Metabolic demands of intense aerobic interval training in competitive cyclists

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ABSTRACT

STEPTO, N. K., D. T. MARTIN, K. E. FALLON, and J. A. HAWLEY. Metabolic demands of intense aerobic interval training in competitive cyclists. *Med. Sci. Sports Exerc.*, Vol. 33, No. 2, 2001, pp. 303–310. **Purpose:** To investigate the metabolic demands of a single session of intense aerobic interval training in highly trained competitive endurance cyclists. **Methods**: Seven cyclists (peak O$_2$ uptake [$\dot{V}O_{2peak}$] 5.14 ± 0.23 L.min^{-1}, mean ±SD) performed 8 × 5 min work bouts at 86 ± 2% of $\dot{V}O_{2peak}$ with 60-s recovery. Muscle biopsies were taken from the vastus lateralis immediately before and after the training session, whereas pulmonary gas exchange and venous blood were sampled at regular intervals throughout exercise. **Results**: Muscle glycogen concentration decreased from 501 ± 91 to 243 ± 51 mmol·kg^{-1} dry mass ($P < 0.01$). High rates of total carbohydrate oxidation were maintained throughout exercise (340 μmol·kg^{-1}·min^{-1}), whereas fat oxidation increased from 16 ± 8 during the first to 25 ± 13 μmol·kg^{-1}·min^{-1} during the seventh work bout ($P < 0.05$). Blood lactate concentration remained between 5 and 6 mM throughout exercise, whereas muscle lactate increased from 6 ± 1 at rest to 32 ± 12 mmol·kg^{-1} d.m. immediately after the training session ($P < 0.01$). Although muscle pH decreased from 7.09 ± 0.06 at rest to 7.01 ± 0.03 at the end of the session ($P < 0.01$), blood pH was similar after the first and seventh work bouts (7.34). Arterial oxygen saturation (%S$_P$O$_2$) fell to 95.6 ± 1% during the first work bout and remained at 94% throughout exercise: the 60-s rest intervals were adequate to restore %S$_P$O$_2$ to 97%. **Conclusion**: Highly trained cyclists are able to sustain high steady state aerobic power outputs that are associated with high rates of glycogenolysis and total energy expenditure similar to those experienced during a 60-min competitive ride. **Key Words:** ACID BASE, CARBOHYDRATE, GLYCOGENOLYSIS, LACTATE

The physiological and performance characteristics of competitive cyclists have been well documented (for reviews see 3,11). However, the scientific literature pertaining to the unique effects of specific training procedures on the performances of previously trained individuals is sparse: sport scientists have found it difficult to persuade elite athletes to experiment with their training regimes (11).

One form of training that has been used by endurance athletes for several decades and is well recognized by sports scientists is interval training. Such training is normally undertaken after an athlete has completed a large volume of endurance work and typically consists of a number of sustained aerobic exercise bouts lasting 3–15 min alternated with shorter rest intervals (60–90 s) of activity at a slower pace. The objective of such training is to expose the physiological power systems to sustained exercise at an intensity (or effort) which corresponds to the athlete's current highest "steady-state pace" (11), the so-called individual "lactate threshold" (17). The benefits ascribed to aerobic interval training include enhanced lactate kinetics, stimulation of specific neurological patterns of muscle fiber recruitment needed at race pace, improved fatigue resistance, and enhanced athletic performance (11,18,32,33).

Although interval training has been a basic element in athletic conditioning since the turn of the century, there have few studies of the effects of interval training on the performances of highly trained competitive athletes (1,11,18,32,33,34). Indeed, it could be argued that sport scientists are currently making recommendations about the role of various training regimens in the preparation of athletes without the precise knowledge of the metabolic demands associated with such interventions. Owing to the scarcity of literature in this area, we have recently undertaken a series of laboratory based investigations using highly trained cyclists to examine time course changes in athletic performance in response to different programs of high-intensity interval training (11,18,32), and the chronic metabolic (33) and muscular (34) adaptations that occur in response to such training. In the present study, we extend this information by determining the metabolic demands of a single session of intense aerobic interval training in competitive cyclists.

METHODS

Subjects. Seven highly trained cyclists (Table 1) who were riding 368 ± 141 km·wk^{-1} (mean ±SD) in Canberra at an altitude of 588 m, and who had not undertaken any high-intensity interval training in the 4 wk before the investigation, were recruited to participate in this study. Subjects, who had all participated in previous studies and were familiar with testing procedures, were fully informed of the

TABLE 1. Subject characteristics.

