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Profiling Substrate Utilization Patterns Using Ventilatory Thresholds as Markers of Exercise Intensity

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Abstract

Background: Profiling substrate utilization patterns at different exercise intensities within the ventilatory threshold (VT) training model is one area of research that may improve nutritional practices, exercise prescription, and body composition management. **Purpose:** The purpose of this study was to examine substrate utilization patterns below VT_1 and below VT_2 in a rested and fatigued state. **Methods:** Twenty-one recreationally active participants completed a total of three test sessions consisting of one maximal graded exercise test and two 20-minute submaximal steady-state exercise tests within a fourteen-day period. The maximal graded exercise test was used for determination of VO_2max and VTs. The submaximal steady-state exercise tests were used to determine substrate utilization patterns at intensities below VT_1 and below VT_2. One of the submaximal steady-state exercise tests was completed in a rested state while the other was completed in a fatigued state. For the fatigued test session, participants were verbally instructed to partake in strenuous exercise (lower body resistance training, lactate threshold, VO_2max, or anaerobic workouts) within twenty-four hours of the test. For the rested test session, participants were verbally instructed to refrain from strenuous or unaccustomed exercise for at least 24 hours prior to testing. A two-way repeated measures ANOVA was conducted to explore the effects of exercise duration on lipid or carbohydrate utilization rates between the fatigued and rested trials. **Results:** There was a significant main effect of exercise duration on carbohydrate utilization rates ($F(1.25, 18.72) = 30.97$, $p = <.001$). There was a nonsignificant main effect of exercise duration on lipid utilization rates ($F(1.67, 33.20) = 3.04$, $p = 0.07$). There was a nonsignificant main effect of fatigue status on lipid ($F(1, 15) = .001$, $p = .971$) and carbohydrate ($F(1, 15) = .005$, $p = .943$) utilization rates. **Conclusions:** Carbohydrate usage increased significantly when comparing exercise below VT_1 to between VT_1 and VT_2. Lipid usage increased gradually over time after steady-state was reached at both intensities.

Key Words: Exercise Prescription, Lactate Threshold, Maximal Oxygen Uptake

Introduction

The traditional approach to profiling substrate utilization during exercise uses percentages of VO_2max to quantify exercise intensity[1,2]. For example, during exercise, free fatty acid flux rates increase until about

55% of VO_2max. Above 55% of VO_2max, free fatty acid flux rates begin to decrease relative to carbohydrates and carbohydrates become the primary source of fuel[2]. Similarly, traditional training paradigms, such as the relative percent method, have long been employed to profile substrate utilization during exercise. This method, favored for enhancing and maintaining cardiorespiratory fitness, relies on percentages of heart rate reserve or oxygen uptake reserve as target training intensities. However, there are numerous approaches to prescribing exercise intensity, although some methods are more effective than others.

In recent years, a growing number of aerobic training studies have utilized a training paradigm that has proven to be effective at standardizing exercise intensities between individuals with varying fitness levels, so that exercise prescription using relative intensities is more accurate. This paradigm uses ventilatory thresholds (VT) as markers for determining exercise intensity[3–6]. The focus of these training studies has been to examine the efficacy of VT-guided training intensities at improving cardiorespiratory fitness relative to traditional methods. This training paradigm follows an exercise intensity prescription model divided into three zones: below ventilatory threshold one (VT_1), above VT_1 but below ventilatory threshold two (VT_2), and above VT_2[4,5,7].

With increasing exercise intensity, VT_1 is marked by an increase in minute ventilation (VE) that is disproportionately higher than increases in oxygen consumption (VO_2). Increases in VE at VT_1 are attributed to accumulating blood lactate levels above baseline and the resulting increases in carbon dioxide (CO_2) production (VCO_2) from its buffering. VT_2 is marked by an increase in VE that is disproportionate to the increase in VCO_2. What results is metabolic acidosis, where an increase in ventilation is needed to remedy CO_2 production by means of exhalation[8]. VTs are useful guides for training because of their relationship to lactate thresholds (LT), which limits the potential for errors in prescribing exercise intensity.

Contrastingly, traditional training paradigms completely ignore VTs for exercise prescription. The relative percent method is a traditional paradigm that utilizes percentages of heart rate reserve or oxygen uptake reserve as target training intensities for exercise. This method is the traditional training paradigm of choice for enhancing and maintaining cardiorespiratory fitness in the general population, whereas a more individualized approach benefits from VT-guided training. The American College of Sports Medicine recommends exercise three to five days a week at an intensity of 40% - 59% and 60% - 89% of heart rate reserve or oxygen uptake reserve for moderate and high intensity exercise, respectively[12]. Because this method ignores LTs, the relative percent concept has shown to be unreliable at equalizing exercise intensity between individuals with varying fitness levels when compared to a VT training paradigm[3–5]. For example, Wolpern et al. found that 100% of participants in an aerobic training program using the VT method experienced an increase in VO_2max greater than 5.9%[4]. In comparison, only 41% of the participants in the heart rate reserve group experienced an increase in VO_2max greater than 5.9%. What makes the VT training paradigm so efficacious is the relationship between lactate thresholds (LT) and VTs.

