Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling

Gregory R. Cox,1,2 Sally A. Clark,3 Amanda J. Cox,3,4 Shona L. Halson,3 Mark Hargreaves,5 John A. Hawley,6 Nikki Jeacocke,1 Rodney J. Snow,2 Wee Kian Yeo,6 and Louise M. Burke1

1Sports Nutrition, Australian Institute of Sport, Belconnen; 2School of Exercise and Nutrition Sciences, Deakin University, Burwood; 3Physiology, Australian Institute of Sport, Belconnen; 4School of Biomedical Sciences, University of Newcastle, Newcastle; 5Department of Physiology, The University of Melbourne, Melbourne; and 6Exercise Metabolism Group, School of Medical Sciences, RMIT University, Melbourne, Australia

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Cox GR, Clark SA, Cox AJ, Halson SL, Hargreaves M, Hawley JA, Jeacocke N, Snow RJ, Yeo WK, Burke LM. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. J Appl Physiol 109: 126–134, 2010. First published May 13, 2010; doi:10.1152/japplphysiol.00950.2009.—We determined the effects of varying daily carbohydrate intake by providing or withholding carbohydrate during daily training on endurance performance, whole body rates of substrate oxidation, and selected mitochondrial enzymes. Sixteen endurance-trained cyclists or triathletes were pair matched and randomly allocated to either a high-carbohydrate group (High group; n = 8) or an energy-matched low-carbohydrate group (Low group; n = 8) for 28 days. Immediately before study commencement and during the final 5 days, subjects undertook a 5-day test block in which they completed an exercise trial consisting of a 100 min of steady-state cycling (100SS) followed by a 7-kJ/kg time trial on two occasions separated by 72 h. In a counterbalanced design, subjects consumed either water (water trial) or a 10% glucose solution (glucose trial) throughout the exercise trial. A muscle biopsy was taken from the vastus lateralis muscle on day 1 of the first test block, and rates of substrate oxidation were determined throughout 100SS. Training induced a marked increase in maximal citrate synthase activity after the intervention in the High group (27 ± 8% vs. 34 ± 16% of pretraining, P < 0.001). Tracer-derived estimates of exogenous glucose oxidation during 100SS in the glucose trial increased from 54.6 to 63.6 g (P < 0.01) in the High group with no change in the Low group. Cycling performance improved by ~6% after training. We conclude that altering total daily carbohydrate intake by providing or withholding carbohydrate during daily training in trained athletes results in differences in selected metabolic adaptations to exercise, including the oxidation of exogenous carbohydrate. However, these metabolic changes do not alter the training-induced magnitude of increase in exercise performance.

Address for reprint requests and other correspondence: G. R. Cox, Sports Nutrition, Australian Institute of Sport, Queensland Academy of Sport, PO Box 956, Nathan, Queensland 4111, Australia (e-mail: greg.cox@ausport.gov.au).

CURRENT SPORTS NUTRITION GUIDELINES recommend that endurance-trained athletes undertake their daily training sessions with high-carbohydrate availability (34). This is based on clear evidence that strategies such as elevating preexercise muscle glycogen stores, consuming a preexercise carbohydrate meal, or eating carbohydrate during exercise increase endurance performance (4, 28, 36) and performance (17, 33, 38) of a single bout of prolonged exercise. However, the results of several recent studies (2, 19, 30, 31, 46) have challenged the idea that this is the best approach to maximize the outcomes of chronic adaptations to training. These investigations have reduced carbohydrate availability during structured training interventions using two different approaches: lowering muscle glycogen concentrations for half of the sessions by scheduling two training bouts in succession on a single day (19, 46) or by undertaking training sessions in a fasted state (2, 15, 31). These strategies are associated with evidence of greater training-induced metabolic adaptations with low-carbohydrate availability compared with high-carbohydrate availability (19, 31, 46) but inconsistent results with respect to improvements in exercise capacity or performance.