	Mean	±SD
Body mass (kg)	76.3	3.1
Age (yr)	26.9	5.4
Sum of 7 skinfolds (mm)	51.9	15.1
Body fat (%)[a]	9.0	2.9
Lean thigh volume (L)	7.9	1.0
$\dot{V}O_{2\ peak}$ (L·min^{-1})	5.14	0.21
Cycling economy (W·L^{-1})	79	2
Power: mass ratio (W·kg^{-1})	5.31	0.31
PPO (W)	405	22
HR$_{peak}$ (beats·min^{-1})	187	6

$\dot{V}O_{2\ peak}$, peak oxygen uptake; HR$_{peak}$, peak heart rate; PPO, peak sustained power output.
[a] Predicted from equations of Withers et al. (35).
All values are mean ± SD for $N = 7$.

potential risks involved before providing their written consent. This study was approved by the Human Research Ethics Committee of RMIT University and the Ethics Committee of the Australian Institute of Sport. There was no "control" or comparative group for the purpose of this investigation: pilot studies revealed that untrained subjects were unable to ride for more than 90 s at the same absolute power output sustained by our highly trained cyclists for 40 min and that, at the same relative exercise intensity, they could not attain the steady state conditions necessary for valid measurements of substrate oxidation (28). More to the point, we have previously reported the effects of chronic (6 sessions) of high-intensity interval training on changes in substrate oxidation (33).

Preliminary testing. On their first visit to the laboratory, each subject was weighed and underwent anthropometric measures for the sum of seven skinfolds (biceps, tricep, subscapular, abdominal, supraspinale, mid-thigh, and medial gastrocnemius) and lean thigh volume (16). Then, after a self-paced warm-up (30 min) they performed a maximal, incremental cycle test to exhaustion on an electromagnetically braked ergometer (Lode, Gronigen, The Netherlands). The test protocol has been described in detail previously (12). A first principles calibration rig was used to evaluate the accuracy and reliability of the ergometer. Expected power output (W) was within ± 2% of actual output from 200 to 800 W when the pedal frequency was between 90 and 140 rev·min^{-1}. Throughout the maximal test and the subsequently described experimental trial, subjects inspired air through a two-way Hans Rudolph valve attached to a custom built automated Douglas bag gas analysis system (Australian Institute of Sport, ACT, Australia) that incorporated O_2 and CO_2 analyzers (Ametek N-22 electrochemical O_2 sensor, model S3A, and Ametek P-61B infrared CO_2 sensor, Applied Electrochemistry, Ametek Instruments, Pittsburgh, PA) and two Tissot gasometers (Warren E. Collins Inc., Braintree, MA) interfaced to an IBM personal computer by Optical Rotary Encoders (RS 341–597, Berne, Switzerland) that calculated the rate of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), minute ventilation (V_{ESTPD}), and the respiratory exchange ratio (RER) every 30 s from conventional equations. Before each maximal test and all subsequent experimental trials, the analyzers were calibrated with commercially available α gases of known O_2

and CO_2 content. Before and after the study an automated high-capacity calibrator for open-circuit indirect calorimetry was used to simultaneously check the gas analyzers, volume device and software of the custom built system (9). This device can calibrate to the high ventilation volumes (100 L·min^{-1}) measured when well-trained athletes work for sustained periods at high (80–90% of maximal oxygen uptake ($\dot{V}O_{2max}$) exercise intensities.

$\dot{V}O_{2peak}$ was defined as the highest O_2 uptake a subject attained during two consecutive 30-s sampling periods. Peak sustained power output (PPO) was calculated from the last completed work rate, plus the fraction of time spent in the final noncompleted work rate multiplied by 25 W (12). The results of the maximal test were used to determine the power output that corresponded to 85% of each subjects $\dot{V}O_{2peak}$ to be used in the subsequently described high-intensity aerobic interval-training session.

Training and dietary control. Training and nutritional status of the subjects was controlled 24 h before an experimental trial to standardize muscle and liver glycogen stores. All subjects reported to the laboratory between 0700 and 0800 h the day before an experiment and completed a 60-min ride at 50% of PPO (60% $\dot{V}O_{2peak}$). They were then provided with a standard diet of 50 kcal·kg^{-1} body mass (BM), composed of 63% carbohydrate (8 g·kg^{-1} BM), 20% fat, and 17% protein, to be consumed over the next 24 h. During this time, subjects refrained from any further training activity.

Experimental trial. On the day of an experiment, subjects reported to the laboratory between 0700 and 0800 h after an overnight fast. They were weighed and then rested quietly in a seated position for 10–15 min. Six of these elite subjects agreed to undergo a maximum of two muscle biopsies. Accordingly, we chose to sample muscle immediately before and after exercise to assess the metabolic demands of the entire interval training session (described subsequently) rather than the response to a single work bout. After local anesthesia had been administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis, a resting biopsy was taken using a 6-mm Bergström needle with suction applied. Approximately 150 mg of muscle was removed, and quickly frozen (18 ± 6 s) in liquid nitrogen. Samples were stored at −80°C until subsequent analysis. Immediately after this biopsy, muscle temperature was measured using a needle thermistor (YSI 525, Yellow Springs Instruments, Yellow Springs, OH) inserted to a depth of 4 cm through the same incision (7). At this time, a separate site on the same leg (5 cm distal) was prepared for the second biopsy to be taken immediately after exercise. A Teflon cannula was then inserted in the forearm antecubital vein for rapid continuous blood sampling via a sterile stopcock. The cannula was regularly flushed with 0.9% sterile saline to keep the vein patent.

