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The Relationship between fitness level, gender, and the percentage of VO₂ max at crossover

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THE RELATIONSHIP BETWEEN FITNESS LEVEL, GENDER, AND THE
PERCENTAGE OF VO₂ MAX AT CROSSOVER

by

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A thesis submitted in partial fulfillment
of the requirements for the

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Christina Louise Pettigrew

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ABSTRACT

The Relationship Between Fitness Level, Gender and the Percentage of VO₂ Max at Crossover

by

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The “crossover” concept is a means of understanding fuel utilization over a range of exercise intensities. According to this concept, certain biochemical adaptations occur in a fit individual allowing for an increase in lipid oxidation. The present study examined whether there was a relationship between fitness level, gender, and the percentage of maximum aerobic capacity (VO₂ max) at crossover. Twenty-five subjects age 18-40 participated in this study. Subjects were divided in half based on their fitness level. After completing a graded exercise test to exhaustion, the crossover point for each subject was determined relative to their VO₂ max. A 2x2 factorial ANOVA was used to assess whether the percentage of VO₂ max at crossover could be predicted from fitness level (top half and bottom half of sample), gender, and the interaction between fitness level and gender. No significant interaction between fitness level and gender was found ($F=1.064$, $p=.314$). The lack of significance shows that beyond certain intensities, gender and fitness no longer have an effect on the percentage of VO₂ max at crossover. It is reasonable to assume that at lower intensities fat oxidation is affected by fitness level and gender, but at higher intensities the sympathetic nervous system causes an increase in glycogen available for use with a reduced dependence on fatty acid oxidation. Further research is needed to determine the effects of gender and fitness level on percentage of

VO₂ max in non-healthy adult populations and to explain the large amount of variability in crossover from one subject to the next.

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CHAPTER 1

INTRODUCTION

The “crossover” concept is a theoretical model of energy utilization which may help explain the balance of carbohydrate and lipid utilization as it relates to exercise intensity. The crossover point is defined as the point at which the body switches from primarily oxidizing lipid to primarily utilizing carbohydrate for energy production during exercise (Brooks et al., 2000).

Recent studies have shown that two of the main training adaptations of endurance exercise are an increase in maximum aerobic capacity (VO_2 max) and an increase in lipid oxidation at the same absolute workload (Friedlander, 1999, 2007; Achten, 2002). Because VO_2 max is an indication of fitness level (and thus whether an individual has engaged in endurance training), it will be used to classify subjects as either fit (trained) or unfit (untrained). Whole body fat oxidation peaks at moderate exercise intensity, generally between 55-65% maximal oxygen uptake (Norby, 2006). Crossover tends to occur shortly after this peak. What is not known, however, is whether the crossover point shifts (as it relates to VO_2 max) after endurance training. It would seem that if fat oxidation and VO_2 max both improve after training, there may simply be no net change in percentage of VO_2 max at which crossover occurs. However, because fat oxidation and VO_2 max may not improve at the same rate, there may be reason to believe the crossover does in fact shift with endurance training. If this shift does occur, numerous people may potentially benefit.

Athletes utilize relatively more energy from carbohydrate because they tend to train and compete at higher intensities. This does not mean that fat utilization is

unimportant, however. Lipid utilization spares glycogen use there by prolonging the time to muscle glycogen depletion; a point at which an athlete is forced to slow down. Lipid is also important for the recovery of an athlete, allowing glycogen to be replenished and the athlete's body to function normally (Brooks, Text, 1996). Thus, if it can be determined that the percentage of VO_2 max at crossover can be improved, there may be training implications for athletes, ideally training them to utilize lipid at as high of an intensity as possible in order to stave off glycogen depletion.

Athletes are not the only ones that may benefit from improved percentage of VO_2 max at crossover. Those with metabolic dysfunction have abnormal lipid utilization (Brun et al, 2007). Lower fat oxidation, compared to their metabolically normal counterparts, as exercise intensity increases appear to be due to "reductions in lipolysis, fatty acid mobilization, as well as fatty acid oxidation by muscle, probably mediated by an insulin effect...insulin is a known potent inhibitor of lipolysis" (Coyle et al, 1997). While exercise is already known to improve lipid oxidation and glucose tolerance, if crossover can improve and subjects can utilize fat over a larger range of intensities, endurance training may have important metabolic ramifications, ultimately allowing the patient to utilize lipid in a normal manner.

Statement of the Problem

"Is there a relationship between fitness level and the percentage of VO_2 max at which crossover occurs?"

"Is there a gender effect on the percentage of VO_2 max at which crossover occurs?"

Research Hypothesis

1. There will be a difference between percentages of VO_2 max at crossover when comparing fit vs. unfit subjects.
2. There will be a difference in percentage of VO_2 max at crossover when comparing males vs. females.
3. The effect of gender is not independent of the effects of activity level.

Null Hypothesis

1. There will be no difference in percentage of VO_2 max at crossover when comparing fit vs. unfit subjects.
2. There will be no difference in percentage of VO_2 max at crossover when comparing males and females.
3. The effect of gender is independent of the effect of activity level.

Definitions of Terms

1. Respiratory Quotient (RQ): The ratio of CO_2 produced to O_2 consumed. This value is an indication of fuel utilization and reflects cellular processes (Brooks Text).
2. Respiratory Exchange Ratio (RER): an estimate RQ during hard exercise. Both RER and RQ are given by the same formula (VCO_2/VO_2), RER is a measurement taking gas exchange ratios at the lungs. Thus changes in CO_2 storage may cause RER not equal to RQ. RQ cannot exceed 1.0, but RER can reach 1.5 or higher. RQ is the ratio of gases in the cell, while RER is the ratio of gases measured at the mouth. Over time these 2 values would be equal (Brooks Text).

3. Indirect Calorimetry: A means of estimating heat produced by determining O₂ consumption and CO₂ production. Open circuit indirect calorimetry is a system that is open to the atmosphere (Brooks Text).
4. VO₂ max: Maximal oxygen consumption. This value is an important determinant of peak power and physical work capacity of an individual. This is an important index of physical fitness (Brooks Text).
5. VO₂ Peak: The maximum VO₂ achieved during an exercise test.
6. Lipolysis: The process of triglyceride hydrolysis (Brooks Text).
7. Glycolysis: the metabolic pathway of glucose breakdown in mammalian cells (Brooks Text).
8. Gluconeogenesis: The making of new glucose (Brooks Text).
9. Fit: According to ACSM guidelines, a subject will be classified as fit if their VO₂ max is in the top 50th percentile based on the subjects' sex and age.
10. Unfit: According to ACSM guidelines, a subject will be classified as unfit if their VO₂ max is in the bottom 50th percentile based on the subjects' sex and age.

Assumptions and Limitations

1. The main assumption of this study is that an indirect measurement of RER is the same as a direct measurement of RQ. Respiratory exchange ratio is measuring respiratory gases at the level of the lungs to determine fat and carbohydrate utilization during exercise. Respiratory quotient is the same as RER, so long as there is not excess CO₂ being exhaled, as would be the case when a person exercises above an RER or RQ of .85. If below an RQ of .85, RER and RQ can only be considered the same value if steady state is obtained.