Despite the interest generated by these studies, it has been difficult to apply findings to the preparation of well-trained athletes; this is in part due to the lack of direct relevance of the study protocols to the characteristics and practices of endurance athletes. For example, studies have been conducted with untrained subjects (2, 15, 19, 31) and have used a low-volume training program (2, 15, 19, 31) compared with the 15–20 h/wk of varied exercise stimulus that is commonly reported by well-trained endurance athletes (18). In addition, real-life training programs typically follow principles such as working to perceptions of effort or of incorporating progressive overload rather than the repetition of a clamped exercise stimulus as has occurred in laboratory studies (2, 15, 19, 31).

A final and important difference is the complexity by which well-trained athletes manipulate carbohydrate availability via combinations of dietary practices, both in training settings and competition. Dietary surveys of endurance athletes typically report the intake of carbohydrate during and after daily workouts (8) as well as the consumption of higher daily carbohydrate intakes than athletes in nonendurance sports (6). These practices are likely to influence muscle glycogen content during periods of intensified training (12) as well as the prevailing utilization of total and exogenous carbohydrate sources during exercise (17).

Accordingly, the aim of the present investigation was to determine whether altering daily carbohydrate intake by providing or withholding carbohydrate during daily training affects metabolic adaptations to endurance exercise. A secondary aim was to determine whether daily training under conditions of high- or low-carbohydrate availability affects subsequent measures of substrate utilization and performance during high-intensity endurance exercise with or without the additional intake of carbohydrate during exercise. The novel aspects of the present investigation were 1) the selection of well-trained subjects, 2) an intensive training program reflecting that of highly competitive athletes, and 3) the interaction of various factors on their responses to high- or low-carbohydrate availability during daily training.
manipulations of carbohydrate availability as they occur in sporting events. We hypothesized that the adaptation to training would be enhanced when training with low-carbohydrate availability but that daily training with high-carbohydrate availability would result in improved performance. In addition, based on previous work in animals (22, 29), we hypothesized that daily training with high-carbohydrate availability would result in an increase in exogenous glucose oxidation during endurance cycling.

METHODS

Subjects and preliminary tests. Sixteen endurance-trained male triathletes or cyclists [body weight: 75.0 ± 6.7 kg, skinfold measurements at 7 sites: 44.7 ± 13 mm, age: 30.7 ± 5.6 yr, peak O₂ consumption (VO₂peak): 64.9 ± 4.7 ml·kg⁻¹·min⁻¹, and peak sustained power output (PPO): 385 ± 23 W (means ± SD)] were recruited to participate in this study. The study was conducted before the competitive season when subjects were undertaking aerobic training with few interval sessions. Subjects had a history of >3 yr of endurance training. This study was approved by the Human Research Ethics Committee of the Australian Institute of Sport (AIS). Subjects were fully informed about the possible risks of all experimental procedures before providing their written consent.

VO₂peak was determined during an incremental test to exhaustion on a Lode cycle ergometer (Groningen, The Netherlands) as previously described in detail (20). In brief, subjects commenced cycling at a workload equivalent to 2 W/kg for 150 s. Thereafter, the workload was increased by 25 W every 150 s until volitional fatigue, which was a workload equivalent to 2 W/kg for 150 s. This workload was maintained by 2 W/kg until volitional fatigue, which was defined as the inability to maintain a cadence of >70 revolutions/min. During the maximal test and the subsequently described experimental trials, subjects breathed through a Hans Rudolph two-way nonbreathing valve attached to a custom-built automated Douglas bag gas analysis system (AIS, Australian Capital Territory, Australia) interfaced to a computer, which calculated the rates of VO₂ and CO₂ production (VCO₂), minute ventilation (VE), and respiratory exchange ratio (RER) every 30 s from conventional equations (32).

Overview of study design. The study involved a 28-day diet and training intervention undertaken in a parallel group design (Fig. 1). Subjects were pair matched according to their physical capabilities (VO₂peak and PPO), training status, and training history and randomly allocated to either a high-carbohydrate availability group (High group; n = 8) or an energy-matched low-carbohydrate availability group (Low group; n = 8). Immediately before this program and during the final 5 days of the intervention (days 24 –28), each subject completed a 5-day test block in which they undertook a cycling protocol incorporating a performance ride on two occasions separated by 72 h. In a counterbalanced design, subjects completed the two experimental trials in each test block while consuming either water (water trial) or a 10% glucose solution (glucose trial).