Despite several studies demonstrating the efficacy of a VT training model at equalizing training intensities between individuals, reducing individual variability in training responses, and improving VO_2max, research on this training model can benefit from more research[3–5]. To further inform the training paradigm and its practical applications for performance enhancement, further research is warranted. Profiling substrate utilization patterns at different exercise intensities within the VT training model is one area of research that may improve nutritional practices, exercise prescription, and body composition management. Therefore, to better understand what is occurring metabolically during VT-guided intensities, the purpose of this study is to analyze substrate utilization patterns at training intensities below VT_1 and below VT_2. A secondary purpose of this study is to analyze substrate usage at the same intensities in a rested and fatigued state.

METHODS

Subjects

Twenty-four recreationally active (having exercised for at least three times a week, for a minimum of thirty minutes, for the past three months) men (n=18) and women (n=6) between 18 and 50 years old were recruited via-email or word of mouth and consisted of Western Colorado University staff and students and Gunnison, Colorado community members. Exclusion criteria included pulmonary and cardiovascular conditions and any physical conditions that may be agitated or exacerbated due to participating in a maximal graded exercise test. Participants were screened for potential health risks using Western Colorado University's medical history questionnaire. Prior to all test sessions, participants were asked to refrain from alcohol consumption for twenty-four hours and caffeine consumption for twelve hours. All participants provided verbal and written consent prior to participation in research.

Experimental Design

In this non-randomized crossover design study, participants completed a total of three test sessions consisting of one maximal graded exercise test and two submaximal steady-state exercise tests within a fourteen-day period. The maximal graded exercise test was used for determination of VO_2max and VTs. The submaximal steady-state exercise tests were used to determine substrate utilization patterns at varying intensities. Anthropometric data was collected before the maximal graded exercise test. Time between test sessions was dependent on participant and researcher availability but were at least one day apart. Data collection took place in Western Colorado University's High Altitude Performance Lab (2,352 m).

One of the submaximal steady-state exercise tests was completed in a rested state while the other was completed in a fatigued state. For the fatigued test session, participants were verbally instructed to partake in strenuous exercise (lower body resistance training, threshold, VO_2max, or anaerobic workouts) within twenty-four hours of the test. For the rested test session participants were verbally instructed to refrain from strenuous or unaccustomed exercise for at least 24 hours prior to testing. Figure 1 outlines the experimental design.

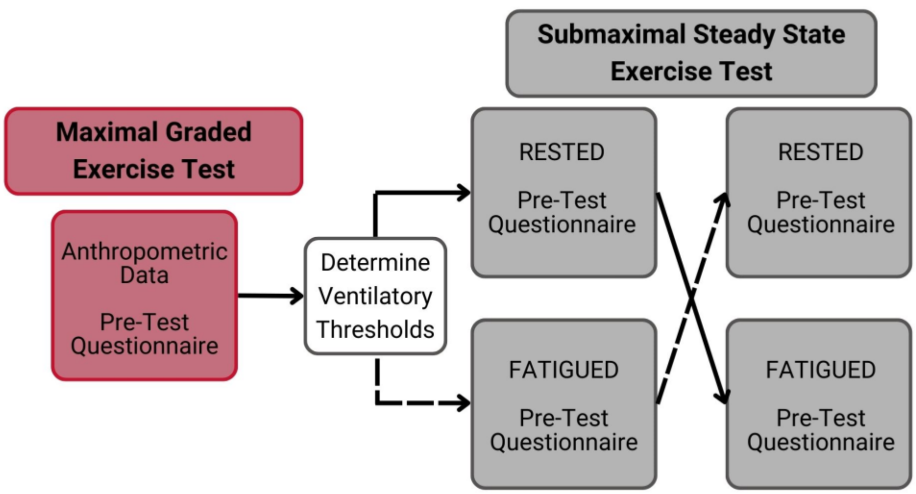

Figure 1. Experimental Flowchart Outlining the Study Design.

Procedures

Anthropometric Data

Participants' height, weight, and age were recorded. Height and weight were measured using a digital stadiometer (WB-3000, Tanita Corporation of America, Arlington, IL, USA).

Outline of Exercise Tests

One maximal and two submaximal exercise tests were completed on a motorized treadmill with an incline of 1% (Fitnex Fitness Equipment, Dallas, TX). Heart rate was continuously recorded with a heart rate chest monitor (WearLink, Polar USA, Worchester, Massachusetts, USA) and a corresponding heart rate monitoring watch (Polar FT1, Polar USA, Worchester, Massachusetts, USA). Heart rate data was also monitored by a Parvomedic TrueOne 2400 Metabolic Cart (TrueOne 2400, Parvo Medics, Salt Lake City, UT), which was used to collect gas exchange data. Prior to exercise tests, participants completed a three-minute walking warm up. Participants completed a self-selected cool down upon completion of the tests.