2. Subjects will not be on any kind of a standard diet prior to testing.
3. Subjects will be testing at different times during the day.
4. Subjects will not have a standardized endurance training background, as this is a cross sectional design.

CHAPTER 2

REVIEW OF RELATED LITERATURE

The Crossover Concept: Basic Carbohydrate and Lipid Utilization Science

The crossover concept is “a model of substrate supply during exercise” (Brooks, 1997). This concept assumes/predicts that lipid is the main fuel for the body at rest. As energy fluctuates (based primarily on exercise intensity), the balance of carbohydrate and lipid also fluctuates. As exercise intensity increases, there is an increased reliance on carbohydrate for fuel with a decreased reliance on lipid oxidation. However, factors such as level of training, dietary intake, and duration of exercise have a secondary effect on the balance of substrate utilization (Brooks, 1997)

Carbohydrate utilization is increased as exercise intensity increases due to a sympathetic nervous system response. An increased dependence on carbohydrate as a fuel source may be due to the relatively greater abundance of glycolytic enzymes versus lipolytic enzyme systems in skeletal muscle (Brooks and Mercier, 1994). Another characteristic of fuel usage and intensity level is that most lactate appearing in the blood is disposed of through oxidation, thus VO_2 is an indirect measure of the energy being used for muscle work (Brooks and Mercier, 1994). However, in ranges of maximal intensity (intensities above lactate threshold), it becomes difficult to measure anaerobic energy production because it is not measurable with current technology. It is assumed that increases in lactate accumulation can be taken to represent increases in carbohydrate utilization (Brooks and Mercier, 1994).

While carbohydrate utilization increases with increases in exercise intensity, lipid oxidation is improved or increased with endurance training. This is a hallmark training

adaptation of endurance exercise; the concept that lipid utilization increases at low to moderate exercise intensities: this would mean that glycogen in the muscle would be spared. However, recent research suggests that fat oxidation may not be the reason for glycogen sparing; rather it may be due to an increased uptake of blood glucose that accounts for the sparing of muscle glycogen (Sumida, 1993). If this is true, there would be no reason to believe that crossover occurs at a different relative percentage of VO_2 max with or without training. Looking further at the problems with assuming lipid utilization increases with endurance training, Brooks and Mercier present several key points. The first of these points is that the relative contribution of free fatty acids (FFA) actually decreased in several studies performed with fit male subjects. Thus, even though there was a small absolute increase in lipid oxidation there was a relative decline at high intensities levels. This again would suggest checks and balances on the two energy sources, producing a net change in crossover that would be minimal at best.

Further support that glycogen is spared not by an increased lipid oxidation, but by increased glucose utilization stems from a study conducted on rats. In this study the capacity for gluconeogenesis is improved after training (Sumida, 1993). In fact, fat oxidation and carbohydrate utilization are hardly separate processes. Carbohydrate metabolism largely regulates fat metabolism during exercise (Coyle, 1997). Similarly, in human studies, muscle glucose uptake actually decreases for a given intensity after training and that hard exercise promotes a glucose “shunt” from inactive tissue to active tissue (Bergman et al., 1999). When participants trained at exercise intensities greater than 60% VO_2 max, it was found that the rate at which glucose appeared and became more concentrated was higher after training. Interestingly, the results were actually

opposite when subjects exercised at 50% of the pre-training VO_2max . This seemingly conflicting data can actually be reconciled when we use the crossover concept. If the relative intensity of the exercise task is reduced, then there would be lower levels of sympathetic nervous system activation. This would dictate lower levels of circulating epinephrine- ultimately leading to lower levels of lactate, a precursor to gluconeogenesis. Thus, high intensity training seems to cause little change in lipid metabolism in comparison to rest (Brooks and Mercier, 1994). However, because crossover is assumed to occur at relatively low-intensity exercise, the effects of increased lipid oxidation at these lower intensities may provide measurable differences in percentage of $\text{VO}_2\text{ max}$ at which crossover occurs.

Fat oxidation can improve with training at any given intensity. Training induces a shift towards higher fat oxidation during exercise done at the same absolute workload before and after training (Norby et al., 2006). Thus if an individual were to run at 8 mph for 30 min today versus running at 8 mph for 30 minutes after endurance training, the individual should theoretically burn more fat after training. The increase in fat oxidation at the same absolute intensity is likely due to an increase in FFA availability in the mitochondria (van Loon et al., 2001). Availability of FFA does not necessarily indicate lipid oxidation however. In fact, under most conditions fatty acids are made available at a rate of two- to threefold more than the actual rate of oxidation (Romijn, 1993). Even though FFA availability exceeds oxidation numerically, availability may still be a limiting factor due to transport limitations (Romijn, 1993). When comparing High-intensity exercise (85% $\text{VO}_2\text{ max}$) with lower intensities (25 and 65% $\text{VO}_2\text{ max}$), plasma FFA concentration is much lower due to a reduction in the rate of appearance of FFA into

the plasma (Romijn, 1993). Although the exact mechanism is not known, increases in FFA availability may be in part due to an increase availability of carnitine, a co-factor that is required for transport of FFA across the mitochondria membrane (van Loon et al., 2001). In addition to carnitine, fat oxidation may increase due to the gene expression of lipoprotein lipase gene expression (Schrauwen et al., 2002) or simply due to an increase in the mitochondrial mass (Brooks, 1997). Free fatty acid may decrease due to a decrease in FFA release from adipose tissue because of a reduction in adipose tissue blood flow (Romijn, 1993).

While research done with regards to changes in carbohydrate after training may suggest there is little reason to believe that percentage of VO_2 max at crossover changes, in a study conducted by Norby et al., researchers found that whole-body peak fat oxidation was higher and occurred at higher absolute and relative exercise intensity in trained versus untrained subjects (Norby et al., 2006). If the peak fat oxidation is shifting out to higher relative exercise intensities after endurance training, it may be reasonable to assume that the crossover point is indeed shifting outwards as well. In contrast, Brooks states that plasma FFA flux rises as exercise moves from mild to moderate intensity with crossover generally occurs 55% VO_2 max (Brooks, 1997). However, he does not discern percentage of VO_2 max at crossover when comparing trained vs. untrained subjects. This may be because a discernable difference simply does not exist.

Gender Differences and Fuel Utilization

In recent research there has been some question as to whether or not men and women utilize substrates the same during exercise. Friedlander and colleagues studied men and women in separate studies to determine the effects of endurance training on fat

oxidation (Friedlander et al, 1998). The first study used females only. Using respiratory exchange ratio (RER), percent of oxidative energy from free fatty acids (FFA) and lipid was calculated. A training protocol for this study included 5 days a week of exercise for 1 hour per session. As expected, VO_2 max improved over the 8-12 weeks of training. This study found a significant reduction in heart rate during exercise at the same absolute workload, but not the same relative workload. Thus participant heart rate remained stable at any given percentage of VO_2 max, while heart rate dropped at any given absolute level of exercise. This is also to be expected and is a classic example of physiological adaptations to exercise. After the 8-12 weeks of training, it was determined that FFA oxidation was significantly higher at the absolute training intensity as well as at the relative training intensity. Thus, VO_2 increased at both absolute and relative intensities, but so did FFA. So it is unclear whether FFA increased to a level that would dictate a shift in percentage of VO_2 max at which cross over occurs. The authors conclude that women increase their reliance on lipid after endurance training (Friedlander, 1998). This data confirmed the results of a similar study by Friedlander showing that RER was lower during steady-state exercise at both the same absolute and relative intensities even though there was no difference in actual workload being performed (Friedlander et al., 1998). However, one wonders, because VO_2 max increased, total energy expenditure would have increased. Thus it is still unclear whether percentage of lipid oxidation increased, as opposed to just the total amount of lipid oxidation, and whether that lipid increase was sustained up to an intensity that would affect cross over. Friedlander (1998) found that the increased reliance on lipids after endurance training was derived primarily from plasma sources.