Experimental trials. Subjects were provided with a standard diet providing a carbohydrate intake equal to 6.7 g/kg body wt for 24 h before each experimental trial and undertook a standard 1-h training session on the day before each experimental trial. Subjects were required to refrain from consuming any caffeine and alcohol-containing foods or fluids during this period. On the day of the experimental trial, subjects reported to the laboratory at 0600 hours, at which time a cannula was inserted to take a fasting blood sample and allow for blood collection throughout exercise. Blood samples (8 ml) were later analyzed for plasma glucose, insulin, free fatty acids (FFA), and lactate (as described below). On the first (pretraining) and third (posttraining) experimental trials, a resting muscle sample (~100 –150 mg) was taken from the vastus lateralis muscle using the percutaneous biopsy technique with suction applied. Muscle biopsies were rapidly frozen in liquid N₂ within seconds of collection and stored at −80°C until subsequent analysis. At this time, a restricted anthropometric profile including body weight, height, and skinfold measurements at seven sites was completed for each subject on the morning of the first (experimental trial 1) and last (experimental trial 4) testing days.

![Fig. 1. Schematic of the study design and experimental trial. Exp Trial, experimental trial; TT, time trial; CHO, carbohydrate; High group, high-carbohydrate availability group; Low group, low-carbohydrate availability group; 100SS, 100-min steady-state cycling; PPO, peak power output; HR, heart rate; RPE, ratings of perceived exertion.](http://jap.physiology.org/)
After resting blood and muscle sample collection, subjects consumed a standard breakfast providing 2.1 g carbohydrate/kg body wt and rested for 2 h before undertaking the experimental trial. At this time point (120 min postbreakfast), subjects were weighed in minimal clothing, and a further blood sample (8 ml) was taken. They then mounted a Lode ergometer and began 100 min of steady-state cycling (100SS) at a work rate equivalent to 63% PPO (≈70% $V_O_{2\text{peak}}$).

At 20-min intervals throughout 100SS, heart rates were recorded using personal telemetry (Polar Accurex Plus, Polar Electro OY, Kempele, Finland), a blood sample (8 ml) and expired respiratory gas data were collected, and subjects provided a rating of their perceived exertion (RPE) (5). Heart rate and RPE data are not reported. Throughout 100SS, subjects ingested 5 ml/kg body wt of their test drink (water or 10% glucose solution) at 20-min intervals. For the glucose trial, the solution contained trace amounts of $^{14}$C-glucose (Amersham Biotech, Sydney, Australia) for the determination of rates of ingested carbohydrate oxidation as previously described (9).

In brief, the glucose solution was prepared on the morning of each trial, and three 1-ml samples were taken from each drink to determine the $^{14}$C enrichment. Before 100SS and at 20-min intervals throughout, a sample of $\sim$1 liter of expired air was passed through a solution containing 1 ml of hyamine hydroxide in methanol, 1 ml of 96% ethanol, and 2 drops of 1% phenolphthalein indicator. The air was bubbled through this mixture for 1–2 min until the phenolphthalein turned from pink to clear, at which point 1 mM of CO$_2$ had been absorbed (37). Liquid scintillation cocktail (10 ml, Ready Safe, Beckmann) was then added to the solution. Numbers of 14CO$_2$ disintegrations per minute were counted in a liquid scintillation counter (1600CA TRI CARB Liquid Scintillation analyzer, Packard, Rungis, France) after the completion of data collection.

Immediately after 100SS, after a standardized 3- to 5-min rest, subjects commenced a 7 kg/kg body wt time trial (TT), which typically lasted 25–35 min. Subjects were instructed to complete this TT “as fast as possible.” The same researcher supervised each TT and provided standardized feedback to each subject. The only information available to subjects was a graphic representation of their performance (i.e., a continuous line graph) each week to enhance endurance exercise performance (Fig. 1).

