 1.25. Maximal oxygen uptake values averaged  $3.65 \pm 0.74$  L/min and  $58.6 \pm 7.94$  ml/kg/min with values ranging from 2.65 to 4.62 L/min and 47.5 to 77.3 ml/kg/min. When corrected for free fat mass,  $VO_{2max}$  averaged  $70 \pm 7.13$  ml/kg/min and ranged from 57.8 to 86.5 ml/kg/min.

Table 4.2 Maximal Oxygen Consumption

	N	Mean	Minimum	Maximum	Standard Deviation	Std. Error
Resting HR	21(11/10)	58.57	42.00	74.00	7.95	1.72
Resting SBP	21(11/10)	109.24	87.00	128.00	12.80	2.79
Resting DBP	21(11/10)	67.90	48.00	80.00	9.91	21.60
Maximal Speed, mph	21(11/10)	9.25	7.00	9.60	0.75	0.16
Maximal Grade, %	21(11/10)	7.61	2.50	10.00	2.77	0.60
Maximal HR	19(11/8)	191.37	178.00	217.00	10.20	2.34
Maximal RER	20(11/9)	1.19	1.09	1.25	0.06	0.01
$VO_{2max}$ , L/min	21(11/10)	3.65	2.65	4.62	0.74	0.16
$VO_{2max}$ , ml/kg/min	21(11/10)	58.64	47.50	77.30	7.94	1.73
$VO_{2max}$ , FFM adjusted	19(10/9)	70.00	57.76	86.50	7.13	1.64

N = (male/female); HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; RER = respiratory exchange ratio, FFM = free fat mass

#### MITOCHONDRIAL FUNCTION

Maximal activities of citrate synthase, cytochrome c oxidase, and beta-hydroxyacyl-CoA dehydrogenase were  $35.2 \pm 19.5$ ,  $57.6 \pm 60.7$ , and  $10.3 \pm 1.0$   $\mu\text{mol}/\text{min}/\text{mg}$  respectively (Table 4.3). CS values ranged from 12.7 to 67.0  $\mu\text{mol}/\text{min}/\text{mg}$ , COX ranged from 3.54 to 156  $\mu\text{mol}/\text{min}/\text{mg}$ , and BHAD ranged from 8.6 to 11.4  $\mu\text{mol}/\text{min}/\text{mg}$ .

Table 4.3 Enzyme Activity

Enzyme	N	Mean	Minimum	Maximum	Standard Deviation	Std. Error
CS	7(4/3)	35.2	12.7	67.0	19.5	7.4
COX	7(4/3)	57.6	3.5	156.0	60.7	22.7
BHAD	6(4/2)	10.3	8.6	11.4	1.0	0.4

N = (male/female); CS = citrate synthase; COX = cytochrome c oxidase; BHAD =  $\beta$ -hydroxyacyl-CoA dehydrogenase

#### RELATIONSHIPS BETWEEN VO<sub>2max</sub>, MITOCHONDRIAL FUNCTION AND ECONOMY

Correlations between maximal oxygen uptake, mitochondrial function and running economy are displayed in Table 4.4. Subject's running economy was determined using the American College of Sports Medicine prediction equation for oxygen cost at a given pace (48). To maintain an oxygen cost of 65% VO<sub>2max</sub> during the 90-minute run, the athletes ran at a steady pace. Given this pace, ACSM predicts an oxygen cost, and deviations from this expected cost give an indication of relative running economy. Mean percent deviation from this prediction was  $-8 \pm 9\%$ , indicating that on average the runners were operating at an oxygen cost 8% lower than predicted for that pace.

Economy ranged from  $-32\%$  deviation from predicted O<sub>2</sub> cost to  $+10\%$ ; 0% would indicate predicted or "average" economy. Running economy negatively correlated with VO<sub>2max</sub>, expressed in either absolute or relative values: L/min ( $r = -.506, p < .05$ ), ml/kg body mass/min ( $r = -.703, p < .001$ ; Figure 4.1) and ml/kg FFM/min ( $r = -.700, p < .001$ ; Figure 4.3). COX activity positively correlated with both CS activity ( $r = .872, p < .05$ ; Figure 4.3) and VO<sub>2max</sub>, L/min ( $r = .789, p < .05$ ; Figure 4.4). There was a trend for CS to correlate with VO<sub>2max</sub>, ml/kg/min but fell just short of reaching a level of significance ( $r = .731, p = .06$ ; Figure 4.5). Non-significant positive correlations were apparent between

both CS and COX activity and all other expressions of  $VO_{2max}$  ( $r > .6$ ) There were no significant correlations between BHAD activity and any measured variable.

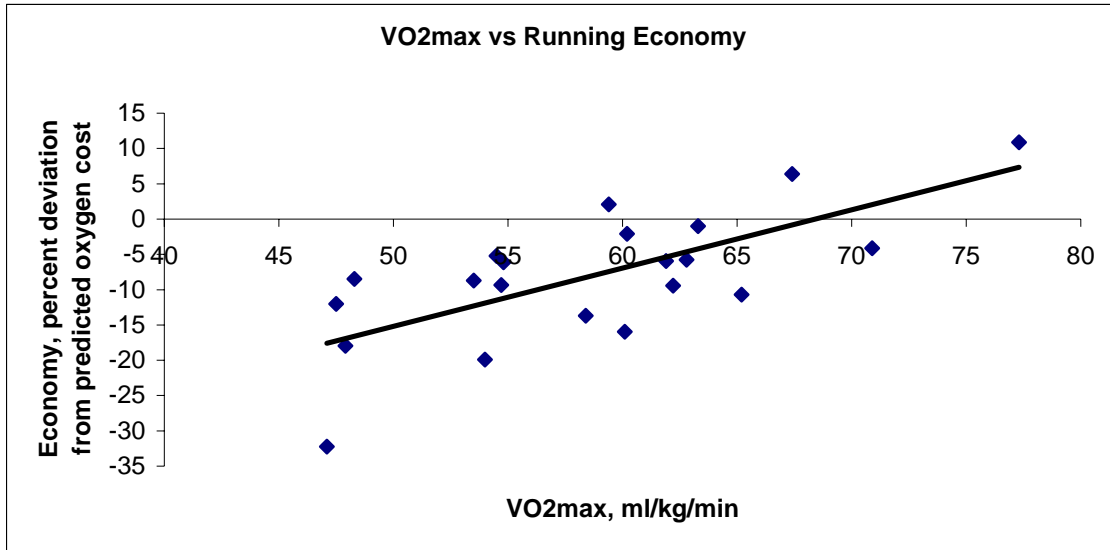


Figure 4.1 Relationship between running economy and  $VO_{2max}$ , ml/kg/min ( $r = -.703$ ,  $p < .0001$ ).