Maximal Graded Exercise Test Protocol

Participants began the test with running at 3.3 mph and the workload increased by 0.3 mph every 30 seconds until volitional fatigue. The last 15 seconds of gas exchange data during the test were averaged and considered VO_2max.

Submaximal Steady State Exercise Test Protocol

Participants began the test by running at a workload (in mph) 10 to 15 bpm below heart rate at VT_1. The second stage was completed at a workload of 10 to 15 bpm below VT_2. Each stage was 20 minutes long.

Determination of Ventilatory Thresholds

Fifteen second averages from gas exchange data were used in determining VT_1 and VT_2. Ventilatory equivalents of O_2 (VE/VO_2) and CO_2 (VE/VCO_2) were plotted on a graph against time. VT_1 occurred when VE/VO_2 deviated from a linear increase independent of VE/VCO_2. VT_2 occurred when VE/VCO_2 increased nonlinearly in concurrence with VE/VO_2. The graphs were visually inspected by the same two trained exercise physiologists and reevaluated in the instance of disagreement. Methods for determining VTs from a submaximal exercise test were consistent with previously published work[4,5,7,9].

Determination of Substrate Utilization

Percentages and rates of substrate utilization during the submaximal steady state exercise tests equations used can be referenced in Table 1. For the percentage equations, RER values used were the average of every five-minute period throughout the 40-minute test. For the rate equations, VCO_2 and VO_2 are the average of every five-minute period throughout the 40-minute test. Gasses are expressed in liters per minute while mass is expressed in grams per minute.

Table 1. Equations Used for Determination of Substrate Utilization.

	Substrate Type	
	Lipid	**Carbohydrate**
Percentage Equation	% = [(1 – RER)/0.29] x 100	% = [(RER - .71)/0.29] x 100
Rate Equation (g/min)	$1.695 \times VO_2 - 1.701 \times VCO_2$	$4.344 \times VCO_2 - 3.061 \times VO_2 - 0.40$* $4.344 \times VCO_2 - 3.061 \times VO_2 - 0.40$**

Note. *Proposed for low intensity exercise (40 – 50% VO_2max), **proposed for high to moderate intensity exercise (51 – 75% VO_2max).

Statistical Analysis

A two-way repeated measures ANOVA was conducted to compare the effects of exercise duration and fatigue status on rate of CHO or Lipid usage. Similarly, a two-way repeated measures ANOVA was conducted to explore the effects of exercise intensity and fatigue status on CHO or Lipid usage. Statistical analyses were conducted using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

RESULTS

All data presented are representative of participants who completed all trials. Three of the twenty-four participants were unable to complete the fatigued submaximal steady-state exercise trial because of scheduling conflicts. Table 1 includes the anthropometric and physiological characteristics of the participants. It is important to note that VO_2max was recorded at moderate altitude (2,352 meters). Our participants likely experienced about a ten percent decrement in VO_2max scores relative to sea level. Mean VO_2max scores for men and women were high when adjusted to sea level values, indicating high cardiovascular fitness amongst our participants[12].

Table 2. Participant Characteristics (M ± SD)

	Female (n = 6)	*Male (n = 15)*	*Combined (n = 21)*
Age (y)	22.83 ± 2.40	25.53 ± 5.99	24.76 ± 5.30
Height (cm)	167.00 ± 5.18	179.17 ± 9.93	175.70 ±10.37
Weight (kg)	60.48 ± 10.67	74.93 ± 11.44	70.80 ± 12.84
VO_2max (ml/kg/min)	47.90 ± 4.47	53.78 ± 7.90	52.01 ± 7.49
VT_1 (% of VO_2max)	65.15 ± 8.91	63.17 ± 5.90	63.74 ± 6.73
VT_2 (% of VO_2max)	81.17 ± 9.44	83.29 ± 6.50	82.68 ± 7.27

Note. M = mean, SD = standard deviation, n = number of participants, y = years, cm = centimeters, kg = kilograms, ml = milliliters, min = minutes.

Section I – Carbohydrates

Figure 2 represents the rate of CHO usage over time during the fatigued and rested 40-minute exercise trials. CHO values represent the average of every five-minutes. There was a significant main effect of exercise intensity on CHO usage ($F(1.28, 25.50) = 46.10$, $p < .001$), meaning that CHO usage increased significantly ($p < .001$) as exercise intensity increased from below VT_1 to below VT_2.

