

# Effect of carbohydrate availability on time to exhaustion in exercise performed at two different intensities

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This study examined the effects of pre-exercise carbohydrate availability on the time to exhaustion for moderate and heavy exercise. Seven men participated in a randomized order in two diet and exercise regimens each lasting 3 days with a 1-week interval for washout. The tests were performed at 50% of the difference between the first (LT<sub>1</sub>) and second (LT<sub>2</sub>) lactate breakpoint for moderate exercise (below LT<sub>2</sub>) and at 25% of the difference between the maximal load and LT<sub>2</sub> for heavy exercise (above LT<sub>2</sub>) until exhaustion. Forty-eight hours before each experimental session, subjects performed a 90-min cycling exercise followed by 5-min rest periods and a subsequent 1-min cycling bout at 125% VO<sub>2max</sub>/1-min rest periods until exhaustion to deplete muscle glycogen. A diet providing 10% (CHO<sub>low</sub>) or 65% (CHO<sub>mod</sub>) energy as carbohydrates was consumed for 2 days until the day of the experimental test. In the exercise below LT<sub>2</sub>, time to exhaustion did not differ between the CHO<sub>mod</sub> and the CHO<sub>low</sub> diets (57.22 ± 24.24 vs 57.16 ± 25.24 min). In the exercise above LT<sub>2</sub>, time to exhaustion decreased significantly from 23.16 ± 8.76 min on the CHO<sub>mod</sub> diet to 18.30 ± 5.86 min on the CHO<sub>low</sub> diet (P < 0.05). The rate of carbohydrate oxidation, respiratory exchange ratio and blood lactate concentration were reduced for CHO<sub>low</sub> only during exercise above LT<sub>2</sub>. These results suggest that muscle glycogen depletion followed by a period of a low carbohydrate diet impairs high-intensity exercise performance.

Key words: Time to exhaustion; Muscle glycogen; Metabolism; Lactate thresholds

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## Introduction

In a classical study of muscle glycogen metabolism, Bergstrom and Hultman (1) demonstrated that muscle glycogen content decreased during exercise and was almost completely depleted when the individual was not able to continue the exercise. In a further study, Bergstrom et al. (2) showed that the pre-exercise muscle glycogen content could be altered by a muscle depletion exercise protocol followed by a 3-day diet with a low or high carbohydrate content. Bergstrom's primary finding was that the performance in subsequent prolonged exercise was related to the pre-exercise carbohydrate availability.

It has been suggested that muscle glycogen is the primary fuel utilized early in exercise, but as exercise duration increases and muscle glycogen is depleted there is a gradual shift toward blood glucose as the predominant carbohydrate energy source (3). This effect could be limited, however, by declining plasma glucose concentrations late in exercise or when exercise is performed after a low carbohydrate diet period, which in turn causes a gradual increase in free fatty acids oxidation. However, fat oxidation may not be sufficient to maintain the power output required during the cycling time-to-exhaustion test. In fact, a number of studies have reported that exogenous carbohydrate supplementation, especially when muscle glyco-

gen concentrations are low, can maintain blood glucose and total carbohydrate oxidation and delay the onset of fatigue (4-9). Therefore, while carbohydrate-loading diets are associated with improved endurance capacity, ascribed mainly to maintenance of a high rate of carbohydrate oxidation throughout exercise, low carbohydrate diets have been shown to impair endurance exercise capacity, which has been ascribed to anticipated reduction of endogenous carbohydrate availability and to a decreased rate of carbohydrate oxidation (4-9).

The reduced carbohydrate oxidation with previous low carbohydrate availability may affect the performance during heavy exercise more than during moderate exercise because fat oxidation could supply energy at a sufficient rate in the latter case, but not the former. Sabapathy et al. (10) have shown that subjects achieved a significantly lower maximal power output during incremental tests performed with reduced muscle glycogen content compared with a normal glycogen state. This suggests that performance during heavy exercise may be impaired by carbohydrate availability. It should be noted, however, that Sabapathy et al. (10) did not investigate the effect of carbohydrate availability on time to exhaustion because subjects did not perform constant-load tests. In addition, muscle glycogen depletion was achieved from prolonged exercise on the night prior to the experiment, followed by a brief fasting until the next morning when the incremental test was performed. This strategy may impair a correct interpretation of the results because the effect of low carbohydrate availability may be confused with the effect of the residual fatigue caused by the protocol of the night before the experimental test. To minimize these effects, the present investigation was designed to determine the effect of low pre-exercise carbohydrate availability, obtained with a muscle glycogen depletion exercise followed by low carbohydrate diet periods, on moderate and heavy constant-load exercise performed until exhaustion. We hypothesized that, when compared to a normal situation, reduction of carbohydrate availability prior to exercise results in a shorter time to exhaustion during heavy exercise, because the increase in fat oxidation may not be sufficient to replace carbohydrate oxidation.