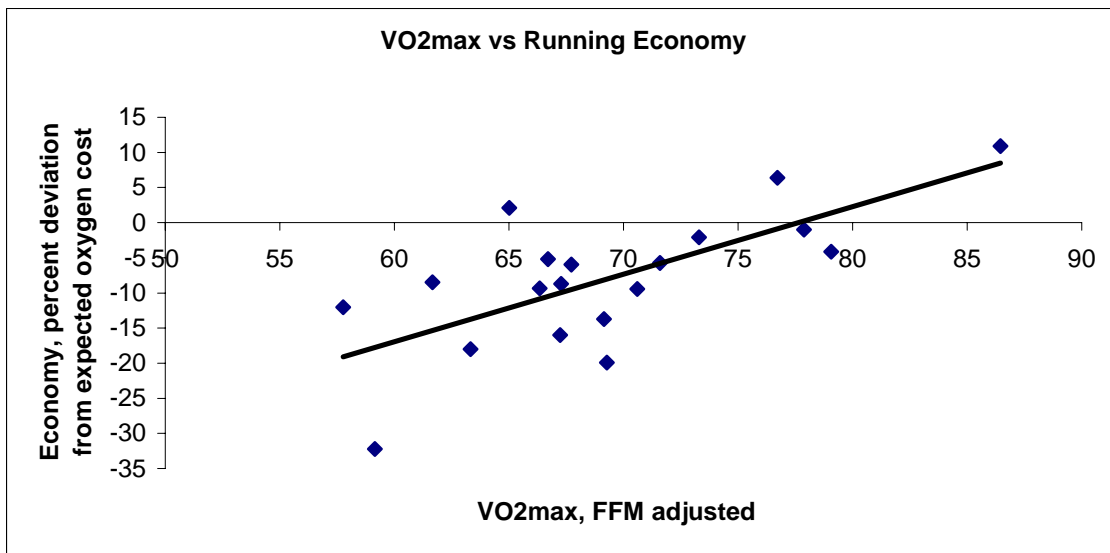


Figure 4.2 Relationship between running economy and  $VO_{2max}$ , FFM adjusted ( $r = -.7$ ,  $p < .001$ ).

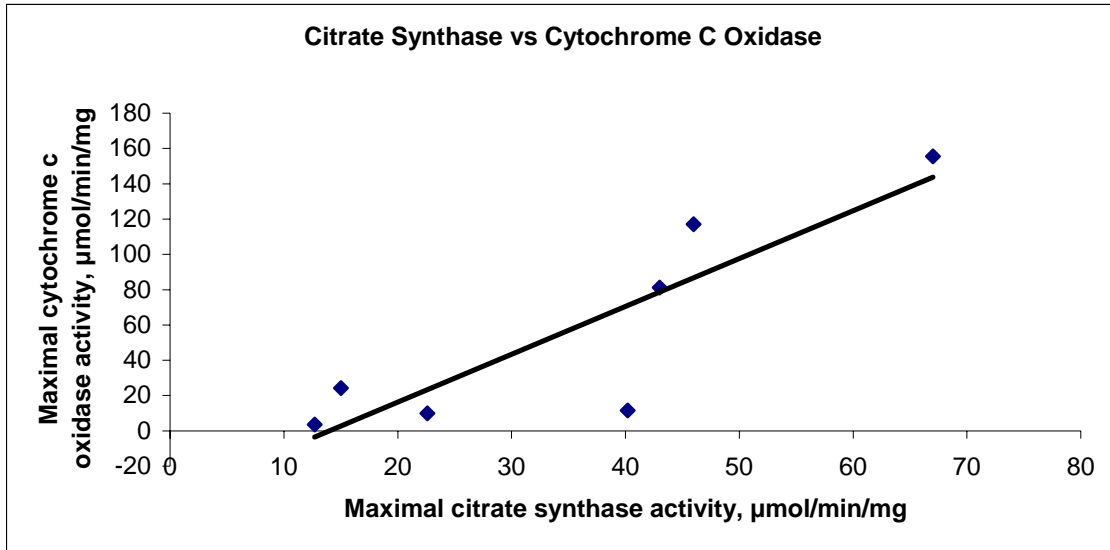


Figure 4.3 Relationship between maximal CS activity and maximal COX activity ( $r = .872$ ,  $p < 0.05$ ).

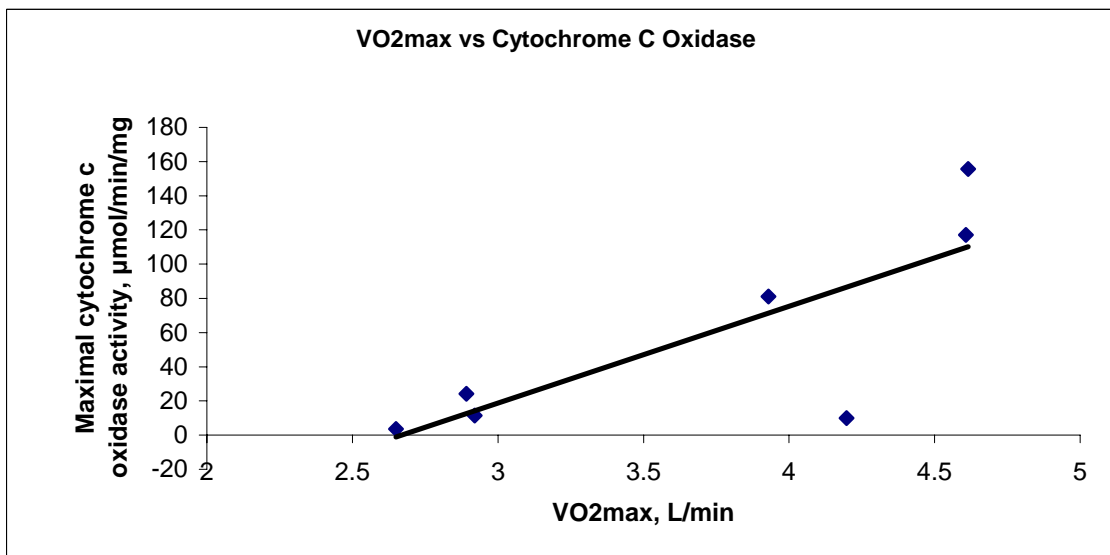


Figure 4.4 Relationship between maximal COX activity and VO<sub>2</sub>max, L/min ( $r = .789$ ,  $p < 0.05$ ).