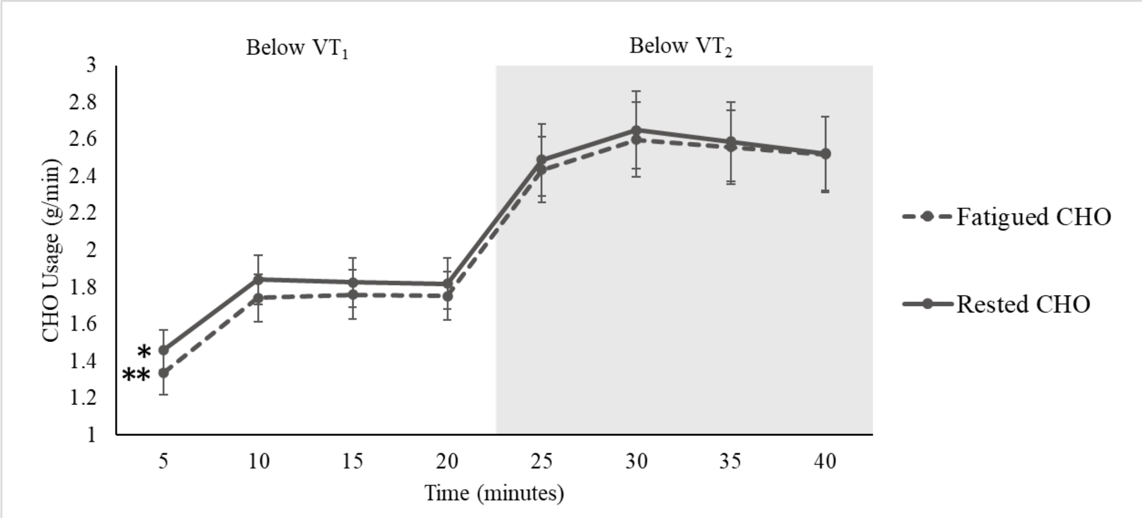

Figure 2. Rate of Carbohydrate (CHO) Usage Between the Fatigued and Rested Exercise Trials Over Time

Note. *P < .001, Rested CHO usage below VT_2 vs. below VT_1; **P < .001, Fatigued CHO usage below VT_2 vs. below VT_1. All values represent the mean ± SE.

Figure 3 represents total CHO usage during the fatigued and rested exercise trials, below VT1 and below VT2. Total CHO usage refers to the sum of all CHO used in grams. Total CHO usage was significantly greater below VT2 when compared to below VT1 ($p<0.001$), in both the fatigued and rested trials ($F(1, 20) = 45.21$, $p < .001$).

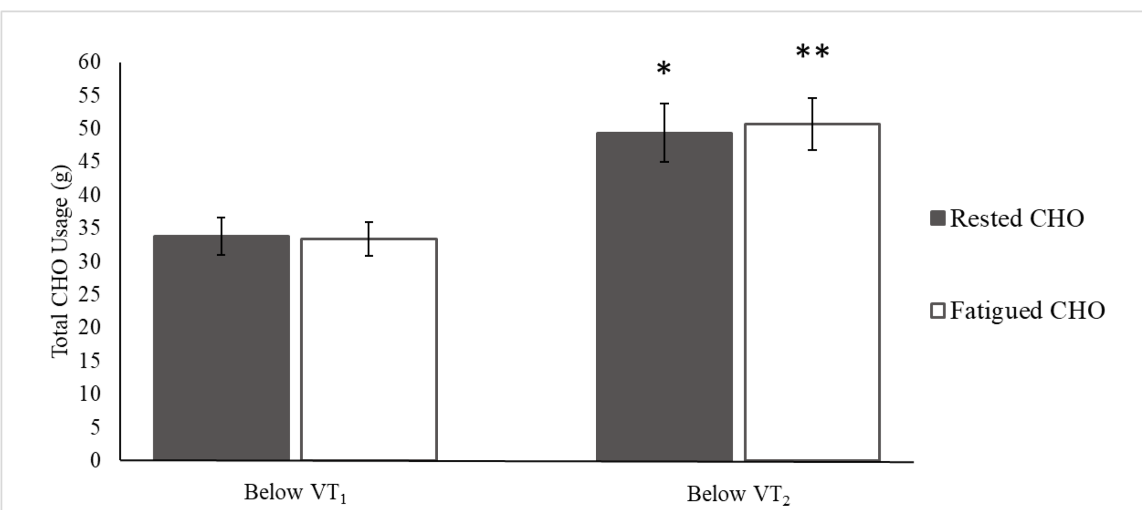

Figure 3. Total Carbohydrate (CHO) Usage Between the Rested and Fatigued Exercise Trials Below VT_1 and Below VT_2.

Note. *P < .001, Rested CHO usage below VT_2 vs. below VT_1; **P < .001, Fatigued CHO usage below VT_2 vs. below VT_1. All values represent the mean ± SE.