An important assumption must be made while trying to determine lipid utilization. This assumption is clearly shown when looking at studies that used rats and humans to determine that, in fact, training induces an increase in FFA binding proteins (Turcotte and Brooks, 1990). While the increase in enzyme capacity seems like a sure precursor to increases in fat oxidation, in reality, due to the complex regulation of substrates an increase in enzyme capacity does not necessarily dictate an increase in lipid oxidation in vivo (Friedlander et al., 1999). This was the case with 22 sedentary young men. After 10 weeks of training, FFA flux increased, but total lipid oxidation was unaffected (Friedlander et al., 1999). This can possibly be explained by a previous study where it was shown that glucose use in men (regardless of lipid oxidation) is directly related to exercise intensity (Friedlander et al., 1997). Further support for Friedlander's work came from Bergman et al. (2000). In a study of nine healthy sedentary males, researchers found that gluconeogenesis increased twofold at rest and threefold during exercise (at given absolute and relative intensities) after 9 weeks of endurance training (Bergman et al., 2000). Bergman's work also confirmed earlier studies with similar findings in rats (Sumida et al., 1993).

Carter et al (2001) noted that while endurance exercise training results in a decrease in carbohydrate utilization and an increase in lipid oxidation in both men and women, there appeared to be slight differences between genders. Carter found that fat oxidation at the same relative workload pre- and post-training resulted in no change in fat oxidation. Fat oxidation did, of course, increase at the same absolute workload for both men and women, but women were shown to oxidize a greater percentage of fat during exercise than males. This was the case for all trials, both pre- and post-training at the

same relative and absolute intensities. This difference in substrate utilization was shown by a lower exercise RER for females at any given intensity (Carter, 2001).

While Friedlander, Carter, and Bergman found differences in fat oxidation between men and women, Roepstorff did not. Roepstorff et al. (2002) conducted a study to determine gender differences in substrate utilization as well. Using endurance-trained subjects, Roepstorff (2002) looked at the utilization of blood glucose, glycogen, plasma FA, and FA from myocellular triacylglycerols using several techniques including muscle biopsy, stable isotope tracer technique, and net balance across the active muscle. The study revealed an equal relative contribution from carbohydrates and lipids as fuel during sub-maximal bicycle exercise when comparing the same relative workload. It was noted that there were no gender differences in RQ or RER during exercise at any point and that the effect of gender occurs in the utilization of different lipid sources (Roepstorff et al., 2002). Keeping this in mind, for the purposes of the present investigation, one of the goals will be to determine if there is a difference of percentage of VO_2 max at the cross-over point when comparing males and females.

Effects of Exercise Training: Differences in Substrate Utilization in Trained and Untrained Subjects

Exercise intensity and duration of exercise are the main regulators of substrate utilization during exercise. But it appears there is a large range of respiratory exchange ratios from individual to individual when comparing RER at 55% max oxygen uptake (Venables et al., 2005). Venables (2005) hypothesized that exercise intensity is of “primary importance to the regulation of fat oxidation and that gender, body composition, physical activity level, and training status are secondary.” In a study conducted by

Venables et al. (2005), researchers were only able to account for 34% of the variance of fat oxidation (at its peak). Fat free mass, VO_2 max, gender, fat mass, and self-reported physical activity levels were the variables that accounted for 34% of the variance in fat oxidation. Venables et al. suggested the 55% max oxygen uptake as a comparison point because this more or less coincides with the crossover point suggested by Brooks. Both VO_2 max and physical activity level are significant predictors of fat oxidation in addition to exercise intensity (Venables, 2005). Thus, it appears that much of the differences in RER at 55% max VO_2 can be contributed to activity level (assuming an active individual has a higher VO_2 max than their sedentary counterpart). A lower RER during exercise indicates a relative increase in lipid oxidation (Bergman and Brooks, 1999).

A hallmark adaptation of training is a decreased reliance on carbohydrate and an increase in fatty acid oxidation during exercise of the same absolute workload. Bergman et al. (1999) conducted a study to determine the effects of exercise and training on muscle lipid metabolism. This study was designed specifically to evaluate whether or not endurance training increases intramuscular free fatty acids (FFA) oxidation at given absolute and relative exercise intensities as well as the effects of exercise training on intramuscular triglyceride oxidation. Inactive male subjects were used for this study. An inactive participant was defined as having a VO_2 max of less than $42 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. In addition, subjects were considered sedentary if they participated in less than 2 hours of regular strenuous exercise for at least the last year (Bergman et al., 1999; Friedlander, 1999). Subjects trained for 5 days per week at 75% VO_2 peak for 9 weeks. Results showed that training significantly decreased RER values at given absolute intensities, but not at relative intensities. Findings also suggested that muscle lipid metabolism, in the

form of FFA, increased at 65% VO_2 Peak, but at high RER and RQ values lipid is of secondary importance as a fuel source. There was no change in intramuscular triglyceride oxidation (Bergman, 1999). Thus the question still remains whether or not the cross-over point occurred at higher VO_2 peak after training than before training. Because FFA uptake increased at 65% VO_2 peak one may easily assume cross-over point has moved upwards. However, that would be assuming that cross-over occurred around 65% VO_2 peak. This information was not determined and so the effect of exercise on cross-over point remains unclear.

In a similar study, Sidossis et al. (1998) studied the effects of exercise on fatty acid oxidation in untrained vs. trained men. As expected, carbohydrate utilization was significantly higher in the sedentary group. FFA oxidation was significantly higher in the trained group. This shift towards an increase in FFA oxidation in endurance trained males was attributed to the acceleration of FFA entry into the mitochondria (Sidossis, 1998). This shift may be occurring due to a low lactate threshold in inactive subjects. Lactate can directly inhibit adipose tissue FFA release (Venables, 2005). In similar studies with both males and females, it was shown that FFA uptake increases with training due to an increase in fatty acid uptake across plasma membrane as well as across the mitochondria membrane. This confirms, once again, that fat oxidation increases with exercises training due to both an increase lactate threshold as well as accelerated entry of FFA into the mitochondria (Venables, 2005; Sidossis, 1998; Helge, 2007, Tunstall, 2002). It still does not, however, give information regarding crossover point. If fat oxidation is increasing (regardless of the mechanism) and VO_2 is increasing during

endurance training, it may be reasonable to assume that crossover point will remain relatively stable and unchanged.