The cyclist then mounted the ergometer and commenced a standardized warm-up, which consisted of 2 min at 25%, 50%, and 60% of PPO. After a 2-min rest during which the subjects remained on the ergometer, they began the interval training session which consisted of 8 × 5 min work bouts at

82.5% of PPO (86 ± 2% $\dot{V}O_{2\,peak}$: 334 ± 18 W) with 60-s active recovery (a work: rest ratio of 5:1). During the 60-s recovery between work bouts, subjects pedalled at a workload of <100 W. We have previously reported that well-trained cyclists who undertake six sessions of this interval training protocol significantly improve their 40-km cycle time trial performance (11,18,32,33). Laboratory conditions were maintained at 22°C and 46% relative humidity. Subjects were cooled with a fan (wind speed of 7 m·s^{-1}) and provided with water *ad libitum* throughout exercise.

During the first, third, fifth, and seventh work bouts and for the subsequent 60-s recovery, subjects breathed through a mouthpiece attached to the previously described automated gas analyzer system. In addition, pulmonary gas exchange data were collected on three subjects throughout the last work bout. Blood samples (10 mL) were taken at rest, immediately after the warm-up, and at the end of the first, third, fourth, fifth, seventh, and final work bout.

Whole body rates of carbohydrate (CHO) and fat oxidation (g·min^{-1}) were calculated throughout bouts 1, 3, 5, and 7 from $\dot{V}CO_2$ and $\dot{V}O_2$ by using nonprotein RER values, according to the following equations (24):

$$CHO\ Oxidation = 4.585\ VCO_2 - 3.226\ VO_2$$

$$Fat\ Oxidation = 1.695\ VO_2 - 1.701\ VCO_2$$

These equations are based on the assumption that $\dot{V}O2$ and $\dot{V}CO_2$ accurately reflect tissue O_2 consumption and CO_2 production. In well-trained subjects similar to those employed in the current investigation, indirect calorimetry has previously been shown to be a valid method for quantifying rates of substrate oxidation during strenuous exercise at 85% of $\dot{V}O_{2\,max}$ (28). The g·min^{-1} rates of substrate oxidation were converted to μmol·kg^{-1}·min^{-1} rates so that they could be compared with the results from previous studies. Rates of fatty acid (FA) oxidation (μmol·kg^{-1}·min^{-1}) were determined by converting the g·min^{-1} rate of triglyceride oxidation to its molar equivalent assuming the average molecular weight of human triglyceride to be 855.26 g·mole^{-1} and multiplying by the molar rate of triglyceride oxidation by three, because each molecule contains 3 mol of FA. Rates of CHO oxidation (μmol·kg^{-1}·min^{-1}) were determined by converting the g·min^{-1} rate of carbohydrate oxidation to its molar equivalent assuming 6 mol of O_2 are consumed and 6 mol of CO_2 produce for each mole (180 g) oxidized.

During all exercise sessions, the subject's heart rate (HR) was continuously monitored via telemetry (Accurex Plus; Polar Electro OY, Kempele, Finland). Arterial oxygen hemoglobin (Hb) saturation (%S_pO_2) was monitored throughout bouts 1, 3, 5, and 7 and the subsequent recovery via a fingertip pulse oximeter (model U.S.-504, Criticare, Waukesha, WI). This technique has previously been shown to provide valid measurements of %S_pO_2 during intense exercise (21). Immediately upon completion of the training session, a second biopsy was taken while the subjects remained seated on the ergometer and rapidly (18 ± 8 s) frozen in liquid nitrogen. Then, postexercise muscle temperature was measured as described above.

Analyses. Blood samples (5 mL) were immediately analyzed for blood glucose and blood lactate concentrations, K$^+$, hemoglobin, hematocrit, and pH on an ABL 700 series blood gas analyzer (Radiometer Medical A/S, Denmark). Changes in plasma volume were estimated from the equations of Dill and Costill (5). Frozen muscle samples were weighed, freeze dried, reweighed, and dissected free of blood and connective tissue. These samples were then divided into two separate aliquots. One aliquot (1 mg dry mass [d.m.]) was extracted in 3 M perchloric acid (PCA) frozen over liquid nitrogen, allowed to thaw to -8°C for 20 min, and finally brought to a temperature of 5°C for 5 min. This extract was then analyzed for muscle lactate using standard enzymatic, fluorometric techniques (19). Muscle glycogen concentration was determined in duplicate from the second aliquot according to the procedures of Passonneau and Lauderdale (23).

Muscle pH was determined from the remaining frozen muscle (5 mg), using the modified technique of Sahlin (31). The muscle sample was homogenized in an ice cold homogenizing solution (145 mM KCl, 10 mM NaCl, and 5 mM sodium iodoacetate; pH 7) and then analyzed using a CIBA Corning 278 blood gas system (CIBA Corning Diagnostic Corps., Medfield, MA).