Section II - Lipids

Figure 4 represents the rate of lipid usage over time during the fatigued and rested 40-minute exercise trials. Lipid values represent the average of every 5-minutes. Mean values of lipid usage are shown in Figure 4.

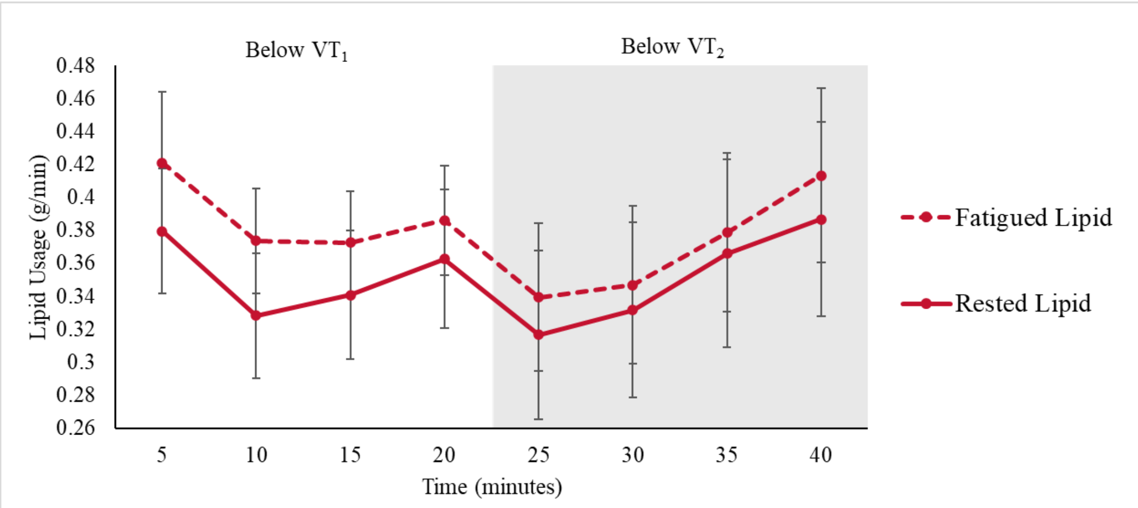

Figure 4. Rate of Lipid Usage Between the Fatigued and Rested Exercise Trials Over Time.
Note. All values represent the mean ± SE.

Figure 5 represents total lipid usage during the fatigued and rested exercise trials. Total lipid usage refers to the sum of all lipids used in grams either below VT_1 or below VT_2. Mean lipid values are shown in Figure 5.

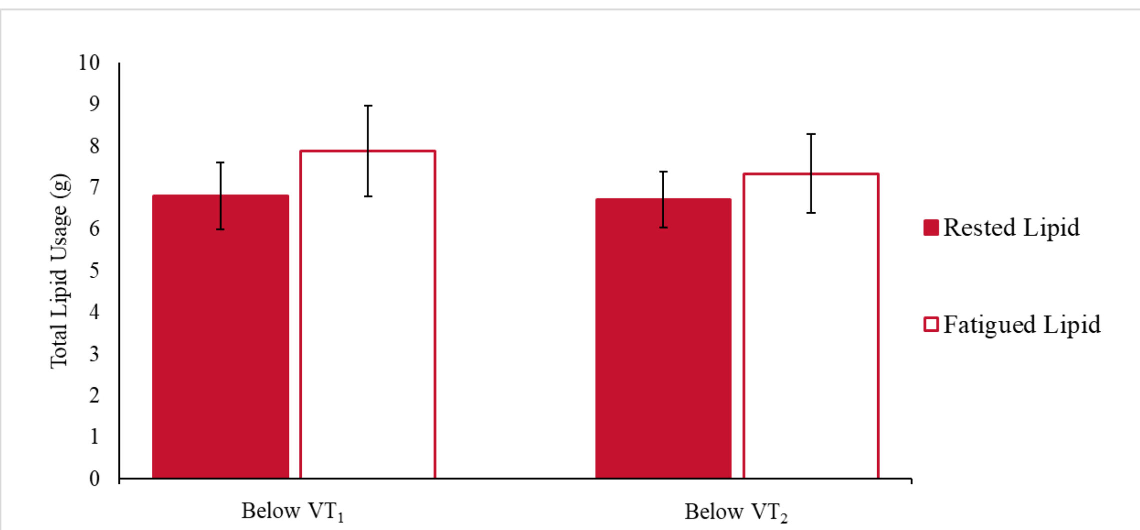

Figure 5. Total Lipid Usage Between the Rested and Fatigued Exercise Trials Below VT_1 and Below VT_2.
Note. All values represent the mean ± SE.

Section III – Relative Contribution

Figure 6 represents the relative contributions of CHOs and lipids to total energy expenditure during the fatigued and rested 40-minute exercise trials. CHO and lipid usage values represent the average of every five-minutes. Although lipids and CHOs have already been displayed as absolute values in previous figures, Figure 6 displays both variables as relative values, as percentages in relation to one another. Both are displayed side-by-side to emphasize their inverse relationship to one another.