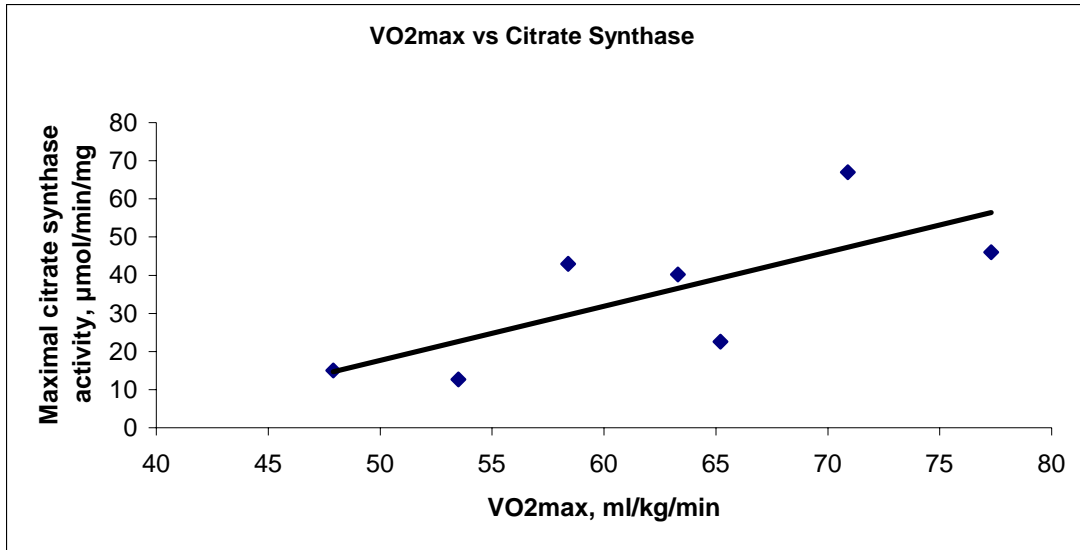


Figure 4.5 Relationship between maximal CS activity and  $VO_{2max}$ , ml/kg/min ( $r = .731$ ,  $p = .06$ ).

Table 4.4 Correlations between maximal oxygen uptake, mitochondrial function, and running economy.

	Economy	VO2max, L/min	VO2max, ml/kg/min	VO2max, FFM adj.	CS	COX	BHAD
Economy							
Pearson Correlation	1	- 0.506*	- 0.703**	-0.700**	-0.51	-0.445	-0.215
Sig. (2-tailed)		0.019	0.001	0.001	0.242	0.317	0.683
N	21	21	21	19	7	7	6
VO2max, L/min							
Pearson Correlation	- 0.506*	1	0.803**	0.595**	0.697	0.789*	0.311
Sig. (2-tailed)	0.019		0.001	0.007	0.081	0.035	0.549
N	21	21	21	9	7	7	6
VO2max, ml/kg/min							
Pearson Correlation	- 0.703**	0.803**	1	0.929**	0.731	0.658	0.361
Sig. (2-tailed)	0.0001	0.0001		0.001	0.062	0.108	0.482
N	21	21	21	19	7	7	6
VO2max, FFM adjusted							
Pearson Correlation	- 0.700**	0.595**	0.929**	1	0.723	0.614	0.383
Sig. (2-tailed)	0.001	0.007	0.001		0.104	0.194	0.524
N	19	19	19	19	6	6	5
CS							
Pearson Correlation	-0.51	0.697	0.731	0.723	1	0.872*	0.099
Sig. (2-tailed)	0.242	0.081	0.062	0.104		0.01	0.852
N	7	7	7	6	7	7	6
COX							
Pearson Correlation	-0.445	0.789*	0.658	0.614	0.872*	1	0.132
Sig. (2-tailed)	0.317	0.035	0.108	0.194	0.01		0.803
N	7	7	7	6	7	7	6
BHAD							
Pearson Correlation	-0.215	0.311	0.361	0.383	0.099	0.132	1
Sig. (2-tailed)	0.683	0.549	0.482	0.524	0.852	0.803	
N	6	6	6	5	6	6	6

\*\* Correlation is significant at the 0.01 level (2-tailed)

\* Correlation is significant at the 0.05 level (2-tailed)

## CHAPTER 5: DISCUSSION

The present study examined the relationship between mitochondrial function and maximal oxygen uptake. Specifically, to better understand the link between oxidative enzyme capacity and  $VO_{2max}$  in endurance trained males and females. Citrate synthase, cytochrome c oxidase and beta-hydroxyacyl-CoA dehydrogenase are key marker enzymes of the citric acid cycle, oxidative phosphorylation, and beta-oxidation, respectively. Measuring the enzyme's maximal activities and responses to stimuli i.e., diet or exercise training, provide researchers with a convenient and proven method of characterizing mitochondrial function. Another goal of this investigation was to observe the relationship between maximal oxygen consumption and running economy in this same population.

The results from this study show significant correlations exist between running economy and maximal oxygen uptake. Strong trends relating mitochondrial oxidative capacity to  $VO_{2max}$  were also observed. In an effort to appreciate these results, this discussion will present and discuss specific findings and relate them to previous research, review limitations and address future considerations.

### MAXIMAL OXYGEN UPTAKE VS. RUNNING ECONOMY

Figure 4.1 shows an inverse relationship exists between running economy and maximal oxygen uptake in a group of male and female endurance runners ( $r = -.703$ ,  $p < .001$ ). This negative correlation was apparent whether  $VO_{2max}$  was expressed in absolute or relative terms.

Similar findings have been reported in walking economy and cycling economy. Hunter (26) investigated walking economy and aerobic capacity in a group of sedentary

women, a subject population very different from that examined in the present study. Yet, the findings of that study revealed data very similar: a significant inverse relationship. Citrate synthase did not appear to relate to walking economy, coinciding with data from the present study showing a lack of significant correlations between oxidative enzyme activities and running economy. Results from the present study are also supported by data illustrating similar relationships in world-class cyclists (23).

Since many endurance performance events incorporate prolonged periods of running, i.e. marathons, whether this relationship is also present in running warrants attention. Three previous studies examined this association in highly trained runners however, two of them studied only males (24, 27), and the other found no correlations in running economy and maximal oxygen uptake (44). The findings from the present study are unique in that they confirm this inverse relationship not only exists but also persists even in a more diversified population, one containing both males and females.

Initially, this relationship may appear counterintuitive since one would expect that athletes that train for endurance events would simultaneously improve both economy and  $VO_{2max}$ . A variety of theories have been proposed to explain the mechanisms responsible for this inverse correlation. Intra-individual differences in muscle fiber type is one hypothesis that has been suggested. A high prevalence of Type I fibers has been associated with better economy (51, 52), while a greater distribution of Type IIA fibers have been linked to greater oxidative capacity (51,53). Type I muscle fibers are generally regarded to be more oxidative in their nature, however when viewed absolutely Type IIA fibers have shown the capacity to consume more oxygen at a given workload. Type IIA fibers have also been linked to inefficiency and inversely related to exercise economy

(54). Thus, it would be conceivable for individuals with a greater proportion of Type IIA fibers relative to Type I to have a higher capacity for maximal aerobic exercise in conjunction with poorer efficiency. Although the present study did not investigate fiber type differences among the participants, based upon this theory, it is predicted that those subjects' with a high  $VO_{2max}$  and poor economy would also have a greater Type IIA/Type I muscle fiber ratio than those subjects with lower  $VO_{2max}$  values and better economy.