In contrast, Bergman and Brooks (1999) compared trained and untrained men in fed and fasted states to evaluate the hypothesis that endurance training increases lipid oxidation over a wide range of intensities. At the higher intensities of 59 and 75 % VO_2 peak, there were no differences in RER between the trained and untrained subjects in either nutritional state (Bergman and Brooks, 1999). This suggests that improvements in lipid oxidation occur primarily at lower intensities. Once certain intensities are reached, training appears to have no impact on lipid oxidation (Bergman and Brooks, 1999; Romijn, 1993). Interestingly, if lipid oxidation does not improve with improved fitness level, the crossover point could theoretical decrease (assuming VO_2 max improves with endurance training). Bergman and Brooks do, of course, state that a major limitation of the study is that is cross-sectional as opposed to longitudinal. Thus, in a longitudinal study conducted by Bergman, it was confirmed that there was no differences in RER values when subjects were tested at the same relative exercise intensities after 9 weeks of endurance training (Bergman et al., 1999).

Substrate Utilization Differences at High- and Low-Intensity Exercise

High and low intensity exercise utilizes different substrates. As already mentioned, there is a shift from fat oxidation to carbohydrate utilization as exercise intensity increases. According to Achten et al., fat oxidation peaks at 64% VO_2 max, although there appears to be substantial variation between subjects (Achten et al., 2002). Indeed, others have suggested a peak in fat oxidation occurring at ranges of 40-65% VO_2 max (Sahlin, 2006). Due to this large variation of VO_2 at peak fat oxidation, it may

be safe to assume that there will be large variations in percentage of VO_2 at crossover as well.

In a study conducted in 1984 by Gaesser and Rich, 16 inactive male subjects completed an 18 week training program in either a high (80-85% VO_2max) or low (45% VO_2max) exercise group. All subjects exercised 3 days per week. The high intensity group exercised for 25 min/session and the low intensity group exercised for 50 min/session, thus both groups had approximately the same energy expenditure. The study was unable to determine a training intensity that induced changes in blood lipid levels (Gaesser and Rich, 1984). While blood lipid values did not readily change, the study did not evaluate whole body lipid oxidation in the form of RER. Thus participants may very well have had an increase in fat oxidation as it relates to exercise even though blood profiling remained the same.

Fatty acid oxidation contributes significantly at low to moderate intensity exercise (Sahlin, 2006). Sahlin notes, there is clear evidence that fatty acid oxidation is enhanced by training and that fatty acid oxidation is impacted by diet, training status, muscle fiber type, and muscle glycogen content. However, there is “no clear effect of exercise intensity on relative fatty acid oxidation at low intensities.” Studying 8 trained and 5 untrained male subjects, Sahlin et al. determined that whole body relative fat oxidation was positively correlated to relative mitochondrial fatty acid oxidation. Thus, we can assume that whole body fat utilization and mitochondrial fat utilization are both dependent on exercise intensity (Sahlin et al., 2006). This is important because previous studies had questioned whether or not glycogen sparing was due to an improvement in fat oxidation (Sumida, 1993; Brooks and Mercier, 1994). It appears that fat oxidation does

indeed improve, since mitochondrial volume increases, or improves, with training (Sahlin et al., 2006). But while fat oxidation may improve with training, it may not have an effect on the crossover point. Sahlin points out that during exercise at moderate- to high-intensity, aerobic training status influences fatty acid oxidation. At lower intensities, “intrinsic mitochondrial characteristics” may play a greater role than training status when evaluating whole body fat oxidation (Sahlin et al., 2006).

Sahlin was focused primarily on low-intensity exercise. When comparing moderate- to high-intensity exercise, the case for an increase in lipid utilization is further supported. Training at a moderate intensity (60-75% VO_2 peak) for 6-12 weeks leads to an improved aerobic capacity as well as an increase in fat oxidation and/or carbohydrate sparing. Sprint training produces similar results in 6-7 weeks. A combination of moderate and high intensity exercise in the form of intervals also produces an increase in fat oxidation in as little as 2 weeks (Talanian, 2007). Thus exercise in general, regardless of intensity, appears to produce improvements in fat oxidation.

Substrate Utilization and Obesity

In a study documenting the effects of exercise training and weight loss in exercise efficiency and substrate oxidation, researchers found that among subjects 64 years of age and older there was no change in efficiency or fat utilization during exercise when subjects lost weight. These overweight to obese subjects had no change in the amount of oxygen being consumed at 50% peak VO_2 after losing weight. Thus, it is not weight alone that dictates fat oxidation and efficiency; it is exercise training that produces beneficial shifts in substrate utilization. Amati (2008) referred to efficiency as a measure of oxygen consumption per unit of work. The idea being that a more efficient individual

would use less oxygen per unit of work than an inefficient individual (Amati, 2008).

This study is important for several reasons. First, the study shows that weight loss may not affect substrate utilization. So we can assume that an overweight- and normal-weight individual have similar substrate utilizations before exercise training takes place.

Second, this study shows that exercise training, either alone or in conjunction with a weight loss diet, increase both exercise efficiency as well as fat oxidation during exercise.

Third, researchers showed that older, previously sedentary individuals can make substantial gains in efficiency, fat oxidation, and peak VO_2 with only moderate-intensity bouts of exercise (Amati, 2008). Amati's research supports earlier an earlier study showing no difference in RER at any given exercise intensity when comparing obese and normal-weight women (Steffan et al., 1999)

In a seemingly opposite studies, researchers suggest that muscle fatty acid metabolism is impaired by obesity and insulin resistance. Researchers found that with endurance exercise the nine previous sedentary, obese subjects increased the rates of mitochondrial fatty acid oxidation (Bruce et al., 2006). Likewise, Venables (2008) suggest that there are elevated intramuscular triglycerides concentrations in obese individuals. This may be due, possibly, to an inability to oxidize lipids within the muscle. Elevated fatty acid within the skeletal muscle is a direct link to insulin resistance (Venables, 2008, Colberg 1995). Thus lowering fatty acid in the muscle via exercise training would be an effective means to improve insulin resistance. While these studies suggests that obesity impairs fatty acid oxidation and Amati's study suggested that weight had no impact on fatty acid oxidation, we can definitively conclude that regardless of somewhat opposing findings, fatty oxidation improves through exercise. In fact, as

stated by Venables, “physical activity is a simple, effective means by which insulin sensitivity can be improved in lean, obese, and diabetic groups.”

In a study comparing exercise intensity in obese men, subjects were divided into either low-intensity group (40% VO_2 max), a high-intensity group (70% VO_2 max), or a non-exercise control group. Total fat oxidation over the last 20 min of exercise for the low-intensity group significantly increased compared with pre-training. There was no difference in the high-intensity or control groups (Van Aggel-Leijssen et al., 2002).

These findings are consistent with studies previously mentioned; Once again fat oxidation appears to improve at lower intensities, but not high-intensity exercise. The exact threshold (in terms of a percentage of VO_2 max) that fat oxidation no longer improves has yet to be determined and it is likely that the threshold will depend on numerous factors, obesity being only one such factor.

Measuring Whole-Body Fat Oxidation: Equipment and Measuring Techniques

For purposes of measuring fat oxidation in this study, a metabolic cart will be used. Thus, substrate utilization will be an indirect measure using the method of indirect calorimetry.