## Material and Methods

### Subjects

Seven healthy fit men, but not competitive athletes, accustomed to ergometric exercises, volunteered for the study. The mean values ( $\pm$  SD) of age, weight, height, % body fat and  $VO_{2max}$  were  $26.6 \pm 6.2$  years,  $78.6 \pm 7.1$  kg,  $180.6 \pm 4.6$  cm,  $10.3 \pm 4.9\%$ , and  $38.5 \pm 6.8$  mL.kg<sup>-1</sup>.min<sup>-1</sup>,

respectively. All subjects gave written informed consent to take part in the study, which was approved by the Ethics Committee of University of Santa Catarina State.

### Experimental design

All subjects were required to participate in 9 laboratory visits until the end of the study. On the first visit, the anthropometric variables were measured and the percentage of body fat was estimated from the measurement of chest, abdomen and thigh skin folds (11). On the same day, the subjects were submitted to a progressive test conducted on an electromagnetically braked cycle ergometer (Ergo Fit 167, Pirmansens, Germany) for  $VO_{2max}$ , and first (LT<sub>1</sub>) and second (LT<sub>2</sub>) lactate breakpoint determinations.

In subsequent visits, subjects exercised under four different conditions, designed in a randomized form, until volitional exhaustion: 1) below LT<sub>2</sub> with moderate carbohydrate availability (CHO<sub>mod</sub>-belowLT<sub>2</sub>), and 2) below LT<sub>2</sub> with low carbohydrate availability (CHO<sub>low</sub>-belowLT<sub>2</sub>). The same procedure was applied to exercise above LT<sub>2</sub> (CHO<sub>mod</sub>-aboveLT<sub>2</sub> and CHO<sub>low</sub>-aboveLT<sub>2</sub>, respectively). These workloads were chosen to represent moderate and heavy exercise, respectively. Subjects were instructed to continue their training programs during the experiment, except before and during the 3 days of the experimental procedures.

### Progressive test

The subjects began pedaling for 5 min at 50 W with a pedaling frequency of 70-80 rpm and thereafter the workload was increased by 20 W every 3 min until volitional exhaustion. At the end of each stage, 25  $\mu$ L blood was drawn from the ear lobe and immediately analyzed for blood lactate determination (YSI 1500 Sport, Yellow Springs Instruments, USA). Blood lactate concentrations were plotted against power and the lactate thresholds determined by linear regression breakpoint analysis (12). A previous study demonstrated that a 20 W/3-min workload increase is the most appropriate for the identification of lactate thresholds due to the blood lactate kinetics (13).

A heart rate (HR) greater than 90% of the maximal heart rate predicted according to age, a respiratory exchange ratio (RER) greater than 1.1 and an increase in  $VO_2$  of less than 150 mL/min over the previous workload were the criteria applied to ascertain that  $VO_{2max}$  was achieved.

### Manipulation of pre-exercise carbohydrate availability

After arriving at the laboratory, a 5-min warm-up was performed and the subject commenced cycling for a fur-

ther 90 min at 50% of the difference between  $LT_1$  and  $LT_2$ , i.e.,  $\Delta LT_{50\%}$  ( $\sim 70\% VO_{2max}$ ). At the end, subjects rested for 5 min and then a series of 1-min bouts at  $125\% VO_{2max}$  separated by 1-min rest periods was carried out until volitional fatigue. Exercise was terminated when subjects could not complete 1 min of exercise at  $125\% VO_{2max}$ . This protocol for muscle glycogen depletion was previously validated by Gollnick et al. (14, 15) and Heigenhauser et al. (16) who demonstrated a decreased muscle glycogen content in both fast and slow-twitch fibers. In addition, Gollnick et al. (15) have demonstrated that, when intermittent heavy exercise is performed until exhaustion, glycogen from both types of fibers is depleted independently of the intensity used (120 to 150% of maximal aerobic power). Therefore, it is very probable that the carbohydrate store was depleted with the protocol used in the present study.

After the exercise-depletion protocol, subjects consumed either a low carbohydrate (10% of carbohydrate, 55% of protein and 35% of lipids) or moderate carbohydrate (65% of carbohydrate, 15% of protein and 20% of lipids) iso-energetic diet for the next 48 h (16-19). A dietician created all the diets, using food plans for each subject considering body mass and food preferences. All subjects were given a list of food choices that described the content permitted for each food group and that would provide the recommended daily energy uptake. For each condition, subjects recorded all food intake for 48 h after the exercise-depletion protocol until arriving for the experimental exercise test. Diet records were subsequently analyzed for caloric contributions of fats, protein, and carbohydrates. No differences were found between recommended and recorded diets in terms of total energy intake and composition. The subjects were asked to abstain from any vigorous exercise during this diet period.