The inverse relationship between  $VO_{2max}$  and economy may also be explained by individual differences in the distribution of mass in the body. Carrying a greater percentage of lean mass in the lower extremities could prove advantageous in the testing of aerobic capacity since  $VO_{2max}$  often requires running or cycling at extremely high workloads. More lean mass in the legs relative to the upper body would be expected to improve ones ability to perform a maximal exercise test since lean upper body mass is an insignificant factor in the modes of exercise associated with  $VO_{2max}$  testing. For the inverse relationship between economy and  $VO_{2max}$  to be caused by differences in body mass distribution, carrying a disproportionate amount of mass in the lower extremities would have to be associated with poorer economy. There is research to suggest that this is in fact the case (55-57). Aerobic demand has been shown to increase 10% for every additional kilogram carried in the shoes (55). In addition, when running at a constant pace of 12 km/h, Jones et al. (56) found that oxygen cost increased by 4.5 % per kilogram of load carried on the feet. Relating to the present study, those individuals with both a high  $VO_{2max}$  and a relatively poor economy quite possibly were carrying more lean mass distally compared to those subjects on the other end of the spectrum. Subjects in the present study were all engaged in regular endurance exercise and hence exhibited similar

body types. Differences in body mass distribution would not figure to vary significantly in this population. When adjusted for free fat mass, the inverse correlations persisted (Figure 4.2). Thus, differences in muscle histology (i.e. fiber type differences) are more likely the dominant mechanism driving this relationship.

The most universal method of measuring economy is by comparing differences in oxygen cost at the same running pace or cycling workload. However, subjects in the present study ran at a pre-determined oxygen cost (65%  $VO_{2max}$ ) and were thus running at different speeds. To overcome this limitation, the American College of Sports Medicine  $VO_2$  prediction equation was used to predict the oxygen cost that each runner would be expected to utilize at a given pace. This predicted  $O_2$  was then compared to their actual cost of running at that pace and deviations from that prediction represented running economy. Extensive reliability testing has validated the ACSM method for predicting oxygen cost at a give running speed (58). Both males and females have been tested using this method and predictions do not differ significantly from actual energy expenditures. Therefore, we remain confident that our assessment of economy is both valid and reliable.

There are a number of variables that have been known cause large intra-individual variation in economy testing (59). Variations in the stability of running economy testing of up to 11% have been reported in environments that neglect to rigorously control confounding variables. The most influential of these variables are footwear, treadmill experience, training status, the time of day of testing, and nutritional status (60). The current study controlled for these variables by implementing an experimental protocol designed to minimize test-retest variability. Each participant consumed a diet identical in

macronutrient content (10% fat, 15% protein, 75% carbohydrate) for 3 days leading up to his or her time trial run. All testing occurred during the same time of day (morning) on the same treadmill. All subjects underwent a light exercise session to become familiar with the treadmill days before any measured exercise testing occurred. All participants were screened for training status and had similar running experience. It can be assumed in a population that regularly endurance trains, that footwear is optimized to fit each individuals needs such that neither subject would experience a significant advantage or disadvantage over another. When similar approaches were taken to minimize testing variability, day-to-day correlations in running economy were high ( $r=0.95$ ) with a coefficient of variation of 1.3% (61).

#### MAXIMAL OXYGEN UPTAKE VS. MITOCHONDRIAL FUNCTION

Data from the current study show a significant correlation between COX activity and absolute  $VO_{2max}$  ( $r = .789$ ,  $p<0.05$ , Figure 4.4). COX also correlated with relative  $VO_{2max}$ , ml/kg/min ( $r = .658$ ) and strongly with maximal CS activity ( $r = .872$ ,  $p<0.05$ , Figure 4.4). This implies that although the two enzymes are responsible for catalyzing reactions involved in separate pathways, stimuli that cause adaptations in COX activity (i.e. increasing abundance/activity) should be reflected in corresponding changes to CS activity, and vice versa. Indeed, we found this to be the case. Apparent correlations were observed between citrate synthase activity and  $VO_{2max}$ . BHAD activity did not correlate with  $VO_{2max}$ , signifying that most likely beta oxidation plays a negligible role in limiting maximal oxygen uptake.

There is continual debate as to what factors limit oxygen consumption. One theory holds that  $VO_{2max}$  is limited solely by the cardiovascular system's capacity to

deliver oxygen to exercising muscles (7-11). It is believed by some that improvements in maximal aerobic capacity that result from training merely reflect adaptations in cardiac output (11). It is proposed that skeletal muscle O<sub>2</sub> extraction and utilization is only critical in improving submaximal performance. Based upon this theory, one would not expect to see distinct correlations between VO<sub>2max</sub> and mitochondrial enzyme activities since oxidative enzyme capacity is relatively unimportant in realizing VO<sub>2max</sub>. The results of the present study suggest differently. Data shown here indicate that increases VO<sub>2max</sub> are associated with paralleled increases in mitochondrial function (Figures 4.4, 4.5 and Table 4.4). The present findings are substantiated by previous literature that has shown similar relationships (7, 17, 18, 21)

The comparative relationships between VO<sub>2max</sub> and mitochondrial function do not appear to be restricted to CS and COX activity. Succinate dehydrogenase and oxogluterate dehydrogenase, both citric acid cycle enzymes, have also been shown to correlate positively with VO<sub>2max</sub> (7, 18). The proportional relationships shown here and previously suggest a few things. It implies that mitochondrial enzyme activity can be used as a measure to estimate whole-body oxygen consumption. Since muscle in the present study was sampled from the vastus lateralis, it appears that metabolic characteristics of the vastus lateralis are representative of whole-body skeletal muscle (62). Also, these data suggest that skeletal muscle mitochondria are operating maximally (Figure 4.4) or very close to maximally (Figure 4.5) as exercise intensities approach exhaustion. Thus, it appears that the enzyme's specific activity is rate-limiting the oxygen flux thru the metabolic pathway. If this is in fact the case, any adaptations that enhance oxidative enzyme capacity will have direct improvements on VO<sub>2max</sub>.