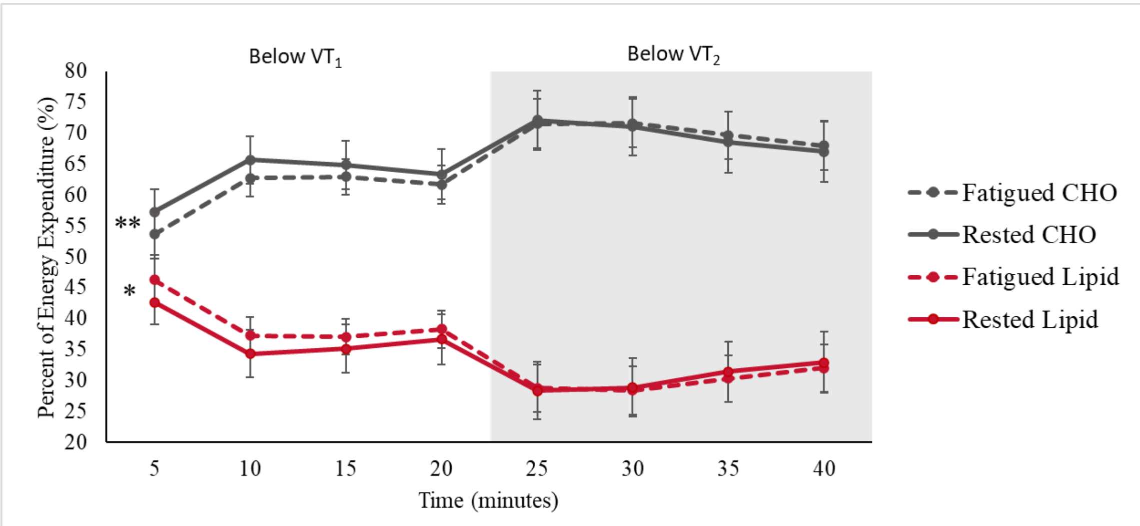

Figure 6. Relative Contribution of Carbohydrates (CHOs) and Lipids Over Time.
Note. *$P < .001$, Lipid usage below VT_1 vs. below VT_2; **$P < .001$, CHO usage below VT_1 vs. below VT_2. All values represent the mean ± SE.

Section IV – Caloric Expenditure

Figure 7 displays the same data in figures 3 and 5, but grams of CHO and lipid were multiplied by 4 and 9 respectively to represent total caloric expenditure, rather than grams. Lipids contain approximately 9 calories per gram, whereas CHOs contain approximately 4 calories per gram[13]. In Figure 7, the CHO and lipid graphs were combined and placed side-by-side on separate scales for visual comparison. Just as in Figure 3, CHO usage increases significantly with increasing exercise intensity.

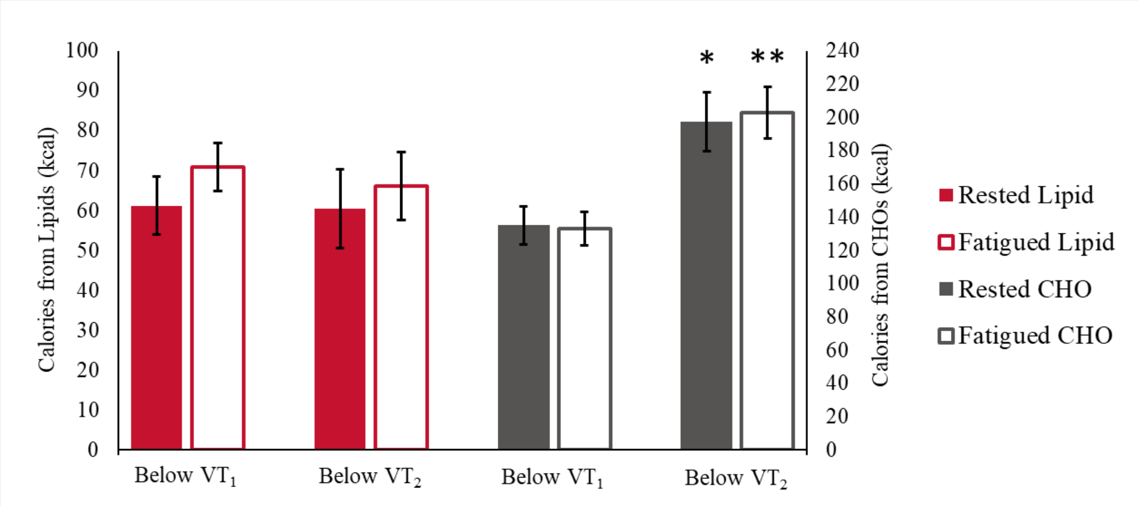

Figure 7. Total Caloric Expenditure from Lipids or Carbohydrates (CHOs) Between the Rested and Fatigued Exercise Trials Below VT_1 and Below VT_2.