Statistical analyses. All values are reported as mean ± SD. Analysis of pre- and post-exercise muscle glycogen concentration, muscle lactate concentration, muscle temperature, and muscle pH were analyzed using a paired Student's *t*-test. The remaining data (HR, rates of substrate oxidation, blood lactate concentration and blood glucose concentrations, HCO$_3$, and K$^+$) were analyzed using a one-way ANOVA with repeated measures. Where a significant *F*-ratio was found, the data were subjected to Tukey's *post hoc* tests. Statistical significance was accepted at $P \le 0.05$.

RESULTS

Pulmonary gas exchange, heart rate, and rates of substrate oxidation. Table 2 shows pulmonary gas exchange measurements and the estimated rates of whole body substrate oxidation measured throughout the first, third, fifth, and seventh work bouts of the interval training session, whereas Figure 1 displays the $\dot{V}O_2$ and HR responses.

Despite similar $\dot{V}O_2$ and $\dot{V}CO_2$ values throughout the training session, V_E increased from 98 ± 7 L·min^{-1} during the first work bout to 106 ± 6 L·min^{-1} after the third bout ($P < 0.05$). By the fifth work bout, V_E had increased to 112 ± 9 L·min^{-1} ($P < 0.001$ compared with bout 1) but did not rise any further during the seventh bout. The time taken to reach a steady-state $\dot{V}O_2$ of 4.45 ± 0.25 L·min^{-1} (the average of the last 3 min of each work bout) took significantly longer during the first compared with the third, fifth, and seventh work bouts (185 ± 30 vs 145 ± 35, 130 ± 25 and 130 ± 36 s, respectively; $P < 0.05$, Fig. 1A). Figure 1B displays the HR response to the high-intensity training session. HR throughout the first work bout (156 ± 8 beats·min^{-1}) was significantly lower than bouts two through

TABLE 2. Pulmonary gas exchange measurements and rates of whole body substrate oxidation determined during the high-intensity interval training session.

| | Work Bout | | | |
	1	3	5	7
$\dot{V}O_2$ (L·min^{-1})	4.1 ± 0.2	4.3 ± 0.2	4.4 ± 0.3	4.4 ± 0.3
$\dot{V}CO_2$ (L·min^{-1})	4.0 ± 0.3	4.1 ± 0.2	4.1 ± 0.3	4.1 ± 0.3
V_{ESTPD} (L·min^{-1})	98 ± 7	106 ± 6*	112 ± 9†	111 ± 10†
RER	0.97 ± 0.04	0.93 ± 0.02	0.93 ± 0.03*	0.92 ± 0.04*
CHO oxidation (μmol·kg^{-1}·min^{-1})	346 ± 62	338 ± 42	332 ± 45	328 ± 61
Fat oxidation (μmol·kg^{-1}·min^{-1})	16 ± 8	21 ± 8	24 ± 10*	25 ± 13*

$\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; V_E, ventilation; RER, respiratory exchange ratio; CHO, carbohydrate.
Values are mean ± SD ($N = 7$).
* Significantly different from bout 1 ($P < 0.05$); † Significantly different from bout 1 ($P < 0.001$).

eight (162 ± 7 to 167 ± 7 beats·min^{-1}; $P < 0.05$). As would be expected, there was a gradual upward drift of exercise HR throughout the interval training session. During each 1-min recovery, HR fell by 35 beats·min^{-1} ($P < 0.01$). The recovery HR for bout one (125 ± 8 beats·min^{-1}) was significantly lower than after all other work bouts (Fig. 1B; $P < 0.05$).

RER values declined progressively throughout the interval training session from 0.97 ± 0.04 during the first bout to 0.92 ± 0.04 by the seventh bout. Accordingly, the average rate of CHO oxidation declined slightly, but not significantly, from the first to the seventh work bout (from 346 ± 62 μmol·kg^{-1}·min^{-1} to 312 ± 58 μmol·kg^{-1}·min^{-1}), whereas the rate of fat oxidation increased (from 16 ± 8 μmol·kg^{-1}·min^{-1} to 25 ± 13 μmol·kg^{-1}·min^{-1}, $P < 0.05$). Pulmonary gas exchange data were collected on three subjects during the eighth work bout. These data revealed there were no differences for $\dot{V}O_2$, $\dot{V}CO_2$, V_E, or RER compared with the seventh work bout. The rate of total energy expenditure was 1252 ± 96 J·kg^{-1}m·min^{-1}.