The relative importance of oxygen supply versus oxygen utilization in limiting  $VO_{2max}$  may depend on training status. It is possible that sedentary individuals are limited by factors different from those that may be limiting oxygen consumption in trained athletes. Likely, untrained individuals are limited aerobically more so by inadequate blood supply, i.e., limitations in stroke volume, rather than by mitochondrial function. Therefore, improvements in mitochondrial function in this population would be futile in improving  $VO_{2max}$ . In contrast, endurance trained individuals may be more sensitive to changes in mitochondrial oxidative capacity and thus their  $VO_{2max}$  will be more responsive to changes in CS or COX activities, for example. Obviously oxygen consumption can be no greater than what is ultimately being supplied to active muscle via the cardiovascular system. However, the endurance trained population has most likely already realized these central adaptations and further improvements in  $VO_{2max}$  must be generated via peripheral adaptations, i.e., improvements in mitochondrial function. This idea is supported by the work of Lortie and Bouchard (63) who compared skeletal muscle characteristics and aerobic performance in sedentary and active individuals. In a large group of sedentary men, mitochondrial enzyme activities did not correlate with  $VO_{2max}$ . However, in the active or well-trained population significant positive correlations existed between all measured enzymes and  $VO_{2max}$ . Thus, it is probable that in sedentary subjects, aerobic capacity is defined by the ability to deliver oxygen whereas in an active population it is the capacity to extract and maximally utilize oxygen that differentiates the group. This would explain why the present study observed significant correlations between enzyme activities and  $VO_{2max}$ , while those studies examining untrained subjects, did not (9,10).

The present study was admittedly limited by the availability of tissue samples for analysis. A total of seven subjects were confidently assayed for mitochondrial function. This inherently made it very difficult for the correlations to prove significant at the  $p < 0.05$  level. Consequently, one must be wary when making conclusions based on these results. Certainly the correlations appear strong; Pearson correlations were all greater than 0.61. With a slightly larger sample size the correlations would almost surely prove significant. Caution is also advised when basing conclusions upon correlations, as they do not imply causation. Although the data herein appear to implicate the role of mitochondrial function in realizing  $\text{VO}_{2\text{max}}$ , it cannot be said with confidence that higher oxygen uptake values in a trained population are the sole result of a greater mitochondrial capacity. Simply, they are highly associated. Nonetheless, the results of the present investigation bear on the long-standing debate about what factors, oxygen delivery or skeletal muscle  $\text{O}_2$  extraction, truly limit maximal oxygen consumption. The answer most likely falls somewhere along a continuum and is dependent on population characteristics, i.e., trained vs. untrained. Here, greater oxidative enzyme capacity was associated with proportionally greater  $\text{VO}_{2\text{max}}$  values. Consequently, it is advised that it would be a mistake to ignore the role mitochondrial function plays in the determination of maximal oxygen uptake in endurance trained populations.

#### FUTURE DIRECTIONS

As mentioned previously, fiber type differences have been suggested to be causal in the relationship between running economy and  $\text{VO}_{2\text{max}}$ . If Type IIA muscle fibers are associated with both increased oxidative capacity (53) and inefficiency (20, 51, 54), this may perhaps explain the inverse relationship. The present study did not characterize

muscle tissue based upon fiber type so our data is unable to provide a mechanism to explain the negative association. Additional research is advised to determine if individuals possessing both a greater aerobic capacity and a poor economy also have a higher proportion of Type IIA fibers relative to Type I.

Although extensive research has been devoted to understanding exercise economy, relatively few interventions have been successful in improving running economy in elite distance runners (28). Preliminary reports indicate that training in certain environmental conditions may be beneficial to running economy. Extensive literature supports the notion that altitude training positively impacts endurance performance (64, 65), however little research has investigated its effect on economy. A few studies have reported no change in running economy following altitude exposure (66, 67) while others have shown modest improvements (68, 69). Training in the heat also has shown promise in improving physiological parameters that may improve one's economy, i.e., elevating core body temperature and decreasing blood viscosity (28). Clearly, further research is needed in this area, as the literature is limited and inconclusive.

The present study also examined diet-gender interactions with mitochondrial function however, the availability of tissues sample for analysis was restricted. Previous investigations have revealed that humans have an extraordinary ability to adapt to changes in diet. Short-term high fat diets are associated with almost immediate adaptations in substrate oxidation (70). Results revealed significant metabolic adaptations occurring after 5 days of diet intervention.

There is a substantial amount of evidence demonstrating the existence of gender differences in substrate metabolism both at rest and during exercise (71-74). Females

appear to have a greater predisposition towards both fat storage and utilization during exercise relative to men (75). Theoretically, it would be sensible to suspect that women will adapt more favorably to a moderate fat diet condition (vs. low fat) compared to men. Thus, in transitioning from a low fat diet to a moderate fat diet, it would be reasonable to suspect that enzymes associated with oxidative metabolism are more responsive in women compared to men. The current study identified trends that appear to support this belief. In a small subset of our subject population (4 males and 3 females), CS and COX activities were assayed following a low fat (10% fat) and a moderate fat (35% fat) diet. There did not appear to be any change in maximal enzyme activity in the males, however the activity of both CS and COX were elevated in females following 3 day moderate fat feeding vs. the low fat condition. These differences also appeared to manifest themselves in the expression of some lipolytic genes including carnitinepalmitoyl transferase-1, a fatty acid transporter. The sample size was small and thus it would be a mistake to draw any conclusion from this finding. Further research is necessary to determine if this trend can be substantiated in a larger and more encompassing population. Whether these apparent gender differences in mitochondrial response to diet intervention are translated into performance advantages for females relative to males is another topic that also warrants investigation.

## CONCLUSIONS

In summary, the findings from the current study suggest that a significant and inverse relationship is present between running economy and maximal oxygen uptake in endurance trained males and females. Also, data herein indicate that maximal activities of key marker enzymes of the citric acid cycle and oxidative phosphorylation are likely a

significant factor in determining maximal oxygen consumption in this same population. Further investigation is warranted to determine if these relationships are replicable in a more diversified population. Furthermore, future inquiries should seek to better understand the mechanisms affecting running economy so that training programs may be more optimally tailored to improve endurance performance in runners.

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## APPENDIX

### CONSENT FORM/SUPPLEMENTAL FIGURES

1. Study Title: Influence of Diet on Recovery of Intramuscular Substrates and Subsequent Performance in Endurance Trained Men and Women.
2. Performance Site: Pennington Biomedical Research Center of Louisiana State University System at Baton Rouge, Louisiana
3. 