**Dietary manipulation and training.** During the intervention period (days 1–28, excluding days 24 and 27), subjects were provided a daily carbohydrate intake of 5 g/kg body wt (185 KJ/kg body wt). Fat and protein intakes were manipulated in an attempt to have athletes remain weight stable. In addition, subjects were provided with nutritional supplements to support the additional energy demands of daily training. Subjects in the High group were supplemented with an additional 1.5 g/kg body wt of carbohydrate (25 KJ/kg body wt) for every hour of exercise performed daily. The carbohydrate supplement was mainly provided in the form of a flavoured glucose drink to match the main form of carbohydrate consumed during experimental trials. Additional foods of equal carbohydrate content (i.e., cereal bar, Vegemite sandwich, slice of bread with jam, glucose confectionary, or PowerBar Gel) were included to meet hourly carbohydrate allowances for each subject in the High group. Subjects in the Low group also received a nutritional supplement providing 25 KJ/kg body wt per hour of exercise performed daily from fat- and protein-rich foods and drinks (macadamia nuts or a formulated cream drink) while limiting additional carbohydrate to 0.1 g/kg body wt. Subjects in the High group were instructed to consume the carbohydrate supplements either immediately before or during training. Any remaining nutritional supplements were consumed immediately after the session, before any further training. Subjects in the Low group were required to fast for 2 h before training sessions and were instructed to consume their additional nutritional supplements after training. During the extended training sessions (>2 h), subjects in the Low group were allowed to consume their nutritional supplements after the initial 90–120 min of exercise.

All meals and snacks were supplied to subjects throughout the dietary control period, with diets being individualized for food preferences and body weights. Dinner was supervised every second day in the laboratory, whereas all other meals, snacks, and nutritional supplements were provided as individual take-home prepackaged items. Subjects were required to keep a food and fluid checklist to note their compliance to the dietary instructions and their intake of any additional food or drinks. No attempt was made to blind subjects from the dietary treatments. The principal investigator of the study advised each athlete of their treatment group and highlighted the potential advantages associated with their treatment to ensure that each subject felt that they would receive a benefit from their dietary intervention. The energy and nutrient composition of all meal plans was estimated using FoodWorks (Professional Edition, version 3.02, Xyris Software, Brisbane, Australia).

The training program was individualized for each subject according to their level of fitness, availability, and current training program. This program was intended to reflect the subject’s habitual training schedule with the inclusion of two high-intensity training sessions each week to enhance endurance exercise performance (Fig. 1).

**Analysis of blood samples.** Blood samples (8 ml) were collected at each sampling time, and $\sim$5 ml of blood were placed in a tube containing lithium-heparin and spun for 5 min at 4,000 rpm to provide 2 ml of plasma. Plasma was stored at $\sim$80°C and later analyzed in duplicate for plasma concentrations of glucose, lactate, and insulin. Glucose and lactate were analyzed in duplicate by a photometric assay using the Hitachi 911 biochemistry automatic analyzer (Boehringer Mannheim). Insulin was analyzed by a solid-phase, two-site chemiluminescent immunometric assay using the Immulite automatic analyzer (Diagnostic Product, Los Angeles, CA). The remaining blood (typically $\sim$2.0 ml) was placed in a tube containing EDTA and spun for 15 min at 1,500 rpm. Plasma was stored at $\sim$80°C and later analyzed in duplicate for plasma FFA by an enzymatic colorimetric assay using the Hitachi 911 biochemistry automatic analyzer (Boehringer Mannheim). All samples were analyzed in a single batch to avoid any day-to-day variations in the performance of the analyzers.

Rates of whole body carbohydrate and fat oxidation as well as ingested carbohydrate oxidation. Whole body rates of carbohydrate and fat oxidation (in g/min) were calculated from $V_{\text{CO}_2}$ and $V_{\text{O}_2}$ measured from the last 5 min of each 20-min block of 100SS using nonprotein RER values according to the following equations (32):

\[
\text{Carbohydrate oxidation} = 4.585 \times V_{\text{CO}_2} - 3.226 \times V_{\text{O}_2}
\]

\[
\text{Fat oxidation} = 1.695 \times V_{\text{CO}_2} - 1.701 \times V_{\text{O}_2}
\]

Rates of carbohydrate oxidation (in $\mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) were subsequently determined by converting the rate of carbohydrate oxidation (in g/min) to its molar equivalent, assuming 6 mol O$_2$ are consumed and 6 mol CO$_2$ are produced for each mole (180 g) oxidized. Rates of fatty acid oxidation (in $\mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) were determined by converting the rate of triglyceride oxidation (in g$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) to its molar equivalent, assuming the average weight of human triglyceride to be 855.26 g/mol and multiplying the molar rate of triglyceride oxidation by 3, because each molecule contains 3 mol fatty acid.