### Experimental protocol

Two of the cycling tests were undertaken at a power output that corresponded to  $\Delta LT_{50\%}$  (below  $LT_2$ ) and the other two at a power output corresponding to 25% of the difference between the maximal workload and  $LT_2$ , i.e.,  $\Delta WL_{25\%}$  (above  $LT_2$ ). The four experimental tests were performed 7 days apart to washout. All cycling tests during experimental regimens were carried out until volitional exhaustion and the criterion for interrupting the test was when the subject could no longer maintain the pedal cadence above 70 rpm.

On the morning of an experiment, subjects reported to the laboratory between 7:00 and 9:00 h after an 8- to 12-h overnight fast. After the subject sat for 5 min in a comfortable chair, a Teflon catheter was inserted into an antecubital vein and a resting blood sample (3 mL) was obtained.

Following this procedure, the cannula was flushed with 1 mL 0.9% saline to maintain patency.

After the resting procedure was completed, the subject was transferred to the cycle ergometer and the gas-collecting equipment was connected. Following a baseline recording of the gases exchanged, the subjects began cycling within approximately 5 min of sitting on the cycle ergometer. Testing was started with a warm-up at 50 W for 5 min, after which the experimental power output was adjusted. In the exercise above  $LT_2$ , blood samples (3 mL) were obtained at rest, at 5-min intervals during exercise and at the end of the trial. When exercise was performed below  $LT_2$ , blood samples were taken after 5 and 15 min, and thereafter at 15-min intervals and at the end of the trial.  $VE$ ,  $VO_2$  and  $VCO_2$  were measured every 5 min on 1 min of exhaled gas collected under each condition. HR and rating of perceived exertion (RPE) were obtained at 5-min intervals using a portable heart rate monitor (Polar Vantage NV, Finland) and the Borg 10-point scale (20), respectively. HR and RPE were measured immediately before the collection of blood samples. The subjects were not informed about HR, power output or time to exhaustion until all experimental tests had been completed.

### Analytical procedures

$VE$ ,  $VO_2$  and  $VCO_2$  were measured using a portable metabolic measurement system (Aerosport Kb1C, Aerosport Inc., USA). Subjects wore a facial mask and breathed through the pneumotach. In the expiratory phase, a sample of air was exhaled past the orifice plate of the pneumotach and later mixed and analyzed for oxygen and carbon dioxide concentration using gas analyzers (galvanic fuel cell and non-dispersive infrared sensors).  $VE$  was determined by the difference in pressure across the orifice plate of the pneumotach where an increase in the pressure difference is indicative of an increased ventilatory volume. King et al. (21) confirmed the validity of this equipment by comparing the  $VO_2$ ,  $VCO_2$ ,  $VE$ , and RER measured by Kb1C with values obtained by the gold standard Douglas bag method. The mean error of the equipment is relatively small (6%) and the Aerosport Kb1C is acceptable for measuring oxygen uptake in the range of 1.5 to 3.5 L/min, which was the interval used in the present study. The medium-flow pneumotach setting (flow-rate range of 10-120 L/min) was used to decrease the error in the volume measurement (21).

The 3 mL of venous blood drained during rest and exercise was placed in a tube containing fluoride EDTA and subsequently centrifuged for 10 min at 3000 g. Plasma was later analyzed for glucose and lactate using the enzymatic colorimetric method (lactate kits: Rolf Greiner

Blochemica, Germany, and glucose kits: Biotécnica, Brazil).

### Data analysis

The rates of carbohydrate and fat oxidation during each experiment were calculated from the  $VCO_2$  and  $VO_2$  values using the following stoichiometric equations (22):

$$\text{CHO oxidation (g/min)} = 4.55 \text{ } VCO_2 \text{ (L/min)} - 3.21 \text{ } VO_2 \text{ (L/min)}$$

$$\text{FAT oxidation (g/min)} = 1.67 \text{ } VO_2 \text{ (L/min)} - 1.67 \text{ } VCO_2 \text{ (L/min)}$$

where CHO and FAT are carbohydrate and fat, respectively. The total carbohydrate and fat oxidized during exercise were calculated from the area under the carbohydrate and fat oxidation versus time curve.

In order to calculate the rate at which the perception of effort increases over time during exercise with low or moderate carbohydrate availability, the RPE was plotted against absolute time (min) and relative to the end time (%) of the test, and adjusted with a linear regression equation. The slope of the functions was thus compared for the different conditions.

### Statistical analysis

Data are reported as the mean  $\pm$  SD and range when appropriate. The Shapiro-Wilk test demonstrated a non-normal distribution of the data and non-parametric statistical analysis was therefore selected. Thus, the time to exhaustion, total carbohydrate and fat oxidized during exercise, and other physiological variables during rest and at the end of the exercise for the different conditions were compared using the non-parametric Friedman test. When a significant difference was found, the Wilcoxon test was used to identify where the difference occurred. The kinetics of the variables was also analyzed using the Friedman and Wilcoxon tests at each intensity.