<u>Investigators:</u>	<u>Role on Project:</u>
Enette Larson-Meyer, Ph.D, R.D.	Principal Investigator
Eric Ravussin, Ph.D.	Co-Principal Investigator
Donna Ryan, M.D.	Co-Principal Investigator
George Argoropolous, Ph.D.	Co-Investigator, Gene Expression
Michael Hamilton, M.D.	Medical Investigator, general
Steve Smith, M.D.	Medical Investigator, biopsies
Jozef Ukropec, Ph.D.	Co-Investigator, Biochemistry
4. Subject Exclusion Criteria:
  - A. Smokers
  - B. Individuals who present with signs/symptoms of an upper respiratory infection (the study visit will be postponed until the infection has passed and the participant is cleared for participation by the study physician)
  - C. Individuals with known allergies to local anesthetics such as Novocain, Lidocain, Bupivacaine, Benzocaine, etc
  - D. Individuals who are diabetic
  - E. Individuals with heart problems or elevated blood pressure >140/90mm Hg
  - F. Individuals who have significant renal, hepatic, endocrine, gastro-intestinal, pulmonary, cardiac or hematological disease
  - G. Individuals with partial syndrome eating disorders (score of >20 on the Eating Attitudes Test)
  - H. Individuals with alcoholism or other substance abuse
  - I. Individuals who use prescription or over-the-counter medication or herbal preparations that can influence metabolism such as  $\beta$ -blockers, caffeine or ephedrine containing medication

- J. Individuals who are not willing to avoid aspirin, NSAIDS or other medicines that may interfere with clotting time during the two 8 day study periods and for up to 24 h after the last muscle biopsy
  - K. Individuals who are not willing to avoid alcohol or caffeine (including caffeinated soft drinks) on exercise testing days
  - L. Individuals who are not willing to consume all foods/beverages provided on the baseline and experimental phases of the study
  - M. Individuals who are not willing to participate in both phases of the study.
  - N. Women who are pregnant (a pregnancy test will be performed before the start of the study)
  - O. Women who are going through or have gone through menopause
  - P. Individuals who have clips or metal plates in their head, wear braces on their teeth, have false teeth or removable bridge work, were ever injured by a metallic foreign body which was not removed, or who have orthopedic pins, screws, or rods will still be eligible for the study but will not have the Magnetic Resonance(MR) scans performed.
5. Eligible Study Population:
- A. Community description and demographics:  
The Pennington Biomedical Research Center is located in Baton Rouge Louisiana, which is the home of the state government, the 31,000-student Louisiana State University, the 9,000-student Southern University. Both Louisiana State University and Southern university have an athletic programs, which include men's, and women's cross country and track and field. Baton Rouge and the neighboring cities and towns (which included New Orleans) have a number of community-based running clubs and triathlon clubs which for example include but are not limited to: Club South Runners and Dream Team Racing in Baton Rouge, Bayou Runners Association in Houma, Cajun Road Runners Club in Lafayette, and the New Orleans Track Club and Team Spot Tees in New Orleans. We will recruit from this large group of local endurance trained men and women.
  - B. Recruitment and Retention:  
Volunteers will be recruited from the Baton Rouge area using the standard procedures utilized at the Pennington Biomedical Research Center (PBRC). This effort is directed by 3 full-time volunteer recruiters and a clinical trials support staff. While newspaper articles and ads, television and radio news stories have all been used at the PBRC as methods for soliciting public

interest, focus for the current study will be on media such as club newsletters and magazines which specifically target endurance runners and triathletes. The specific criteria for volunteer selection for this study will be clearly communicated in all media information, and all advertisements are approved by the Institutional Review Board prior to publishing.

Interested individuals will be instructed to contact a volunteer recruiter. At this point, an initial telephone screening will take place to identify any exclusion criteria that could eliminate the person from the study pool. Following phone screening, all eligible candidates will be seen at the PBRC Clinic for an initial screening visit. At Screening Visit 1, information related to medical and surgical history and medication use is collected, height, weight and blood pressure are measured, a resting EKG is obtained, and a fasting blood sample is collected for blood chemistry and lipid profiles (Chem-15) which includes glucose, creatinine, potassium, uric acid, albumin, calcium, magnesium, CPK, ALT, alkaline phosphatase, iron, triglycerides and total, HDL and LDL cholesterol. A screening informed consent, approved by the IRB, (please see attached) is discussed and signed by the volunteer, a clinic representative, and a witness. Potential volunteers will then undergo a physical exam by the medical investigator or another PBRC staff physician, and a variety of psychological measures are completed, including measures of eating and dieting behaviors, alcohol and drug use, depression and anxiety, and physical activity and diet histories. Additionally, volunteers meet with a clinical psychologist to discuss any concerns or questions, determine the volunteer's time availability for participation in the study, and evaluate whether there are any lifestyle or personality factors that might interfere with the volunteer's ability to participate. The clinical psychologist then makes a recommendation for continuation or exclusion of the volunteer. The Eating Attitudes Test will be administered at this time to help screen for eating disorders or partial syndrome eating disorders. Potential volunteers who are no longer interested in participating can withdraw from the screening at any point.

Those volunteers who are still eligible and interested after the initial screening visit are scheduled for a second appointment, where study-specific screenings are performed (including aerobic fitness testing and body composition analysis as described below). Additionally, they meet with the study dietitian to review the foods that will be included on the menus and identify any items they cannot or prefer not to eat. They will also meet with the study coordinator, the medical investigator and/or the PI for any additional questions that have not been unanswered. At this time, the volunteer will have the opportunity to sign an approved informed consent form for the study. Consenting takes place in a clinical examination room. First the consent form is provided to the volunteer, then explained by the study coordinator who asks for and answers questions about this consent. The study coordinator will then leave the room, allowing as long as is needed for the volunteer to privately

review the consent form. Then the volunteer summons the study coordinator who will answer any pending or additional questions the subject has. A witness, who is part of the PBRC nursing staff (RN or LPN) but not in any way involved in the study, will be present during the entire process. A copy of the consent form, with contact phone numbers, is provided to each volunteer. Documentation of consent is assured by requiring a witness signature on the consent form. No modification or waiver of informed consent has been sought from the IRB. If all study-specific eligibility criteria are met and the psychologist and dietitian approve the volunteer's participation, they are then officially enrolled in the study.

We anticipate that the major barriers to recruitment will be the invasiveness of the study and the time commitment during the 8 days of both crossover trials (i.e., subjects will need to have flexible work schedules, take off time from work, or not be employed full-time). The major barriers to subject retention will be overuse injuries (common to distance runners) that could develop between the crossover trials. It has been the experience of the Principal Investigator that screening for partial syndrome eating disorders and history of overuse injuries will help minimize problems with retention. It is also the experience of the Principal Investigator that endurance athletes enjoy participating in this type of study and learning more about their body and their performance. Following the completion of their participation, they will receive information about their aerobic fitness and body composition. Following the completion of the trial, they will receive a summary of the study results, how they performed under both diet treatments, and how the study results are important to the performance of endurance athletes (see Benefits to the Study for further detail). Volunteers will receive an appropriate cash incentive for their participation.