A vital assumption when using indirect calorimetry to determine substrate utilization is that respiratory exchange ratio (R or RER) equals tissue respiratory quotient (RQ). Simply put, gas exchange at the lungs is the same as gas exchange within the tissue. The assumption that RER is equal to RQ is further enhanced by the use of steady-state conditions with stable RER's and lactate concentrations. Assuming RER and RQ are the same value only holds true at levels below a lactate threshold and providing an individual is not hyperventilating. At intensities above LT, RER is no longer an accurate

measure of gas exchange at a muscular level. This could theoretically present a problem, however for the purposes of this study RER is only used up to the crossover point, which tends to occur well before the LT (Brooks and Mercier, 1994). Indirect calorimetry has been validated as an accurate way of measuring substrate utilization up to 85% of VO_2 max (Romijn, 1993). Again, because crossover will occur well before 85% of VO_2 max, indirect calorimetry is an accurate, noninvasive method for determining percentage of VO_2 max at crossover.

In Achten's (2002) study to determine the exercise intensity that elicits maximal fat oxidation, it was noted that a general problem with graded exercise testing as it relates to substrate utilization is that previous stages can effect substrate utilization in later stages. This occurs because the longer one exercises, the greater the reliance on fat as a fuel source. Thus, later stages may artificially show a higher rate of fat oxidation when compared to the same intensity done at an earlier stage, or when the body is fresh. As Achten concludes, "it may be advantageous if the duration of each stage could be reduced." By reducing the stage duration from 5 minutes to 3 minutes, there appears to be no impact on fat oxidation or VO_2 max (Achten, 2002). Three minutes is long enough to produce a steady state effect, thus insuring RQ is accurate (Astrand et al., 1986).

It is important to note that whole-body fat oxidation measurements (as determined via indirect calorimetry) are not the same as working muscle fat oxidation rates. In a study conducted by Friedlander et al., it was determined that leg muscle lipid metabolism was lower than whole-body lipid metabolism. The patterns of lipid use by the leg do in fact mimic whole-body lipid oxidation, but the leg tends to use more carbohydrate regardless of intensity (Friedlander et al., 2007). While this does not present a problem

for this study, when trying to determine where the lipid oxidation is originating from, this would certainly need to be factored in. Friedlander points out another key assumption when using indirect calorimetry and that is that contributions of protein oxidation are considered negligible relative to total energy expenditure. Thus, a non-protein RQ can be assumed to be equal to RER (Friedlander et al., 2007).

Summary

Lipid oxidation improves with training at low- and moderate- intensities of exercise (Brooks, 1998; Sahlin, 2006). This improvement likely occurs due to increases in lipolysis via an increase in mitochondria as well as an increase in FFA availability (Coyle, 1997). Glucose utilization appears to affect lipid utilization by inhibiting fatty acid oxidation (Coyle, 1997). Thus, the reasons for decreases in lipid oxidation as exercise intensity increase are not related to a single substrate; rather, there is an interaction between substrates that is responsible for overall fuel utilization. This relationship between substrates is still somewhat unclear and remains under investigation. Just as lipid utilization improves with training, so does VO_2 max. This is considered a hallmark training adaptation and is under no dispute. The question remains whether the percentage of VO_2 max at crossover is affected by the increases in lipid oxidation and VO_2 max improvements. If the rate of improvement is similar and linear, there is no reason to believe that crossover would change. This remains unclear, however, and is open to interpretation.

Men and women utilize substrates differently. Women tend to have lower RER's at any given intensity. While both men and women increase lipid oxidation with training, the source of lipid appears to be different. Women primarily derive lipid from plasma

sources, while men's utilization of lipid seems to be related more to carbohydrate utilization (Friedlander et al., 1998; Friedlander et al., 1997). These differences in lipid oxidation may not impact the percentage of VO_2 max at crossover however, since crossover is not affected by the type of lipid being oxidized, only lipid oxidation as a whole.

Metabolic dysfunctions are commonly found in individuals with obesity and type 2 diabetes. Sedentary obese subjects oxidize lipid at a lower rate than their non-obese counterparts (Brun et al., 2007). While weight alone does not dictate fat oxidation, it does appear to play an important role in the initial dysfunction of lipid oxidation (Amati et al., 2008). Because obese subjects oxidize less fat at any given intensity, if improvements in crossover can occur, the obese population may be a group that benefits greatly from endurance training, specifically endurance training that improves percentage of VO_2 max at crossover. It is not known if crossover will improve in a healthy adult population. Thus, this will be necessary to determine first so as to have a baseline for comparison of normal metabolic response vs. metabolic dysfunction.

By determining if endurance training has an effect on the percentage of VO_2 max at crossover, we will be contributing to the base of knowledge regarding substrate utilization and exercise. If the percentage of VO_2 max at crossover does in fact go up, the next step would be to determine the most effective training program to elicit said response. Doing so would allow athletes to spare glycogen and those with metabolic dysfunction to gain control over their metabolism. Even if there is no change in VO_2 max at crossover, we will have still gained valuable knowledge about energy utilization in the human body.

CHAPTER 3

METHODS

Subject Characteristics

Twenty-five healthy, nonsmoking, injury-free adults participated in this study. Participants were assessed as healthy based on a medical questionnaire. All subjects were informed of the purpose and nature of the study, after which a written informed consent was given to them to sign. Protocol for this study was approved by the University of Nevada, Las Vegas Committee for Protection of Human Subjects. Subjects were between the ages of 19-40 years. Physical characteristics of the participants are shown in Table 1. A comparison of fitness levels was done by dividing the sample in half based on VO₂ max percentiles for sex and age (ACSM's Guidelines for Exercise Testing and Prescription, 2000). Thus, there was a top half and a bottom half for males as well as a top and bottom half for females. Subjects were considered unfit if they had a VO₂ max percentile in the bottom half of the sample for their sex, as determined by a graded exercise test. Fit participants had a VO₂ max in the top half of the sample for their gender. To qualify for this study, participants had to be non-obese as defined by BMI. Female subjects were not menstruating or pregnant at the time of the graded exercise test. Gender specific characteristics are shown in Table 2. Characteristics based on fitness level are shown in Table 3.

Volunteers were disease and injury free as determined by a medical questionnaire. Specifically, subjects did not have any known metabolic condition including, but not limited to diabetes, obesity, metabolic syndrome, and/or thyroid dysfunction.

Participants were asked to avoid strenuous activity and alcohol 24 hours prior to testing.

A graded exercise test was performed after fasting for at least 4 hours. Volunteers were recruited from Red Mountain Resort and Spa via team member meetings and via word of mouth. All recruitment was done verbally. Subjects read and signed an informed consent prior to beginning any part of this study.