## Results

### Progressive test and depletion protocol

The maximal power output and  $VO_{2max}$  were  $254.4 \pm 23.9$  W and  $3.1 \pm 0.3$  L/min ( $38.5 \pm 6.8$  mL.kg<sup>-1</sup>.min<sup>-1</sup>), respectively.  $LT_1$  and  $LT_2$  occurred at  $131.9 \pm 26.0$  W ( $1.7 \pm 0.3$  L/min) and  $204.4 \pm 29.6$  W ( $2.3 \pm 0.4$  L/min), respectively. The calculated  $\Delta LT_{50\%}$  and  $\Delta WL_{25\%}$  were  $168.1 \pm 24.4$  and  $217.2 \pm 27.9$  W, respectively. All subjects completed 90 min at  $\Delta LT_{50\%}$  in the muscle glycogen depletion protocol. On average, subjects performed a total of  $6 \pm 3$  work bouts at 125%  $VO_{2max}$  ( $318.2 \pm 29.9$  W). Unfortunately, 2 subjects were unable to complete all experimental procedures due to illness. Thus, data are reported for only 5 subjects.

### Time to exhaustion

The time to exhaustion for exercise performed with  $CHO_{mod}$ -below $LT_2$  was not different from that performed with  $CHO_{low}$ -below $LT_2$  ( $57.2 \pm 24.2$  vs  $57.2 \pm 25.2$  min). However, when the exercise was performed above  $LT_2$  there was a significant difference between conditions ( $CHO_{mod}$ -above $LT_2$ :  $23.2 \pm 8.8$ ,  $CHO_{low}$ -above $LT_2$ :  $18.3 \pm 5.9$  min;  $P < 0.05$ ). Individual reduced time to exhaustion above  $LT_2$  as a result of muscle glycogen manipulation ranged from 1.2 to 36.6% ( $19.1 \pm 13.7\%$ ).

### Total fuel utilization

There was no effect of pre-exercise carbohydrate availability or exercise intensity on the total carbohydrate and fat oxidized (Table 1). However, total carbohydrate tended to be higher and total fat to be lower ( $P = 0.08$ ) for  $CHO_{mod}$ -above $LT_2$  compared with  $CHO_{low}$ -above $LT_2$ . Individual data demonstrated that the total carbohydrate oxidized during exercise above  $LT_2$  decreased with low carbohydrate availability in 4 of the 5 subjects investigated.

### Kinetics of variables during exercise below $LT_2$

A significant effect of time on  $VO_2$ , RER, lactate, glu-

**Table 1.** Estimated carbohydrate (CHO) and fat oxidized during exercise performed below and above the second lactate breakpoint by subjects with normal or glycogen depletion.

	$CHO_{mod}$ -below $LT_2$	$CHO_{low}$ -below $LT_2$	$CHO_{mod}$ -above $LT_2$	$CHO_{low}$ -above $LT_2$
Total CHO (g/min)	$134.3 \pm 89.9$ (81.7-238.1)	$99.3 \pm 84.3$ (20.3-188.0)	$45.1 \pm 23.3$ (23.9-83.5)	$20.0 \pm 15.5$ (1.0-36.0)
Total fat (g/min)	$7.8 \pm 2.4$ (6.4-10.6)	$13.8 \pm 12.4$ (0.6-25.2)	$7.0 \pm 4.8$ (3.2-13.1)	$11.6 \pm 2.7$ (8.2-15.0)

Data are reported as means  $\pm$  SD for 5 men. Ranges are reported in parentheses.  $CHO_{mod}$  = moderate carbohydrate diet;  $CHO_{low}$  = low carbohydrate diet;  $LT_2$  = second lactate breakpoint.

cose, and RPE was observed when exercise was carried out below  $LT_2$ .  $VO_2$  increased exponentially with time up to ( $P < 0.05$ ) and remained relatively constant thereafter throughout the exercise. Low carbohydrate availability had no significant effect on  $VO_2$ , except at min 20 when values in the case of  $CHO_{mod}$ -below $LT_2$  were higher than  $CHO_{low}$ -below $LT_2$  ( $P < 0.05$ ). In addition, the RER also increased from rest to exercise and remained relatively stable until exhaustion under both conditions (Figure 1A). A similar response was observed for rates of carbohydrate and fat oxidation.

Blood lactate concentration increased progressively from rest to min 15 ( $P < 0.05$ ) and then remained largely unchanged until exhaustion under both conditions. Blood glucose concentration was stable until min 5 and then fell at min 15 ( $P < 0.05$ ), while no further decrease was observed after this time. A difference between situations was found only in the case of min 5 when blood glucose

concentration was higher for  $CHO_{mod}$ -below $LT_2$  than  $CHO_{low}$ -below $LT_2$  (Table 2).