Blood parameters. Figure 2 displays the venous blood measures at rest, immediately after the warm-up, and during the first, third, fifth, and seventh work bouts. Blood lactate concentration (panel A) increased from 1.0 ± 0.3 mmol·L^{-1} at rest to 4.7 ± 1.7 mmol·L^{-1} ($P < 0.05$) after the first bout. Thereafter, it remained constant between 5–6 mmol·L^{-1} for the remainder of the training session. In contrast, blood HCO$_3$ concentration (panel B) declined from 26.1 ± 2 mmol·L^{-1} at rest to 22.2 ± 1.1 mmol·L^{-1} ($P < 0.05$) after the first work bout. Thereafter, HCO$_3$ concentrations remained at 21 mmol·L^{-1}. Blood pH (panel C) was 7.35 ± 0.03 at rest, declined to 7.32 ± 0.02 after the first work bout ($P > 0.05$), after which is rose slightly so that by the fifth work bout it had recovered to close to resting values. Blood K$^+$ concentrations (panel D) was 4.0 ± 2 mmol·L^{-1} at rest and increased to 4.8 ± 0.2 mmol·L^{-1} ($P < 0.05$) by the end of work bout 1. The K$^+$ concentration rose to 5.4 ± 0.4 mmol·L^{-1} ($P < 0.05$) after the third work bout, where it remained until the end of the training session. Blood glucose concentration was 5.02 ± 0.41 mmol·L^{-1} at rest and only rose to 5.6 ± 0.6 mmol·L^{-1} after the seventh work bout. Blood samples taken from three subjects revealed no difference for any blood variable between the seventh and eighth work bout. Plasma volume declined by 9.8 ± 2.8% from rest immediately after the warm-up and only fell a further 2% (to 12.1 ± 3.4%) by the end of exercise.

Muscle parameters. Figure 3 displays the data from the pre- and post-exercise muscle samples. Resting muscle glycogen concentration (panel A) was 501 ± 91 mmol·kg^{-1} d.m. and decreased to 243 ± 51 mmol·kg^{-1} d.m. after the interval training session ($P < 0.01$). Resting muscle lactate concentration (panel B) and muscle pH (panel C) were 6.2 ± 0.8 mmol·kg^{-1} d.m and 7.09 ± 0.06, respectively. Muscle lactate concentration increased significantly to 32.7 ± 12.2 mmol·kg^{-1} d.m. ($P < 0.01$), whereas muscle pH de-
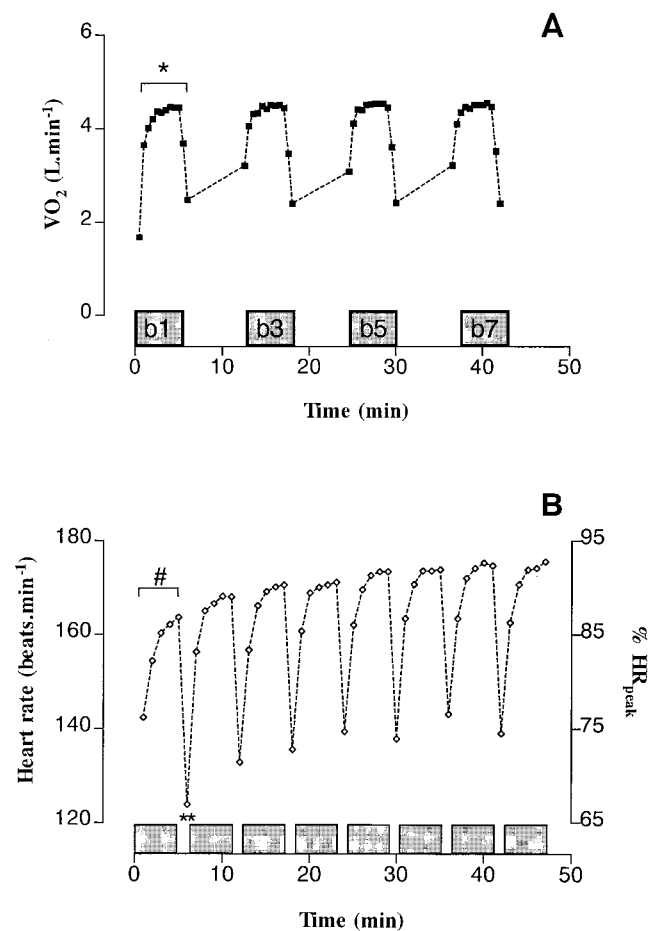