Rates of total exogenous glucose oxidation were calculated from the following equation:

\[
\text{Gluox} = \left( \frac{S_{\text{carb}}}{S_{\text{carb}} + S_{\text{glu}}} \right) \times V_{\text{CO}_2}
\]

where $\text{Gluox}$ is the rate of exogenous glucose oxidation (in mmol/min; later converted to g/min), $S_{\text{carb}}$ is the specific (radio)activity of expired $^{14}$CO$_2$ (in disintegrations$\cdot$min$^{-1}$$\cdot$mmol$^{-1}$), $S_{\text{glu}}$ is the corresponding specific (radio)activity of the drink (in disintegrations$\cdot$min$^{-1}$$\cdot$mmol$^{-1}$), and $V_{\text{O}_2}$ is the volume of expired CO$_2$ (in mmol/min), which was calculated from the liter per minute $V_{\text{CO}_2}$ value and the 22.4 ml/mmol gas volume.

**Muscle glycogen content.** Approximately 10–15 mg of muscle were freeze dried and powdered with all visible blood and connective
tissue removed under magnification. The freeze-dried muscle sample was then incubated in 250 μl of 2 M hydrochloric acid at 100°C for 2 h before being neutralized with 750 μl of 0.67 M sodium hydroxide. Glycogen content was determined via enzymatic analyses (50 mM Tris, 25 mM HCl, 1 mM MgCl2, 0.5 mM DTT, 0.3 mM ATP, 0.05 mM NADP, 1 U/ml hexokinase, and 0.1 U/ml glucose-6-phosphate dehydrogenase) with glucose standards by fluorometric detection and was expressed as millimoles of glycogen per kilogram dry weight.

Glucose transporter 4 protein content. Approximately 30 mg of wet muscle were homogenized (50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM NaF, 5 mM Na pyrophosphate, 10% glycerol, 1% Triton X-100, 10 μg/ml trypsin inhibitor, 2 μg/ml aprotinin, 1 mM benzamidine, and 1 mM PMSF), and the homogenates were then centrifuged at 20,000 g for 30 min. The supernatant was aliquoted, and the total protein concentration in the aliquots was determined by the bicinchoninic acid method (Pierce).

Forty micrograms of total protein were electrophoresed by 10% SDS-PAGE and detected by immunoblot analysis with antibodies specific for glucose transporter 4 (GLUT4; ab654, Abcam, Cambridge, UK) and α-tubulin (T 6074, Sigma). The immunoreactive proteins were then detected with enhanced chemiluminescence (Amersham Biosciences) on a Bio-Rad Chemidoc XRS system and quantified by densitometry (Quantity one, Bio-Rad). Total GLUT4 protein was expressed relative to total α-tubulin content.

Citrate synthase and β-hydroxyacyl CoA dehydrogenase activity. Approximately 5–10 mg of wet muscle were weighed and homogenized in 50 μl of buffer (0.175 M KCl and 2 mM EDTA) per milligram wet weight. The sample was freeze thawed twice and spun at 10,000 rpm for 1 min. Samples for the analysis of citrate synthase activity were individually prepared in a cuvette to a final volume of 500 μl and contained 330 μl of 100 mM Tris buffer, 50 μl of 1 mM DTNB (D-1830, Sigma), 80 μl of 3 mM acetyl CoA (A-2897, Sigma), and 10 μl of muscle homogenate. Samples were placed in a Shimadzu UV-1601 spectrophotometer for 10 min to warm to 25°C before the addition of 30 μl of 10 mM oxalacetate (O-4126, Sigma). The change in absorbance was recorded every 15 s for 3 min at 412 nm. Samples for the analysis of β-hydroxyacyl CoA dehydrogenase (β-HAD) activity were individually prepared in a cuvette to a final volume of 490 μl and contained 430 μl of assay mixture (1 M Tris HCl, 200 mM EDTA, and 5 mM NADH), 50 μl of muscle homogenate, and 10 μl Triton X-100 (10%). Samples were placed in a Shimadzu UV-1601 spectrophotometer for 10 min to warm to 25°C before the addition of 30 μl of 10 mM acetoacetyl CoA (A-1625, Sigma). The change in absorbance was recorded every 15 s for 3 min at 340 nm.