RPE increased consistently until the end of exercise ( $P < 0.05$ ) and the RPE values at exhaustion were always higher compared to the preceding time points. Carbohydrate availability had no effect on RPE. In addition, the rate at which RPE rose over absolute ( $CHO_{mod}$ -above $LT_2$ :  $0.13 \pm 0.09$ ,  $CHO_{low}$ -above $LT_2$ :  $0.13 \pm 0.04$  RPE units/min) and relative time ( $CHO_{mod}$ -above $LT_2$ :  $0.06 \pm 0.03$ ,  $CHO_{low}$ -above $LT_2$ :  $0.07 \pm 0.02$  RPE units/%) did not differ under different conditions ( $P > 0.05$ ).

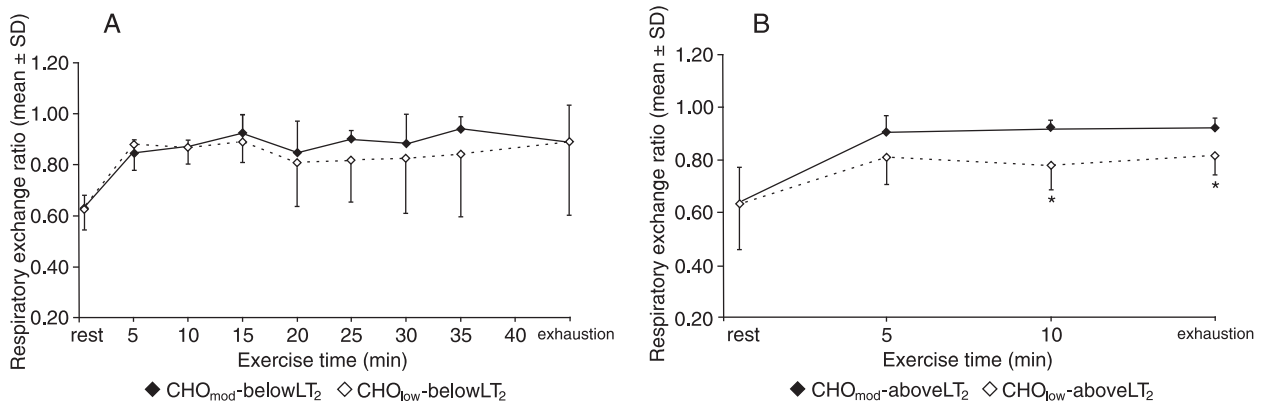
#### Kinetics of variables during exercise above $LT_2$

A significant effect of time on  $VO_2$ , RER, lactate, glucose, and RPE was also observed when exercise was performed above  $LT_2$ . The effect of low carbohydrate availability was more evident for this exercise condition.  $VO_2$  increased exponentially with time until min 10 ( $P < 0.05$ )

**Table 2.** Changes in blood lactate and glucose concentration as a function of time during exercise performed below the second lactate breakpoint by subjects with normal or glycogen depletion.

	Rest	5 min	15 min	30 min	Exhaustion
Lactate (mM)					
$CHO_{mod}$ -below $LT_2$	$1.2 \pm 0.1$ (1.1-1.4)	$2.6 \pm 0.6^*$ (1.8-3.5)	$3.6 \pm 0.8^{**}$ (2.6-4.6)	$3.6 \pm 1.1^*$ (2.5-5.0)	$3.3 \pm 1.0^*$ (2.2-4.7)
$CHO_{low}$ -below $LT_2$	$1.2 \pm 0.3$ (0.9-1.7)	$2.1 \pm 0.5^*$ (1.6-2.8)	$3.3 \pm 0.7^{**}$ (2.7-4.2)	$3.8 \pm 0.8^*$ (3.0-5.0)	$3.7 \pm 1.1^*$ (2.5-5.1)
Glucose (mM)					
$CHO_{mod}$ -below $LT_2$	$4.9 \pm 0.4$ (4.5-5.4)	$4.8 \pm 0.4$ (4.3-5.4)	$4.2 \pm 0.7^*$ (2.9-4.8)	$4.3 \pm 0.4^*$ (3.8-4.7)	$4.3 \pm 0.6^*$ (3.6-5.1)
$CHO_{low}$ -below $LT_2$	$4.7 \pm 0.4$ (4.2-5.1)	$4.4 \pm 0.3^{\#}$ (4.0-4.7)	$4.3 \pm 0.3^*$ (3.9-4.7)	$4.1 \pm 0.4^*$ (3.7-4.6)	$4.3 \pm 0.5^*$ (3.7-4.7)

Data are reported as means  $\pm$  SD for 5 men. Ranges are reported in parentheses.  $CHO_{mod}$  = moderate carbohydrate diet;  $CHO_{low}$  = low carbohydrate diet;  $LT_2$  = second lactate breakpoint. \* $P < 0.05$  compared to values at rest; \*\* $P < 0.05$  compared to values for the preceding time; # $P < 0.05$  compared to values for  $CHO_{mod}$ -below $LT_2$  (Wilcoxon rank test).