Table 1
Physical Characteristics

	Minimum	Maximum	Mean	Std. Deviation
Age	19	40	30.76	6.19
Height	62.0	74.0	68.18	3.65
Weight	107	218	161.28	30.04
BMI	17.54	29.40	24.03	3.10
BodyFat	9.03%	32.20%	19.59%	6.79
VO2	32.70	63.60	47.08	7.52
Crossover	32%	78%	55%	12

Table 2
Gender Specific Physical Characteristics

Gender		Minimum	Maximum	Mean	Std. Deviation
Male	Age	27	40	33.36	3.80
	Height	67.0	74.0	71.32	2.35
	Weight	160	218	187.45	19.32
	BMI	21.11	29.01	25.38	2.33
	BodyFat	9.03%	20.91%	14.89%	4.26
	VO2	40.50	63.60	50.92	6.89
	Crossover	33%	71%	53%	13
Female	Age	19	40	28.71	7.03
	Height	62.0	71.0	65.71	2.33
	Weight	107	166	140.71	18.54
	BMI	17.54	29.40	22.96	3.28
	BodyFat	14.08%	32.20%	23.28%	6.14
	VO2	32.70	54.50	44.06	6.76
	Crossover	32%	78%	57%	12

Table 3
Physical Characteristics Based on Fitness Level

Fitness		Minimum	Maximum	Mean	Std. Deviation
Top 50%	Age	23	40	32.62	5.55
	Height	65.0	73.0	68.96	3.29
	Weight	112	218	162.38	29.22
	BMI	17.54	28.76	23.87	2.96
	BodyFat	9.03%	28.70%	18.32%	5.82%
	VO2 max	44.60	63.60	52.37	5.22
	Crossover	32%	78%	59%	14%
Bottom 50%	Age	19	39	28.75	6.45
	Height	62.0	74.0	67.33	3.96
	Weight	107	208	160.08	32.17
	BMI	18.79	29.40	24.19	3.37
	BodyFat	10.81%	32.20%	20.96%	7.73
	VO2 max	32.70	49.10	41.35	4.99
	Crossover	36%	68%	51%	8

General Experimental Design

A cross sectional design was used to evaluate differences in percentage of VO₂ max at crossover between sex and fitness level. Subjects performed a graded exercise test to exhaustion on a treadmill (TRUE 750 S.O.F.T System). Fat and carbohydrate utilization was determined using indirect calorimetry and was plotted as a function of exercise intensity as shown in Figures 2 and 3. Body mass, height, and body fat were determined prior to the start of the treadmill test. Body fat was determined using the four-site skinfold technique according to the methods of Jackson Pollock. Lange calipers were used to measure skinfold thickness at each of the four sites. Subjects self-selected a comfortable walking or running pace prior to the start of the test. The volunteers began the graded exercise test on the treadmill by exercising at 3.0 mph with no gradient. The speed was increase by 1-1.5 mph every 3 minutes until the self-selected speed was

reached. Gradient was then increase by 2% every 3 minutes until an RER of .85 was reached. At this point the gradient was increased every minute until exhaustion. Stages after crossover (RER=.85) no longer needed to be 3 minutes in duration. RER is assumed to equal RQ during steady state exercise, but only at low- to moderate-intensity exercise (Brooks and Mercier, 1994). Thus, steady state was no longer necessary as RER was no longer a measure of RQ beyond moderate intensity exercise (defined here as RER=.85). In addition, beyond crossover, substrate utilization is no longer of relevance to this study. Therefore, there was no reason to prolong the test duration by using 3-minute stages, when 1-minute stages enabled a participant to reach maximum effort faster. Peak VO_2 's obtained were considered a VO_2 max if a RER of 1.1 was reached, an age-predicted max heart rate was reached (within 5 beats), and a leveling off of O_2 occurred. Breath-by-breath analysis was taken during the entire duration of exercise using an automated gas analysis system (Newleaf Active Metabolic Training System, exerSmart 6.1). The Newleaf metabolic cart uses a neoprene face mask, which has been shown to be effective in previous studies (Byard and Dengel, 2002). Heart rate was also measured continuously using a heart rate monitor strap (Polar T31, N2965).

Indirect calorimetry and calculations

According to previous protocol, VO_2 and VCO_2 during the last 2 minutes of each stage (prior to RER= .85) were averaged to ensure a steady state value (Venables, 2005). Fat and carbohydrate utilization as well as total energy expenditure were calculated using standard stoichiometric equations (Frayn, 1983).

Statistical Analysis

An examination of a histogram was conducted to insure variables were normally distributed. Levene's test was used to assess whether the assumption of homogeneity across groups was violated. A factorial analysis of variance (2x2) was used to determine whether there was a main effect for gender and fitness level, as well as whether there was a gender/fitness level interaction. Independent t-tests were used to compare males vs. females and fit vs. unfit. Significance was set at $p \leq .05$.

CHAPTER 4

RESULTS

Analysis of data

A 2 x 2 factorial ANOVA was performed using PASW Statistics 18 (formerly SPSS) to assess whether there was a cause and effect relationship between percentage of VO₂ max at crossover, fitness level (top half and bottom half of sample), gender, and the interaction between fitness level and gender. Preliminary data screening was done to assess whether the assumptions for ANOVA were violated. Levene's test indicated no significant violations of homogeneity ($p=.104$). The Shapiro-Wilk test indicated that there were no significant violations of normality in the data (see Table 4).

Table 4

Tests of Normality

	Gender (IV)	Shapiro-Wilk		
		Statistic	df	Sig.
Percentage VO ₂ at crossover (DV)	Male	.945	11	.585
	Female	.960	14	.730

Descriptive Statistics of Subject Characteristics

The age, body weight, height, body mass index, and percentage of body fat were similar in the fit and the unfit group. Maximal oxygen uptake was higher in the fit than

in the unfit group at $52.4 \text{ ml.kg}^{-1} \text{ min}^{-1}$ and $41.4 \text{ ml.kg}^{-1} \text{ min}^{-1}$ respectively ($p < .05$). Table 4 shows t-tests for all variables when comparing fitness level.

Males had significantly higher maximal oxygen uptake when compared to females ($p < .05$) and were significantly leaner than their female counterparts with an average body fat of 14.9% body fat versus 23.3% body fat respectively ($p < .05$). Additionally, there was a significant difference between males and females when comparing height and weight (Table 5).