Statistical analysis. Descriptive statistics (means ± SD) were used to summarize the physical and performance characteristics of the subjects, dietary analysis data, and weekly training volume. Group and drink effects were analyzed using three-factor (group, drink, and time) repeated-measures ANOVA post hoc analysis performed using the Bonferroni adjustment. Within-group changes from before to after training were also examined using t-tests for dependent measures. For blood parameters, repeated measures throughout 100SS were averaged and compared with resting values and post-TT values for each variable. For carbohydrate and fat oxidation, the total area under the curve (AUC) was calculated to estimate total oxidation during 100SS. Data were analyzed using SPSS 15.0 (SPSS, Chicago, IL), and all values are expressed as means ± SD, with significance at *P* < 0.05.

RESULTS

**Dietary manipulation and training.** Dietary intake data for the High and Low groups for the intervention period (excluding days 24 and 27) are shown in Table 1. Daily carbohydrate intake ranged from 4.7 to 14.3 g/kg body wt in the High group to reflect daily training, whereas daily carbohydrate intake remained relatively constant (4.3–6.1 g/kg body wt) in the Low group, despite fluctuations in daily training. Changes in body weight from days 1 to 24 revealed a mean loss of 1.3 ± 0.7 kg for the Low group and 1.8 ± 1.3 kg for the High group, with a concurrent decrease in skinfold measurements from seven sites of 5.1 ± 3.5 and 5.5 ± 3.1 mm for the Low and High groups, respectively. Subjects experienced a mild energy deficit during the intervention period. Subjects in the Low and High groups completed a similar number of training hours each week throughout the intervention period (16.1 ± 3.5 and 16.3 ± 2.3 h, respectively).

**Glycogen content.** There was a main effect of carbohydrate intake over the intervention period. This effect was, however, only significant in the High group (*P* < 0.001; Fig. 2B). While citrate synthase activity increased in the Low group, such changes failed to reach statistical significance (*P* = 0.08). There was a trend for maximal β-HAD activity to increase in both the Low (*P* = 0.23) and High (*P* = 0.18) groups after the intervention period (Fig. 2C). There were no apparent differences in the increase between groups (*P* = 0.87).

**GLUT4 protein content.** There were no changes in GLUT4 protein concentration after the diet and training intervention period for both Low and High groups (Fig. 2D).

**Substrate oxidation during exercise.** Estimated rates of carbohydrate and fat oxidation during 100SS at 20, 40, 60, 80, and 100 min for the water and glucose trials are shown in Fig. 3A. There was a main effect of drink on carbohydrate oxidation during 100SS, as measured as the total area under the carbohydrate oxidation versus time curve (AUC), with higher values for the glucose trial compared with the water trial before (*P* < 0.01) and after (*P* < 0.05) the intervention period (Fig. 3A). There was a main effect of time as carbohydrate oxidation was similarly reduced after training in both Low and High groups in the water (−6.9 ± 7.5%, *P* < 0.01) and glucose (−9.4 ± 7.5%, *P* < 0.01) trials.