**Figure 1.** Respiratory exchange ratio (mean  $\pm$  SD) during exercise below (A) and above (B) the second lactate break point ( $LT_2$ ). RER = respiratory exchange ratio;  $CHO_{mod}$  = moderate carbohydrate diet;  $CHO_{low}$  = low carbohydrate diet. \* $P < 0.05$  compared to moderate carbohydrate diet condition (Wilcoxon rank test).

**Table 3.** Changes in blood lactate and glucose concentration as a function of time during exercise performed above the second lactate breakpoint by subjects with normal or depleted glycogen.

	Rest	5 min	10 min	Exhaustion
Lactate (mM)				
CHO <sub>mod</sub> -aboveLT <sub>2</sub>	1.3 ± 0.2 (1.0-1.5)	3.2 ± 0.6* (2.8-3.6)	5.8 ± 1.0** (5.1-6.5)	10.3 ± 2.3** (7.8-13.5)
CHO <sub>low</sub> -aboveLT <sub>2</sub>	1.2 ± 0.2 (1.0-1.4)	3.5 ± 0.5* (3.0-4.3)	6.6 ± 1.8** (4.4-9.3)	8.3 ± 3.1** (5.1-13.4)
Glucose (mM)				
CHO <sub>mod</sub> -aboveLT <sub>2</sub>	5.9 ± 2.1 (4.7-9.6)	5.0 ± 0.4 (4.7-5.3)	4.8 ± 0.7 (4.3-5.3)	5.5 ± 0.8 (4.8-6.7)
CHO <sub>low</sub> -aboveLT <sub>2</sub>	4.4 ± 0.6 (3.4-5.2)	4.0 ± 1.0 (2.7-5.0)	4.4 ± 0.7 (3.3-5.1)	5.3 ± 1.7 (4.1-8.2)

Data are reported as means ± SD for 5 men. Ranges are reported in parentheses. CHO<sub>mod</sub> = moderate carbohydrate diet; CHO<sub>low</sub> = low carbohydrate diet; LT<sub>2</sub> = second lactate breakpoint. \*P < 0.05 compared to values at rest; \*\*P < 0.05 compared to values for the preceding time (Wilcoxon rank test).

and thereafter the values did not differ from the values at exhaustion under either condition ( $P > 0.05$ ). RER increased in the first 5 min and remained elevated until exhaustion (Figure 1B). An effect of low carbohydrate availability was observed whereby RER during CHO<sub>mod</sub>-aboveLT<sub>2</sub> at 10 min was higher than during the CHO<sub>low</sub>-aboveLT<sub>2</sub>, and these differences persisted until exhaustion ( $P < 0.05$ ). Accordingly, the rate of carbohydrate oxidation was higher and fat oxidation was lower at 10 min for the CHO<sub>mod</sub>-aboveLT<sub>2</sub> compared to CHO<sub>low</sub>-aboveLT<sub>2</sub> (carbohydrate:  $2.4 \pm 0.5$  vs  $0.8 \pm 1.1$  and fat:  $0.3 \pm 0.1$  vs  $0.9 \pm 0.5$  g/min;  $P < 0.05$ ).

Blood lactate concentration increased progressively at the end of exercise for both conditions, but at exhaustion it tended to be higher for the CHO<sub>mod</sub>-aboveLT<sub>2</sub> trial than for the CHO<sub>low</sub>-aboveLT<sub>2</sub> trial ( $10.3 \pm 2.3$  vs  $8.2 \pm 3.1$  mM;  $P = 0.08$ ). In contrast, blood glucose concentration remained stable during exercise and did not change in relation to that at rest. No effect of the conditions was observed on blood glucose concentration in the exercise performed above LT<sub>2</sub> (Table 3).

Similar to exercise below LT<sub>2</sub>, RPE increased progressively until the end of exercise ( $P < 0.05$ ), but no effect of low carbohydrate availability was observed. However, when the slope of the functions was compared for different conditions, a significant difference was found (CHO<sub>mod</sub>-aboveLT<sub>2</sub>:  $0.33 \pm 0.19$ , CHO<sub>low</sub>-aboveLT<sub>2</sub>:  $0.42 \pm 0.23$  RPE units/min;  $P < 0.05$ ). The differences were not significant when the values were expressed relative to the end of exercise (CHO<sub>mod</sub>-aboveLT<sub>2</sub>:  $0.06 \pm 0.02$ , CHO<sub>low</sub>-aboveLT<sub>2</sub>:  $0.07 \pm 0.02$  RPE units/%).