Note. *P < .05, Rested CHO usage below VT_2 vs. below VT_1; **P < .05, Fatigued CHO usage below VT_2 vs. below VT_1. All values represent the mean ± SE.

Figure 8 represents the sum of all calories expended from lipids and CHOs. There was a significant main effect of exercise intensity ($F(1, 20) = 73.00$, $p < .001$) on total caloric expenditure, meaning that calories expended were greater below VT_2 when compared to below VT_1. Mean caloric expenditure values are shown in Figure 8.

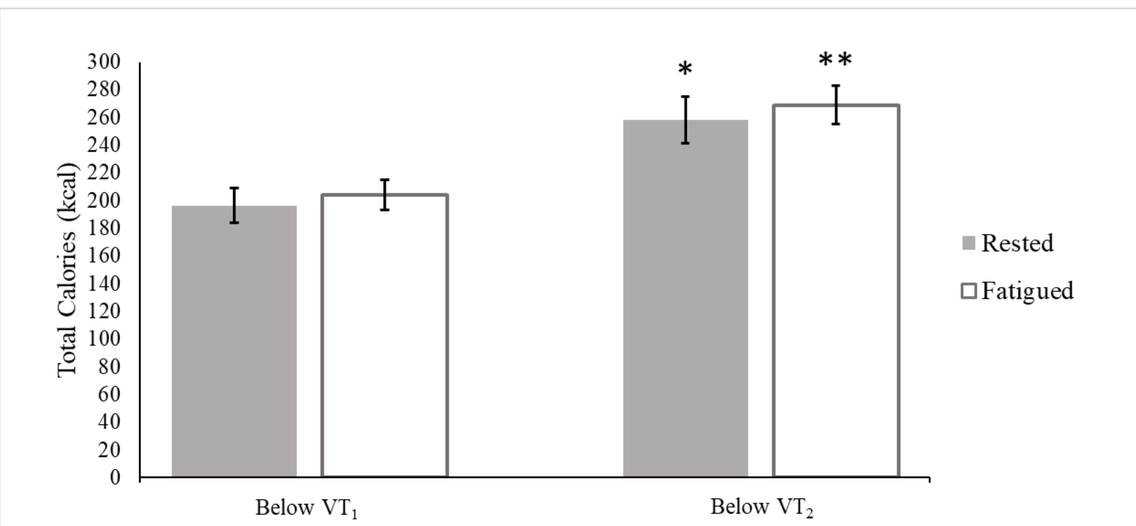

Figure 8. Total Caloric Expenditure Between the Rested and Fatigued Exercise Trials Below VT_1 and Below VT_2.

Note. *P < .05, Rested caloric usage below VT2 vs. below VT1; **P < .05, Fatigued caloric usage below VT2 vs. below VT1. All values represent the mean ± SE.

DISCUSSION

The purpose of this study was to profile substrate utilization patterns below VT_1 and below VT_2. This study also examined how substrate usage may differ between rested and fatigued exercise trials. During the first five minutes of exercising below VT_1, participants were already beyond the crossover point. It was hypothesized that substrate utilization rates would be greater in the fatigued trials compared to the rested trials. However, the findings of this study indicate that there were no significant differences in substrate usage between rested and fatigued exercise trials.

Profiling Substrate Utilization Patterns

Crossover Point

After the first five minutes of exercise in both the rested and fatigued trials, the relative contribution of lipids was 42% and 47%, respectively. However, the difference between those two values was not statistically significant. Contribution of CHOs was 58% and 53% in the rested and fatigued trials, respectively. Similarly, this difference was not statistically significant. CHOs contributed more to energy production than lipids by the first five minutes in both trials. This indicates that the crossover point occurred before steady state was even reached. These findings suggest that an intensity just below VT_1 is great enough for crossover to occur in this sample. VT_1 occurred at about 63% of VO_2max in our participants. Brooks suggests that the crossover point commonly occurs around 55% of VO_2max, though we know this value can vary depending on training status[2]. Purdom et al. reports that the crossover point can occur in a range of 47 – 75% of VO_2max[14]. These authors support our findings, as crossover occurred below 63% of VO_2max in our participants.