FIGURE 1—The oxygen uptake ($\dot{V}O_2$) responses (A, upper panel) during the first, third, fifth, and seventh work bouts, and the heart rate (HR) responses (B, lower panel) during each work bout (▨) and subsequent 60-s recovery determined during the high-intensity interval training session. Values are mean ($N = 7$); * significantly different to bouts 3, 5, and 7 ($P < 0.05$); ** significantly different to all other recoveries ($P < 0.05$). # Significantly different to all other work bouts ($P < 0.05$).
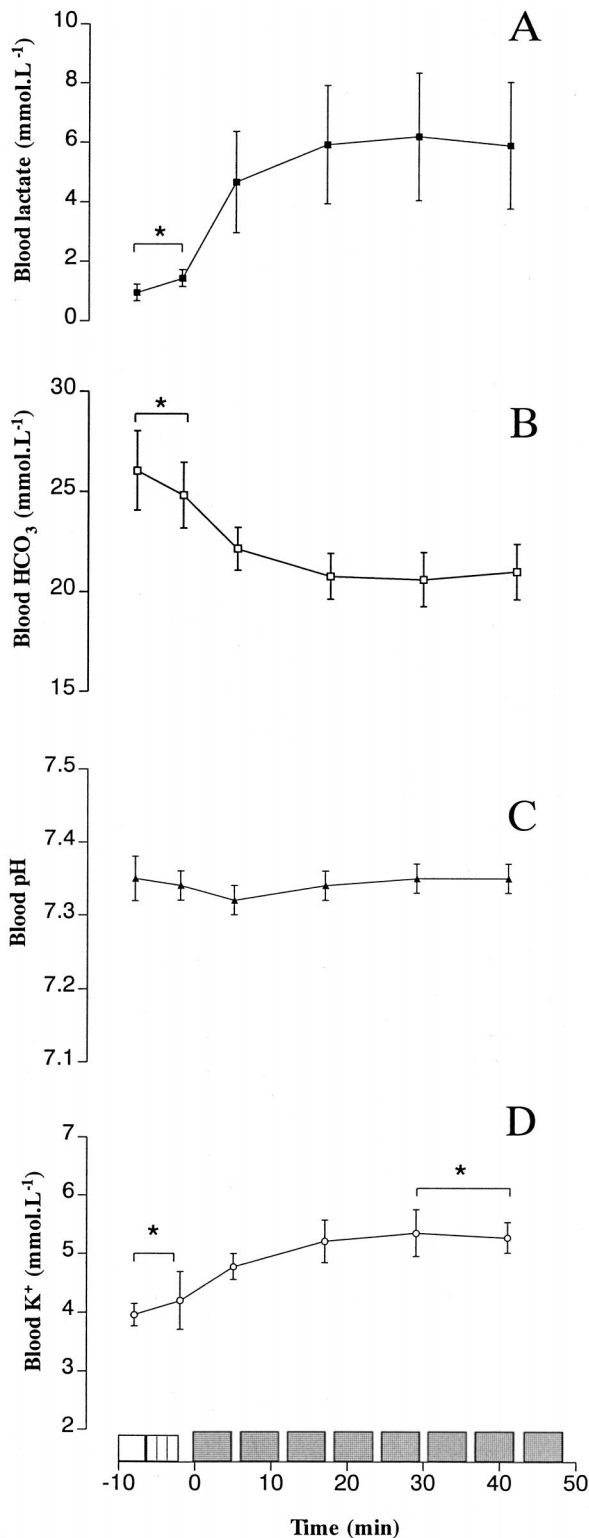
FIGURE 2—Venous blood lactate concentration (A), HCO₃ concentration (B), blood pH (C), and blood K⁺ concentration (D) measured at rest (□), after the warm-up (▦), and during the first, third, fifth, and seventh work bouts of the high-intensity interval training session (▩). Values are mean ± SD ($N = 7$); * significantly different to bout 1 ($P < 0.05$).

clined to 7.01 ± 0.03 ($P < 0.01$) immediately after the final work bout. There was a significant correlation between the average blood lactate concentration during exercise and the post exercise muscle lactate concentration ($r = 0.91$, $P <$

0.05). Muscle temperature (panel D) was $33.6 \pm 2°C$ at rest and had increased to $36.8 \pm 2°C$ ($P < 0.001$) by the end of the interval training session.

% S_PO_2. Arterial oxygen saturation (%SPO₂) was 95.6 $\pm$ 1% during the first work bout and fell to $94 \pm 2\%$ ($P < 0.05$) for the third, fifth, and seventh work bouts. The 60-s rest intervals were adequate to restore %S_PO_2 to 97% throughout the whole high-intensity interval training session.

DISCUSSION

The physiological and metabolic responses of well-trained individuals to prolonged, moderate-intensity, continuous endurance cycling have been well documented (3). There is also substantial information on the changes in skeletal muscle substrate utilization after repeated bouts of short-duration (<30 s) maximal sprinting in both cycling and treadmill running (for review see 22). However, little data exist that document the metabolic perturbations that occur in response to repeated bouts of aerobic exercise that are similar in intensity (i.e., 85% $\dot{V}O_{2\ peak}$) and duration (i.e., 1 h) to those encountered by competitive athletes during training and racing (33). Here, for the first time, we report the metabolic demands associated with the high (340 W) absolute work rates that competitive endurance cyclists can sustain during a single bout of interval training. Our
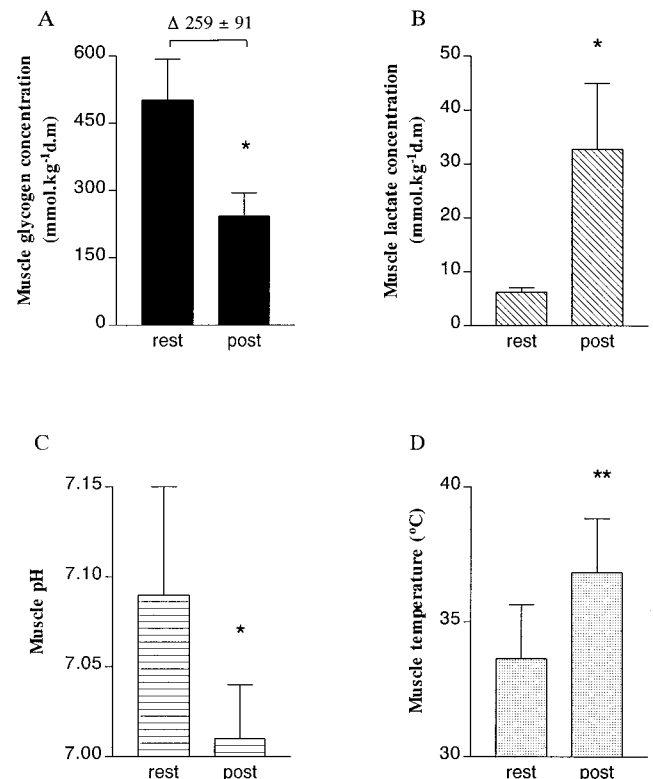
FIGURE 3—Muscle glycogen concentration (A), muscle lactate concentration (B), muscle pH (C), and muscle temperature (D), before and after the high-intensity interval training session. Values are mean ± SD ($N = 6$); * significantly different from rest ($P < 0.01$); ** significantly different from rest ($P < 0.001$).