## Discussion

The primary purpose of this study was to determine the effect of low pre-exercise carbohydrate availability on the

time to exhaustion for moderate and heavy exercise. It was hypothesized that when compared with a normal situation, reduction of carbohydrate availability prior to exercise should result in a shorter time to exhaustion during heavy exercise. This was to be expected because when the exercise is performed with a reduced pre-exercise carbohydrate store there is a lower carbohydrate oxidation during exercise and the counter-regulatory increase in fat oxidation may not provide a satisfactory substitute for the supply of energy necessary for contraction. In fact, we observed a greater use of fat and a lower use of carbohydrate as fuel during heavy exercise. Pre-exercise carbohydrate availability had no significant effect on the performance or rate of substrate oxidation in the case of moderate exercise.

The validation of these findings is dependent on the successful manipulation of the carbohydrate store. Although we did not directly measure the pre-exercise carbohydrate store, several observations made during the experimental exercise strongly suggested that the carbohydrate stores of the subjects were significantly reduced under the CHO<sub>low</sub> condition: 1) the RER values during the CHO<sub>low</sub>-aboveLT<sub>2</sub> trial in this study were significantly lower than those for the CHO<sub>mod</sub>-aboveLT<sub>2</sub> trial, indicating a greater utilization of fat as fuel; 2) blood lactate tended to be lower during the CHO<sub>low</sub>-aboveLT<sub>2</sub> trial than during the CHO<sub>mod</sub>-aboveLT<sub>2</sub> trial, and 3) all subjects confirmed that they followed the recommended diets as demonstrated by their own records. These results are in accordance with other studies that utilized a similar protocol intended to reduce the carbohydrate store (10,14-16).

However, the present study is not free of the carryover effect due to the number of repeated measurements taken for the same subject. In fact, a learning effect could occur since we used a randomized rather than counterbalanced design. In the study, 3 subjects tested first the conditions of

low carbohydrate availability and moderate exercise and the other two tested first moderate carbohydrate availability. In the case of heavy exercise, 3 subjects tested low carbohydrate availability first while the other two tested moderate carbohydrate availability. For heavy exercise, all 5 subjects significantly reduced their time to exhaustion with a previous low carbohydrate availability, suggesting that a potential carryover effect probably did not occur. In addition, in the present study, a reduced sample size was used because 2 subjects were ill during the experiments. We calculated the power effect on time to exhaustion obtained for heavy exercise and found a relatively low value. However, the number of subjects required to produce a power effect of 0.80 was estimated to be 28. This number could be considered to be unrealistic because investigations of this nature involve invasive procedures, many measurements and many days of tests. This may explain the fact that a series of other studies have been conducted with similarly reduced sample sizes (10,16,23). Nevertheless, the lower sample size decreases the statistical power, even with the non-parametric statistics applied in the present study, indicating that future studies should be carried out with a larger sample size to better clarify this question.

Sabapathy et al. (10) assessed the ventilatory and lactate response during an incremental exercise test with and without a prior muscle glycogen protocol. The authors found that maximal power output attained in the test and maximal blood lactate accumulation were significantly reduced after muscle glycogen depletion compared to the normal muscle glycogen condition. These results demonstrate that carbohydrate metabolism could be significantly affected by muscle glycogen depletion. However, Sabapathy et al. (10) did not investigate the effect of carbohydrate availability on time to exhaustion and, principally, the muscle glycogen depletion was achieved from prolonged exercise the night prior to the experimental tests followed by an 8- to 12-h fast until the next morning. In the present study, we investigated the time to exhaustion after the muscle glycogen depletion protocol followed by 2 days on a low carbohydrate diet to split the effect of the residual fatigue caused by the protocol and that caused by low carbohydrate availability (17). Therefore, our results demonstrated that low carbohydrate availability *per se* could affect performance during heavy exercise.

The data of the present study suggest that time to exhaustion can be decreased by a previous low carbohydrate availability only during high intensity exercise. These findings are consistent with those reported by Havemann et al. (24) who demonstrated a decrease in power output only during high intensity exercise with 7 days of high fat

feeding followed by 1 day of carbohydrate load. In the study by Havemann et al. (24), the power output during a 100-km time trial as well as four 4-km sprint distances (~78-84% of maximal power output) performed after 20, 40, 60, and 80 km covered was not compromised after a short-term high fat diet. However, the power output during 1-km sprint distances (>90% of maximal power output) performed after 10, 32, 52, and 72 km was significantly reduced with a high fat dietary strategy. This suggests that after muscle glycogen depletion followed by a low carbohydrate diet, or after a high fat diet period as in the study by Havemann et al. (24), fat oxidation could satisfactorily replace muscle glycogen oxidation during low and moderate intensity exercise but not high intensity exercise. This is compatible with the present results where a higher fat oxidation was found during exercise performed above  $LT_2$  as demonstrated by a lower RER (Figure 1B) but carbohydrate oxidation was not sufficiently substituted to maintain the performance of exercise. These results indicate that fatigue would most likely be the result of a loss of the ability to oxidize the available carbohydrate store at a rate sufficient to fuel the high intensity exercise.