Table 5
Independent Samples Test for Fitness

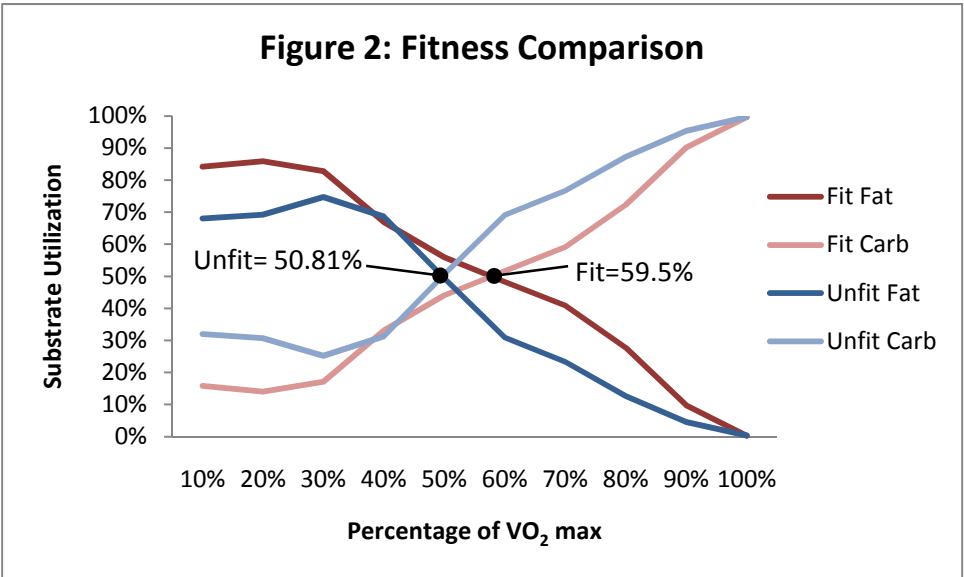
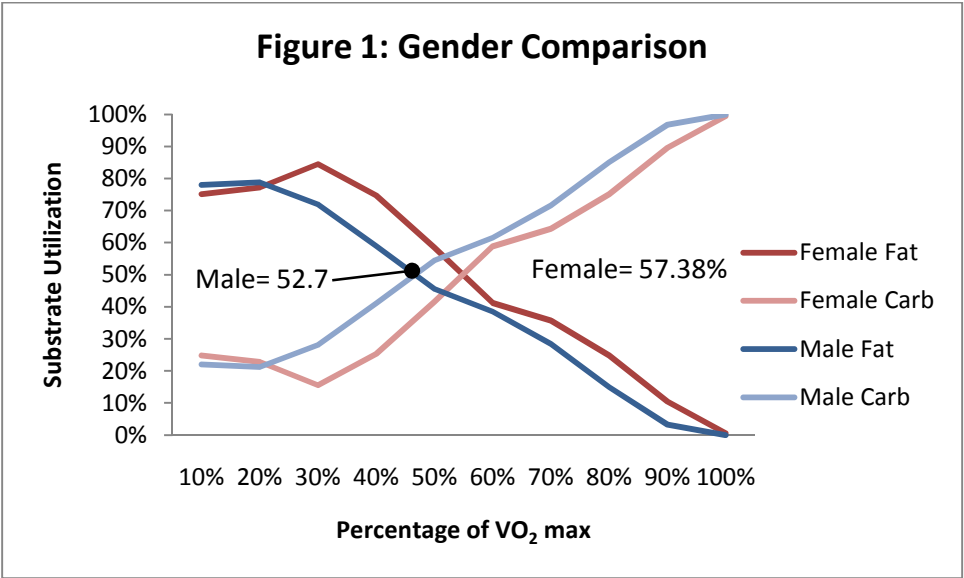
	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Age	1.610	23	.121	3.865	2.401
Height	1.122	23	.274	1.6282	1.4516
Weight	.187	23	.853	2.301	12.276
BMI	-.249	23	.805	-.31514	1.26512
BodyFat	-.971	23	.341	-2.64327	2.72128
VO2	5.386	23	.000	11.01923	2.04604
Crossover	1.852	23	.077	.08683	.04688

Table 6**Independent Samples Test for Gender**

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Age	1.972	23	.061	4.65	2.36
Height	5.954	23	.000	5.6	.94
Weight	6.142	23	.000	46.74	7.61
BMI	2.073	23	.050	2.43	1.17
BodyFat	-3.853	23	.001	-8.39	2.18
VO2	2.495	23	.020	6.85	2.75
Crossover	-.944	23	-.355	-.05	.05

Gender and Fitness Interaction

Data from indirect calorimetry show there was no significant interaction between gender and fitness ($F=1.064$, $p=.314$). An independent t-test was done to compare males vs. females and fit vs. unfit. There was no significant difference ($F= 1.288$, $p= .269$) in the percentage of VO_2 max at crossover between the genders. The average percentages of VO_2 max at crossover for males and females equal to 52.7% and 57.38% respectively (see Figure 2). There was also no significant difference ($F= 4.089$, $p= .056$) in the percentage of VO_2 max at crossover between fitness levels. An average percentage of VO_2 max at crossover for fit subjects was equal to 59.49% and an average percentage of VO_2 max at crossover for unfit subjects was equal to 50.81 % (see Figure 3).



CHAPTER 5

DISCUSSION

The purpose of this study was to determine if there is a difference in the percentage of VO_2 max at which substrate usage crosses over from predominately fat to carbohydrate between men and women or between fit and unfit individuals. For the population studied, no significant differences were found either between genders, between fitness levels, or in interactions between the variables. However, it may be premature to suggest that no differences existed. While VO_2 max values were significantly different between the fit and unfit groups, the range of VO_2 max values may not have been large enough. The average VO_2 max for the fit group was better than average according to ACSM guidelines (American College of Sports Medicine, 2000). However, the average VO_2 max for the unfit group was also higher than the age group average. Even among subjects that classified themselves as “inactive,” VO_2 max values ranged from 40-50 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. In order to truly evaluate whether fitness affects the percentage of VO_2 max at crossover, it may be necessary to compare an elite athlete group to an unfit, non-elite group of subjects.

Fat and Carbohydrate Metabolism during Exercise

Another possibility to explain the failure to detect a difference between the variables, and likely the more reasonable explanation, is simply that none exists. It is well documented that fat oxidation increases in trained individuals as a consequence of training. Van Loon, et al. (2001) suggest that an increase in free fatty acid (FFA) levels could stimulate fat oxidation. Van Loon further hypothesized that levels of carnitine (a factor that is required for shuttling FFA into the mitochondria) decrease causing a shift

from fat oxidation to carbohydrate utilization (Van Loon, et al., 2001). Brooks suggests that another reason for the decline in fat oxidation is a decrease in arterial FFA due to an increase in lactic acidosis, which inhibits lipolysis (Brooks et al., 2000). Brooks further suggests that an endurance trained individual will have a decreased epinephrine response at any given absolute workload which limits the amount of glycogen breakdown, thereby reducing carbohydrate oxidation (Brooks, et al., 2000). Factors such as a decrease in carnitine, an increase in lactic acidosis, and a suppression of epinephrine explain why a trained individual may have a higher crossover point at a given absolute work rate, but not a higher crossover point at the same relative intensity. Additional explanations for an increase in fat oxidation (in terms of absolute amount) include an increase availability of epinephrine and nor-epinephrine, which stimulate lipolysis, an increase in growth hormone which stimulates fat metabolism during exercise, an increased blood flow to adipose tissue, and decreased levels of insulin (McArdle et al., 2007). But while the absolute fat oxidation rate does indeed increase as an individual trains and becomes fitter, so does VO_2 max. Thus fat oxidation and VO_2 max are both shifting to higher levels, creating a minimal effect on the percentage of VO_2 max at crossover. While these checks and balances within the body create little impact on the crossover point, there is substantial variability from one individual to the next. This variability does not appear to be related to gender, fitness level, or a combination of the two. This largely unexplained variability confirms earlier research (Venables et al., 2005) and is likely predetermined by genetics.

Venables (2005) found a significant difference when comparing crossover of males to that of females. Men had lower fat oxidation and an earlier shift in using

carbohydrate as the dominant fuel source (Venables et al., 2005). In the present study a slightly higher percentage of VO_2 max at crossover was determined; however, it was not significant (Venables crossover was between 48-53%, our crossover was 55%). Other research shows that women do indeed oxidize more fat than their male counterparts, but only at low intensity exercise (Kang et al., 2007). This difference disappears at intensities above 40% VO_2 max. The fact that we only observed a significant difference in the unfit sample is likely due to the small sample sizes of this study or sampling error. These differences in fat oxidation can be accounted for by differences in sex-specific hormones, muscle fiber type proportions, regulation of FFA mobilization, and the effect of hormone sensitive lipase (Venables et al., 2005).