previous investigations have determined changes in the patterns of substrate oxidation (33), performance (18,32), and selected muscle parameters (34) that occur in response to a chronic (6 sessions) intervention.

The first finding was the high average rate of total CHO oxidation (340 μmol·kg^{-1}·min^{-1}) sustained throughout exercise (Table 2). Such rates of oxidation are similar to those reported previously (350 μmol·kg^{-1}·min^{-1}) for well-trained cyclists ($\dot{V}O_{2peak}$ 5.2 L·min^{-1}) riding at 325 W for 50 min (10) and are likely to be close to the upper limit for steady state rates of fuel utilization that can be sustained for 1 h. We are aware of only three other investigations who have reported steady-state rates of substrate utilization during intense aerobic exercise (4, 29, 30). Romijn et al. (30) found that during the last 10 min of a 30-min work bout at 85% of $\dot{V}O_{2max}$, the rate of CHO oxidation was 260 μmol·kg^{-1}·min^{-1}. Although this figure is some 35% lower than the *average* rate of CHO oxidation found in the present study, this is probably due to the 25 W higher absolute work rate sustained by our subjects than those of Romijn et al. (30). Coyle et al. (4) report an average rate of only 170 μmol·kg^{-1}·min^{-1} for total CHO oxidation during 30 min of cycling at 80% of $\dot{V}O_{2max}$ in a group of seven competitive cyclists ($\dot{V}O_{2max}$ 4.87 L·min^{-1}) with a "high lactate threshold." If we assume these subjects sustained a work rate of 300 W (12), then not only is this rate of substrate utilization extremely low but the prevailing RER value of 0.85 means that the contribution from CHO to total energy was only 52%. In the present study, the RER value during the latter stages of exercise was 0.92 (74% of energy from CHO), which is in good agreement with the figure of 0.91 reported by Romijn et al. (29) for similarly well-trained cyclists ($\dot{V}O_{2max}$ 5.04 L·min^{-1}) during the last 10 min of a 30-min ride at 85% of $\dot{V}O_{2max}$. Indeed, not only were the proportions of CHO and fat oxidized similar between the present investigation and the study of Romijn et al. (29) at the same relative exercise intensity, but the absolute rates of fat oxidation (25 vs 27 μmol·kg^{-1}·min^{-1}) were also comparable during the later stages of exercise.

It is well established that glycogen and glucose utilization scale exponentially to the relative exercise intensity, with a greater gain in glycogen than in glucose oxidation at higher power outputs (for review see 2). In the current study, the absolute amount of glycogen oxidized during the 40 min of exercise was 260 mmol·kg^{-1} d.m., which equates to an average rate of glycogen disappearance from the biopsied muscle of 6.5 mmol·kg d.m.$^{-1}$·min^{-1}. As would be expected, such a rate of glycogen utilization is somewhat higher than the 4.6 mmol·kg·d.m.$^{-1}$·min^{-1} rate calculated for trained cyclists during a 1-h time trial (13) and considerably higher than the estimated figure of 4 mmol·kg d.m.$^{-1}$·min^{-1} calculated from the data of Coyle et al. (4). In the present study, glycogen utilization accounted for 90% of total CHO oxidation. This proportion is slightly higher than the 85% contribution from glycogenolysis to overall CHO utilization estimated by Romijn et al. (29) using a combination of stable isotope tracers and indirect calorimetry, and notably higher than the 77% reported by Coyle et al. (4) from direct measurements of muscle glycogen disappearance and RER data.

There are several possible reasons for such a discrepancy. The most likely is an overestimation of the active muscle mass of our subjects. The equations of Katch and Katch (16) assume that the thigh is a truncated cone that includes the active muscle and all other tissue including bone (the femur) but excluding the subcutaneous fat and skin. Second, our muscle measurements made before and after exercise represent the total disappearance of glycogen throughout the entire training session, including the transition from rest and incorporating the initial 10 min of exercise, a time when the rate of glycogenolysis is known to be very rapid (6). On the other hand, the estimations of muscle glycogen utilization in the study of Romijn et al. (29) were made during the latter stages of exercise at a time when the contribution from muscle glycogen would be expected to be lower and the contribution from the oxidation of blood glucose higher to the overall CHO requirements of exercise (2). In the study of Coyle et al. (4), both the absolute and relative exercise intensity was lower than the present investigation, conditions that would be expected to result in an increased lipid utilization and a decreased rate of total CHO oxidation. Despite these differences, it is clear that even in highly trained cyclists, whose musculature would be expected to have an enhanced capacity for the uptake and oxidation of plasma FFA and/or intramuscular triglyceride, substrate selection during intense exercise is dominated by CHO oxidation (2).