The volunteer's primary care physician will not be notified of enrollment, and no medical clearance will be obtained

6. Emergency Procedures:

While all subjects participating in the study will be well-trained athletes, the exercise and clinic staff is prepared to handle unexpected medical emergencies. All exercise testing and procedures will be conducted at the "Health and Fitness Center" on the Pennington Campus, located just a short distance (~1000 feet) from the Clinic. The Health and Fitness Center houses an exercise testing lab, two exercise intervention rooms and Men and Women's locker rooms. In case of an emergency, the PBRC has an Emergency Response Policy and a one page "Life Threatening Emergency – Action Flow Chart". The exercise laboratory is equipped with a Life Pak 9 defibrillator, a fully stocked crash cart that has first and second line code drugs, supplemental oxygen, and anaphylaxis kits. Every room in the fitness center also has An American Red Cross Res-cue mask, all purpose first aid kit, and Emergency Action Plan Notebook. Each phone in the

facility has a sticker that gives the phone number for EMS, security and the front desk, and the Life Threatening Emergency – Action Flow Chart” is hanging nearby for easy viewing. All the exercise staff is Basic Life Support (BLS) certified and there is always an Advanced Cardiac Life Support (ACLS) certified physician at the center during business hours when exercise procedures take place. The PBRC staff is knowledgeable about the PBRC Emergency Response Policy. The response time has been determined to be 2-4 minutes. The nearest hospital is 6 blocks from the PBRC and the EMS station is ~3/10 of a mile.

To ensure safety during submaximal exercise and performance testing, it is standard policy at the PBRC Health and Fitness Center that an exercise staff member monitor every exercise session and not leave a subject unattended (even for a second) while they are using the exercise equipment. This policy is enforced at all times and will help to ensure that the participants in this study are not injured from treadmill associated falls.

7. Subject Benefit and Compensation:

The results of this study will help in understanding how nutrient intake can influence performance in endurance trained athletes, including military troops, during excessively demanding endurance events and provide useful data for determining whether the nutritional needs of extremely active women are different than those of active men. Individuals volunteering for the study will learn whether the fat composition of the diet will influence their individual performance. In addition, they will receive information about their body composition, blood cholesterol and other blood lipids, lipid and carbohydrate metabolism, and fitness level.

Participants will be paid \$ 1000,- upon completion of the study. \$1000 was rounded up from \$978. If she is or has been an employee of LSU within the current calendar year, the normal employee payroll deductions will be withheld. Also, volunteers will be offered prize incentives (as external motivators) during their 10-K performance test. Specifically, they will be given a workout towel for coming within 90 sec of their 10-K personal record (PR) (set at any competitive 10-K event over the last year), and a runner’s PR package (\$50 gift certificate to a local running store, and certificate for aerobic and body composition analysis at PBRC) for meeting or exceeding their PR. PR times will be verified from race records. In the case that the participation in the study will end early they will receive \$300 for completion of the first 8-day period and \$500 for completion of the final 8-day period.

8. Risks for Procedures:

A. Physical Exam:

The risks involved in the physical exam include a breach in confidentiality of the personal data obtained from the subjects. To minimize this risk subjects will be assigned ID numbers for most records. Therefore personal

identifiers are not usually visible.

B. DEXA Scan:

The risks involved in the DEXA scan include an exposure to a low dose of radiation. However, the scan takes only 10 minutes and the radiation dose to the subject is less than 1.0 mrem, equal to about 12 hours of background radiation from the sun while outside.

C. Phlebotomy:

The risks involved in phlebotomy include discomfort with the needle stick, bruising, bleeding, infection, fainting, pain, and venous scarring. To minimize this risk proper techniques will be employed, using an i.v. to prevent having to stick at each blood draw.

D. Maximal aerobic capacity:

The risks involved in testing maximal oxygen uptake include muscle tightness or soreness 1 to 2 days after the exercise, difficulty breathing through a mouthpiece-nose clip system, cardiac arrhythmia, and death. To minimize these risks subjects are screened for cardiovascular system problems. Also, the Medical Investigator will be available to handle any medical emergency that may arise during testing.

E. Endurance exercise:

The risks involved in submaximal tests are minimal in endurance trained athletes. Potential risk factors include muscle tightness or soreness 1 to 2 days after the exercise, difficulty breathing through a mouthpiece-nose clip system, boredom exercising indoors, cardiac arrhythmia, and death. Steps to minimize these risks involve: selection of trained athletes who perform similar exercise bouts during their normal training, screening for cardiovascular system problems, and the Medical Investigator available to handle any medical emergency that may arise during testing.

F. Skeletal muscle biopsy:

The risks involved in the skeletal muscle biopsy include pain at the biopsy site, bleeding, infection, bruising, damage to superficial sensory nerve, and keloid forming. To minimize these risks the administration of topical antibiotics upon completion and careful follow-up procedures will be employed.

9. Minimizing Potential Risk:

Although there are potential risks involved in the participation of the presented study, we will make all attempts to prevent the occurrence of any adverse event. This starts with the exclusion of any endurance athlete who might be at higher risk due to a medical history as described in the exclusion criteria, and the regulations established by the American College of Sports Medicine (ACSM).

The efforts to minimize the risks involved with every individual procedure are presented in the table above. Basically they include use of sterile techniques, periodic monitoring of vital signs, and the continuous presence of BLS-trained personnel during procedures. In addition, all abnormal medical screening and lab results will be reviewed by both a clinical chemist and a physician who will notify the P.I. of abnormalities in blood work.

All study volunteers will be assured of the anonymity and confidentiality of their data. This will be done both verbally and in the consent form. The clinical facilities are strictly limited to Pennington staff and research volunteers. This is accomplished by a variety of security measures. All medical records are and will continue to be stored in a locked area in the clinic. Access to this area is limited to the clinical support staff, director of the clinical facilities, and the P.I. of the study. Records are filed according to an assigned volunteer ID number. All forms in the chart, with the exception of the consent form, will be identified by this number only. Access to the electronic data storage is also restricted, with only the P.I. and authorized personnel having access to the data base containing confidential clinical records. The one exception is that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

All clinical procedures performed at the Pennington Center require the presence of a licensed physician on site all the time. In addition, an emergency on-call telephone number is available 24 hours per day, 7 days a week, with a physician on call. If necessary, short-term psychological counseling is also available to research volunteers through faculty of the Psychological Services Center at Louisiana State University.

Adverse events occurring during the participation in the study will be documented in the volunteers record, whether they are related to the study or not. Adverse events include illnesses and injuries, and worsening of preexisting conditions. Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the USAMRMC Deputy for Regulatory Compliance and Quality (301-619-2165) (non-duty hours call 301-619-2165 and send information by facsimile to 301-619-7803). A written report will follow the initial telephone call within 3 working days. Address the written report to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RCQ, 504 Scott Street, Fort Detrick, Maryland 21702-5012. An unbiased written report of the event by the Medical Monitor for all serious and unexpected adverse events (per ICH definitions) will be provided within 10 calendar days of the initial report. The Medical Monitor will be a qualified physician, other than the Principal Investigator, otherwise not associated with this protocol, able to provide medical care to research subjects for conditions that may arise during the conduct of the study, and who will monitor the subjects during the conduct of the study. The Medical Monitor is required to review all serious and unexpected adverse events (per ICH definitions) associated with the protocol and provide an unbiased written

report of the event within 10 calendar days of the initial report. At a minimum, the Medical Monitor should comment on the outcomes of the adverse event (AE) and relationship of the AE to the test article or intervention. The Medical Monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator.