Recommendations for Further Studies: Metabolic Irregularities and Fat Oxidation

Our study compared variables within healthy adults. The results of this study can therefore, not be applied to individuals with metabolic abnormalities. It may be worth investigating the way in which a metabolically abnormal individual's crossover might differ from that of a metabolically normal individual's crossover since an inability to effectively oxidize fat is factor in both obesity and diabetes (Achten and Jeukendrup, 2004). There is some research that suggests that exercise done at moderate- and high-intensities improves lipid mobilization in overweight men and women (Pillard et al., 2007). However, this mobilization during exercise does not appear to affect energy expenditure over a 24 hour period of time (Saris and Schrauwen, 2004). Determining differences in crossover between normal and metabolic impaired subjects may lead to a better understanding of the effects of exercise on the obese and/or diabetic patient.

Ultimately if the goal is increased fat oxidation it may be necessary to evaluate the post-exercise state. Specifically, a high level of exertion during exercise leads to greater calorie utilization, both fat and carbohydrate, after exercise in the form of a greater excess postexercise oxygen consumption, or EPOC (McArdle, et al., 2007). Thus for individuals with metabolic irregularities, such as diabetes or obesity, improved fat utilization may be found not during exercise, but after. The results of a recent study indicate that by training at too low of an exercise intensity (which is often recommended as a means of stimulating fat oxidation) there are negative effects on endurance (Meyer et al., 2007). In an evaluation of obese men, Van Aggel-Leijssen et al. found that low intensity (40% VO_2 max) exercise increased fat oxidation, but not high intensity (70% VO_2 max) exercise (2002). Although this may apply to non-obese subjects as well, the results of our study indicate that crossover does not shift beyond a certain percentage of VO_2 max, regardless of training. Thus it may be reasonable to have metabolically impaired individuals training at higher intensities if their current fitness level will allow. A suggested follow up study would be a comparison of the percentage of VO_2 max at crossover for healthy subjects and metabolically abnormal subjects.

Recommendations for Further Studies: Aging and Fat Oxidation

While this study did not specifically look at an elderly population, it is worth noting that carbohydrate and fat utilization does seem to be impacted by the aging process. Carbohydrate and fat oxidation are significantly affected by exercise intensity in the elderly male (much like in a non-elderly male), but only carbohydrate appears to be affected by training (Bassami et al., 2007). This is interesting, as there is still not a clear consensus on why there is a shift in fuel usage during exercise regardless of age. Some

researchers maintain that it is glycogen sparing that causes a shift in absolute fat oxidation during exercise, while others suggest that it is an increase in fat oxidation itself that causes the shift (Jeukendrup and Achten, 2001). Either way, more research is needed in both an elderly and non-elderly population to determine the exact mechanism by which fat oxidation decreases and carbohydrate utilization increases with an increase in exercise intensity and with training.

Conclusion

This study demonstrated that there are no significant differences in the percentage of VO_2 max at crossover when comparing males and female, fit and unfit, and an interaction between variables. These findings can, of course, only be applied to the healthy, adult population. It is worth noting that previous research has found differences in fat oxidation between men and women. However, these differences exist only at low intensity exercise. Thus, findings from this study demonstrate that while fat oxidation can be improved in an absolute sense, it does not improve relative to VO_2 max. This suggests that the sympathetic nervous system, regardless of training, will at some point regulate fuel utilization. Further research is still needed to assess the effects of exercise and gender on populations other than the healthy, adult population.

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APPENDIX I

SUBJECT CHARACTERISTICS

Subject	Fitness	Gender	Age	Height	Weight
1	Bottom 50%	Female	28	63	107
2	Top 50%	Female	25	66	146
4	Bottom 50%	Female	39	65	155
5	Bottom 50%	Female	21	62	139
6	Bottom 50%	Female	19	67.5	156
7	Top 50%	Female	29	71	157
8	Top 50%	Female	40	68	150
9	Bottom 50%	Male	32	73	180
10	Bottom 50%	Female	32	63	166
11	Top 50%	Male	40	69	176
12	Top 50%	Female	39	65	161
13	Top 50%	Male	35	68.5	165
14	Top 50%	Male	29	73	195
15	Top 50%	Female	31	65	131
16	Top 50%	Male	37	73	200
17	Bottom 50%	Male	34	67	195
18	Top 50%	Female	31	67	112
19	Top 50%	Male	35	73	160
20	Bottom 50%	Female	21	65.5	130
21	Bottom 50%	Male	27	70	165
22	Bottom 50%	Female	24	67	120
23	Top 50%	Female	23	65	140
24	Bottom 50%	Male	36	74	200
25	Bottom 50%	Male	32	71	208
26	Top 50%	Male	30	73	218

Subject	BMI	Body Fat	VO2 max	Percentile Ranking	Crossover percentage
1	19	19.1	37	60	0.71
2	23.6	23.9	45.5	90	0.33
4	25.8	30.3	32.7	40	0.58
5	25.4	32.2	40.7	80	0.64
6	24.1	28.8	37.5	65	0.59
7	21.9	21.75	50	90	0.71
8	22.8	28.7	45.7	90	0.53
9	23.8	18.68	40.5	45	0.68
10	29.4	29.38	34.9	60	0.78
11	26	19.84	55.7	90	0.32
12	26.8	26.33	44.6	90	0.5
13	24.7	14.6	55.5	90	0.71
14	25.7	11.9	54.6	90	0.66
15	21.8	17.5	52.6	90	0.47
16	26.4	13	55.6	90	0.36
17	23.7	11.29	46	80	0.45
18	17.5	14.8	54.5	90	0.51
19	21.1	9.03	63.6	90	0.45
20	21.3	22.5	44.3	90	0.47
21	23.7	10.81	43.2	55	0.57
22	18.8	14.08	45.6	90	0.52
23	23.3	16.6	51.3	90	0.68
24	26.4	20.91	44.7	70	0.49
25	29	13.5	49.1	85	0.57
26	28.8	20.2	51.6	90	0.55

APPENDIX 2

PERCENTILE VALUES FOR MAXIMAL AEROBIC POWER

Percentile	Age				
	20-29	30-39	40-49	50-59	60+
Men					
90	51.4	50.4	48.2	45.3	42.5
80	48.2	46.8	44.1	41	38.1
70	46.8	44.6	41.8	38.5	35.3
60	44.2	42.4	39.9	36.7	33.6
50	42.5	41	38.1	35.2	31.8
40	41	38.9	36.7	33.8	30.2
30	39.5	37.4	35.1	32.3	28.7
20	37.1	35.4	33	30.2	26.5
10	34.5	32.5	30.9	28	23.1
Women					
90	44.2	41	39.5	35.2	35.2
80	41	38.6	36.3	32.3	31.2
70	38.1	36.7	33.8	30.9	29.4
60	36.7	34.6	32.3	29.4	27.2
50	35.2	33.8	30.9	28.2	25.8
40	33.8	32.3	29.5	26.9	24.5
30	32.3	30.5	28.3	25.5	23.8
20	30.6	28.7	26.5	24.3	22.8
10	28.4	26.5	25.1	22.3	20.8

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