It is well established that muscle glycogen utilization increases with the relative intensity of exercise (3). The exercise above the  $LT_2$  was performed at an intensity during which muscle glycogen is the predominant fuel source (3). Other studies that investigated the effects of muscle glycogen content on performance during short-term high-intensity exercise have obtained conflicting results. Vandenberghe et al. (25) demonstrated that a super-compensation strategy had no effect on time to exhaustion for exercise performed at 125%  $VO_{2max}$  (~175 s). In contrast, Casey et al. (26) reported that during the first three of four bouts of 30-s maximal isokinetic cycling exercise, initial low muscle glycogen levels caused a significant reduction in the total work produced when compared to control conditions. A possible reason for these discrepancies includes differences in the strategy for manipulating carbohydrate availability, intensity and type of exercise, and heterogeneity of the groups. In fact, in the present study, the standard deviation of the time to exhaustion was very high, suggesting that the group was heterogeneous in terms of performance. However, Pitsiladis and Maughan (27), in a study on 6 well-trained cyclists, also found a high variability in time to exhaustion after exercise with low and high carbohydrate availability performed at 10 or 30°C. Recently, Claassen et al. (28) showed in a homogeneous group tested for training status that the maintenance of euglycemia in the carbohydrate-depleted state with glucose infusion had an ergogenic effect, but the effect was highly variable between individuals. These results suggest

that carbohydrate manipulation could affect the performance in a specific manner and could be linked to the existence of a metabolic phenotype.

In the exercise below  $LT_2$ , there was no effect of carbohydrate availability on the rate of carbohydrate and fat oxidized. In addition, time to exhaustion was also similar for different conditions. Several lines of evidence have suggested that pre-exercise muscle glycogen depletion causes a decrease in carbohydrate oxidation and a counter-regulatory increase in fat oxidation during prolonged exercise (5,27). We are unable to explain why the total substrate oxidation during exercise below  $LT_2$  was not altered, although the mean values of carbohydrate oxidation tended to be lower after a low carbohydrate diet. Because the time to exhaustion was not different between conditions, we speculate that the exercise performed below  $LT_2$  may have been interrupted before a total depletion of fuel occurred. In fact, in the present study, the subjects cycled for about one hour and this may have been insufficient for oxidation of the entire carbohydrate store. Denadai and Denadai (29) found the time to exhaustion at an intensity 10% below the  $LT_2$  to be around 32 min without caffeine and 46 min with caffeine ingestion. As caffeine could have multiple effects on performance, it cannot be ruled out that mechanisms other than carbohydrate availability may also be involved in the fatigue process during prolonged exercise (27,30).

It was found in the present study that the rate at which RPE rose over time was significantly higher during  $CHO_{mod}$ -above $LT_2$  compared to  $CHO_{low}$ -above $LT_2$ , which is indicative of the subjects' anticipation of the rise in perception of effort during high intensity exercise with low pre-exercise carbohydrate availability. When the data were normalized by plotting the RPE against relative time, these differences were not observed. These results are in agreement with the findings of Noakes (31) who plotted the data for the RPE reported by Baldwin et al. (23) against the absolute and relative duration of exercise for both conditions. The author observed that the slope of the RPE-time relationship, but not the RPE-relative time relationship, is increased in the case of glycogen depletion and could serve

as a marker of the time left to exhaustion. These results corroborate the idea of a complex and system integrative model regulating fatigue during high intensity exercise. Thus, changes in peripheral physiological systems, such as substrate depletion, could act as afferent signals to the subconscious brain that "calculates" the duration of exercise that can be carried out safely, and interrupts the exercise prior to the total depletion of energy (32). Accordingly, the subjects of the present study were not informed about the duration of exercise and we found that the slope between the RPE and relative time did not differ across all intensities and conditions, which supports the hypothesis that the subjects unconsciously knew the time left to exhaustion (comparison not shown). The time to exhaustion and the rate at which the RPE rose with time did not differ between conditions for exercise below the  $LT_2$ , suggesting that other mechanisms besides muscle glycogen or alterations caused by diets could act as afferent signals and cause the interruption of exercise.

In summary, low pre-exercise carbohydrate availability results in impairment of performance of the exercise carried out above, but not below,  $LT_2$ . The mechanisms underlying this effect are unclear, but may be related to an inability of fat oxidation to act as a substitute for muscle glycogen oxidation at high exercise intensities without a decrease in time to exhaustion. In addition, our data showed that exercise performed above  $LT_2$  under low carbohydrate availability caused by exercise and diet manipulation may result in greater fat oxidation, lower blood lactate concentration and a greater rate of increase in effort perception over time.

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