As noted previously, few studies have reported rates of substrate utilization during high-intensity aerobic exercise because it has been assumed that at intensities above the so called "lactate threshold" $\dot{V}CO_2$ is not entirely a function of tissue production but also reflects to a depletion of the bicarbonate pool, to maintain acid-base homeostasis. However, indirect calorimetry can provide valid rates of substrate oxidation during intense exercise in those endurance trained subjects who are capable of sustaining high absolute (and relative) work rates with a stable acid–base balance, as reflected by the prevailing blood lactate concentration (28). In the current study, $\dot{V}CO_2$ values remained steady throughout exercise (Table 2), whereas blood lactate concentration rose to 5 mM after the first work bout and thereafter was stable for the remainder of the training session (Fig. 2). These blood lactate concentrations are similar to the 7 mM steady state values reported by others for highly trained endurance cyclists during intense (85–88% of $\dot{V}O_{2peak}$) exercise (4, 30). The perturbations in acid–base balance are most marked after the first bout work bout (Fig. 2). Thereafter, the initial metabolic acidosis is presumably compensated for by an efflux of H$^+$ ions that cause an initial drop in blood pH and HCO$_3$ concentration (Fig. 2). Once this H$^+$ ion efflux has been buffered by the HCO$_3$, blood pH returns to near resting levels for the remainder of the exercise bout showing good metabolic control in these cyclists.

Measures of muscle acid–base status determined from the biopsy samples taken immediately post exercise revealed a significant correlation (r = 0.91, $P < 0.05$) between blood

lactate concentration and the prevailing muscle lactate concentration, as has been reported previously (15). Changes in arterial O_2 saturation during the high intensity training session were commensurate with previous reports that have determined $\%S_PO_2$ during trained athletes capable of sustaining steady state metabolic rates approaching 4.5 L·min^{-1} (27). Typically $\%S_PO_2$ falls to around 94% during short-term, high-intensity exercise (27) and, depending on the rest interval between work bouts, recovers to between 96 and 97%. In the current study, 60 s of active recovery was sufficient to restore $\%S_PO_2$ to 97% throughout the entire exercise session.

The $\dot{V}O_2$ kinetics at the onset of each work bout were rapid (Fig. 1A) taking 3 min to reach the high (4.3–4.4 L·min^{-1}) steady state oxygen uptake values our subjects were capable of sustaining for the duration (40 min) of the training session. Previous studies have reported that $\dot{V}O_2$ responds to a stepped transition from rest to submaximal intensities more rapidly after training (25), and that the rise in $\dot{V}O_2$ is more rapid in trained compared to untrained individuals (26). In the present study, the rise in $\dot{V}O_2$ in response to the first work bout was significantly longer than after the subsequent work bouts. This agrees with the findings of MacDonald et al. (20), who reported that a single bout of high-intensity exercise improved the rise time in $\dot{V}O_2$ for the subsequent bout of (high-intensity) exercise.

Despite the high work rates, there was only a 10 beat·min^{-1} drift in exercise HR throughout the training session with the 60-s recovery resulting in a 30–40 beat·min^{-1} drop in HR (Fig. 1B). Cardiac drift can be as much as 20 beats·min^{-1} during intense exercise lasting 20–60 min in trained individuals despite unchanged work rates (14) and/or steady or declining plasma lactate concentrations (17). This limitation should be recognized when prescribing high-intensity interval training sessions based solely on HR (14).

We are unaware of any previous studies that have determined the rises in muscle temperature after intense exercise. Febbraio et al. (8) have previously reported a 3.7°C rise in muscle temperature in well-trained male subjects after only 40 min of low-intensity cycling (225 W). Another measure of the metabolic control exhibited by our highly trained cyclists during the training session was the smaller (3.2°C) rise in muscle temperature after exercise of the same duration of as employed in the study of Febbraio et al.(8), but at a markedly higher absolute and relative work rate (i.e., 340 W, 85% $\dot{V}O_{2\text{ peak}}$). A possible mechanism underlying such an observation is likely to be an increased muscle blood flow during high-intensity compared with low-intensity cycling in our well-trained subjects (7).

In conclusion, this is the first study to report the metabolic demands associated with an intense aerobic interval exercise session in competitive cyclists. Our results reveal that these athletes are able to sustain high steady-state aerobic power outputs, which are associated with high rates of glycogenolysis and total energy expenditure. The absolute fuel requirements and physiological responses associated with this type of interval training session are similar to those observed when trained cyclists compete in events lasting 60 min (13). Accordingly, it is not surprising that such training has become a basic element of conditioning for cycling and is associated with improvements in performance.

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