At the end of the first diet phase, the participants will be scheduled for the next visit and asked to maintain body weight and similar training habits and asked to avoid giving blood. They will also be given specific instructions as described elsewhere on caring for their muscle biopsy. At the end of the second diet phase, the participants will be given only the muscle biopsy information. They will not be officially debriefed until the closure of the study when the randomization code is broken and the data is analyzed. At this time, a special letter will be sent to all volunteers, describing in lay language the major study findings of the study. This form will be developed after the study is closed.

10. Procedures for Early Withdrawal from the Study:

The Study P.I., Dr. Larson-Meyer, or the study sponsor can withdraw the participant from the study for any reason or for no reason. The participant may also elect to withdraw from the study at any time without penalty. Possible reasons for withdrawal by the P.I. include not showing up for appointments or exercise training sessions, not complying with the exercise prescription, not completing all procedures, not complying with the assigned study diet, not being willing to eat the all study food provided and/or developing an over-use injury that would prevent full participation in exercise testing. After a participant requests for early withdrawal from a study, the participant will be contacted to determine the reason for the request. This information is documented in the participant's study chart. If the participant is asked to withdraw from a study, the reason for withdrawal will be communicated to the participant and documented in the participant's study chart. Participants who are withdrawing early from a study will be paid, if applicable, by the schedule of payments outlined in the study consent form signed at the beginning of their participation. Any abnormalities in lab, ECG, physical exam or other obtained results will be discussed with the participant by the Study Physician or designee to ensure adequate follow-up.

## SUPPLEMENTAL FIGURES

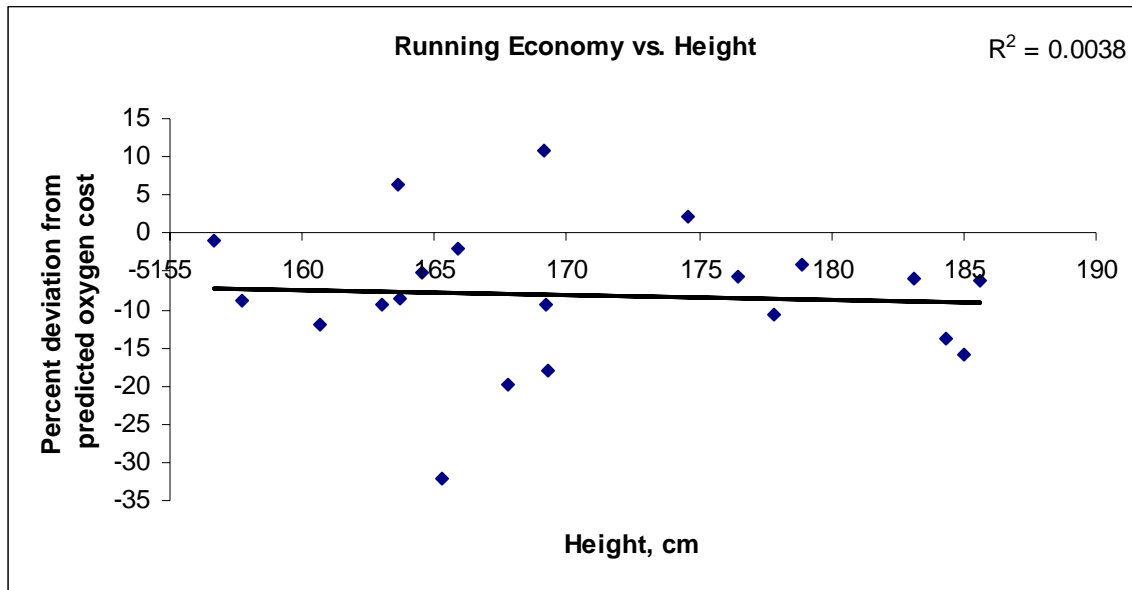


Figure 1. Relationship between running economy and subject height ( $r = 0.06$ )

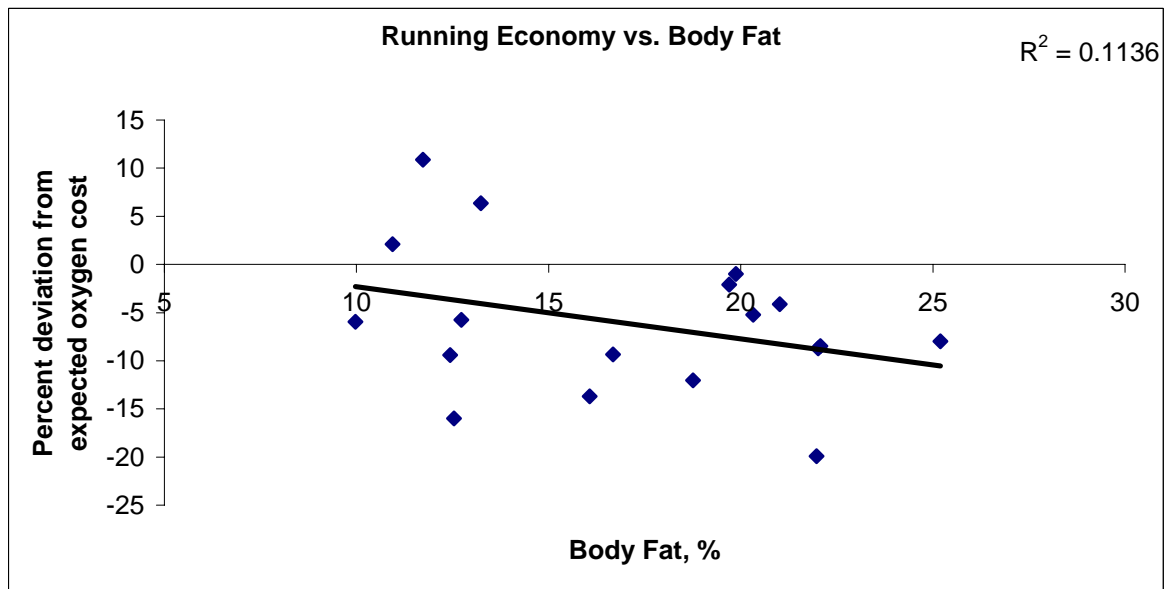


Figure 2. Relationship between running economy and subject body fat ( $r = 0.33$ )

## **VITA**

Ryan P. McMillan was born in Houston, Texas, on November 5, 1980. He graduated from Bellaire Senior High School in Bellaire, Texas, in May of 1999. He received his Bachelor of Science degree in kinesiology from the University of Colorado at Boulder in May of 2003. The following fall semester he began work towards his master's degree in kinesiology at Louisiana State University in Baton Rouge. He will complete the requirements for the degree of Master of Science in April of 2006.