Carbohydrate Utilization

During steady state exercise in both the fatigued and rested exercise trials below VT_1, CHO utilization rates varied between 1.7 and 1.8 g/min. Manetta et al. observed similar rates of 2 g/min, while participants cycled 15% below VT_1 for 50 minutes[15]. Rate of CHO usage was slightly higher in Manetta et al.'s study even though intensity was similar. Individuals in Manetta et al.'s study had mean VT_1 values of 60.8%, which were expressed as a percentage of VO_2max. Similarly, our participants' mean VT_1 scores were 63%. It is unclear whether this difference is related to fitness level. Our participants had higher VT_1 scores (expressed at a greater percentage of VO_2max), however Manetta et al.'s participants had greater VO_2max scores. Manetta et al. observed mean VO_2max scores of 65.5 ml/kg/min[15]. Our participants experienced about a 10% decrement in VO_2max, with a mean score of 52 ml/kg/min, or an estimated 57 ml/kg/min when adjusted to sea level. Nonetheless, Manetta et al. observed no differences in rate of CHO usage between untrained and trained individuals, suggesting that VO_2max scores have no effect on CHO usage.

Lipid Utilization

According to Purdom et al., maximal fat oxidation rates have been observed at intensities ranging from 45 – 75% of VO_2max[14]. Maximal fat oxidation is influenced by factors such as training status, intensity, duration, and pre-exercise nutrition. Individuals in our study were exercising just below VT_2, which as a mean occurred at 82% of VO_2max. This intensity is well beyond the threshold that elicits maximal fat oxidation rates. Lima-Silva et al. observed maximal fat oxidation rates of 0.29 g/min in highly trained runners with mean VO_2max scores of 58.6 ml/kg/min[16]. Maximal fat oxidation rates in our study were observed at 0.42 g/min. There is a large discrepancy between our studies, though it is unclear why.

Lipid usage rates in our study did not vary significantly between rested and fatigued trials. However, although statistically insignificant, it may be worth noting that the fatigued trials elicited a greater reliance on lipids compared to the rested trials.

Comparing Fatigued and Rested Trials

Although it is unclear why lipid utilization rates were slightly greater in the fatigued trial compared to the rested trial, we can speculate that it may have been related to substrate depletion. However, because we did not control for nutrition, exercise types, or exercise intensities for the fatiguing exercise bouts, there was no way to know for certain whether these exercises were intense or long enough to deplete muscle glycogen stores. In a glycogen depleted state, high intensity exercise is difficult to maintain and a greater reliance on lipids for energy may be observed[17]. Coupled with neuromuscular fatigue and thus poorer running economy, it is reasonable to expect a greater cost of running accompanied by greater rates of lipid usage, as well as a decrease in CHO usage. Nonetheless, running economy was not measured.

Fatiguing Protocol

One possibility for our findings may be attributed to our fatiguing protocol. Our participants were advised to perform a self-reported fatiguing exercise protocol of their choice before the fatigued exercise trial that was used to collect substrate usage data. Compared to Glace et al., our fatiguing protocol was much shorter and less precise[22]. Glace et al. was attempting to quantify muscle fatigue by measuring maximal force production in a variety of muscle groups after a fatiguing run[22]. They had all participants run at VT_1 (66 – 69% of VO_2max) for two hours to induce fatigue. In addition, the long duration fatiguing run was completed shortly after VO_2max was recorded with a maximal graded exercise test. In comparison, because our fatiguing protocol was self-prescribed and self-reported, it may have been inadequate at fatiguing our participants sufficiently. We had our participants engage in lower body resistance training, threshold, VO_2max, or anaerobic workouts, which all have the potential to fatigue an individual in varying ways. Standardizing the fatiguing protocol adds the benefit of normalizing fatigue across all participants, reducing the possibility for fatigue variability. Furthermore, the non-randomized nature of the fatiguing protocols could introduce some bias. Running below VT_2 followed by and exercise bout below VT_1 could yield different substrate usage patterns. It is worth noting that participants were not advised to follow a particular diet pre or post protocols.

Future Research

Future research should prioritize the refinement of fatiguing protocols so that fatigue variability is reduced. Additionally, studies of this nature may benefit from monitoring pre-and post-exercise nutrition to ensure that nutritional practices are standardized. This standardization could exclude nutrition as a confounding variable, as we know that nutritional practices have an influence on metabolic responses during prolonged exercise bouts. Lastly, analyzing the data based on sex would likely yield drastically different results.

Conclusions

In conclusion, this study aimed to assess substrate utilization patterns in rested and fatigued individuals below VT_1 and VT_2. Despite the initial hypothesis that substrate utilization rates would be higher in the fatigued trial, the results indicate no statistically significant differences in substrate usage rates between the fatigued and rested trials. However, it is worth noting that CHO usage was lower while lipid usage was higher in the fatigued trials, contributing to a greater total caloric expenditure. The increase in caloric expenditure might be attributed to decreased running economy and an increase in cost of running due to potential changes in running mechanics, strength production, and minute ventilation. Although we did not directly examine running economy, its potential impact on our findings are acknowledged. Another influencing factor could be the fatiguing protocol, with the self-reported nature potentially contributing to variability in fatigue. Greater lipid utilization rates in the fatigued trials may also be linked to muscle glycogen depletion, further influencing substrate preferences during submaximal exercise.

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