As expected, there was a main effect of drink on fat oxidation, as determined as the total area under the fat oxidation versus time curve (AUC), with higher values for the water trial compared with the glucose trial before (*P* < 0.01) and after (*P* < 0.05) the intervention (Fig. 3B). There was a main effect of time as fat oxidation increased significantly in the glucose intake ranged from 4.7 to 14.3 g/kg body wt in the High group to reflect daily training, whereas daily carbohydrate intake

### Table 1. Dietary intake data

<table>
<thead>
<tr>
<th>Energy</th>
<th>Low Group</th>
<th>High Group</th>
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<tbody>
<tr>
<td>In kcal</td>
<td>17,593 ± 2,862</td>
<td>18,417 ± 2,832</td>
</tr>
<tr>
<td>In kg/kg body wt</td>
<td>242 ± 38</td>
<td>243 ± 34</td>
</tr>
</tbody>
</table>

| Carbohydrate |          |          |
| In g         | 383 ± 39 | 640 ± 132 |
| In g/kg body wt | 5.3 ± 0.4 | 8.5 ± 1.7 |

| Fat, g       | 223 ± 62 | 134 ± 29 |
| Protein, g   | 151 ± 25 | 143 ± 23 |

Values are expressed as means ± SD; *n* = 8 subjects in the low-carbohydrate availability group (Low group) and 8 subjects in the high-carbohydrate availability group (High group). Mean energy and carbohydrate, fat, and protein intakes during the 28-day diet and training intervention period (excluding days 24 and 27) for the Low and High groups are shown.
trial after training (P < 0.05). While fat oxidation increased after training in the water trial, it failed to reach statistical
significance (P < 0.15). No differences were noted between
groups.

**Exogenous glucose oxidation.** Accumulated oxidation of
exogenous glucose during 100SS increased from 54.6 to 63.6
g for the High group (P < 0.01; Fig. 4) after the diet and
training intervention such that the change in posttraining ac-

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**Fig. 2.** Muscle glycogen concentration (A), maximal citrate synthase activity
(B), maximal β-hydroxyacyl CoA dehydrogenase (β-HAD) activity (C), and
glucose transporter (GLUT)4 protein concentration (D) before (Pre) and after
(Post) the 28-day diet and training intervention period. Values are means ±
SD. *Significantly different from before the intervention period (P < 0.001).

**Fig. 3.** Rates of whole body carbohydrate (A) and fat (B) oxidation at 20, 40,
60, 80, and 100 min during 100SS for water and glucose trials before and after
the 28-day diet and training intervention period, respectively. Values are
means ± SD. *Significantly different between water and glucose trials before
the intervention period (P < 0.01); †significantly different between water and
fat trials after the intervention period (P < 0.05).

**Fig. 4.** Accumulated exogenous glucose oxidation before and after the 28-day
diet and training intervention period. Values are means ± SD. *Significantly
different from before the intervention period (P < 0.01); †significantly
different compared with Low group after training (P < 0.05).
cumulate oxidation of exogenous glucose was significantly higher than that observed in the Low group ($P < 0.05$; Fig. 4). The postraining accumulated oxidation of exogenous glucose remained unchanged in the Low group (Fig. 4).

**Plasma metabolites.** Figure 5, A–D, shows concentrations of blood glucose, plasma insulin, plasma FFA, and blood lactate, respectively, for the water and glucose trials during the 2 h before and throughout exercise. Consumption of the carbohydrate-rich meal 2 h before exercise caused blood glucose concentrations to decrease by the start of 100SS (Fig. 5A). There was a main effect of drink on blood glucose concentrations throughout 100SS ($P < 0.001$; Fig. 5A). Blood glucose concentrations remained unchanged from resting values in the water trials, whereas blood glucose rose quickly at the onset of exercise and remained higher throughout 100SS during the glucose trials ($P < 0.001$; Fig. 5A). No differences in this response were observed between the High and Low groups.

As expected, plasma insulin concentrations increased in response to the carbohydrate-rich preexercise meal in all trials ($P < 0.01$; Fig. 5B). There was a significant decrease in plasma insulin from resting values throughout 100SS ($P < 0.001$) except in the postraining glucose trials (Fig. 5B). No differences were observed in this response between groups. At the onset of 100SS, plasma FFA concentrations decreased significantly in response to the carbohydrate-rich preexercise meal in all trials ($P < 0.01$; Fig. 5C). Plasma FFA concentrations increased during 100SS from resting values in the water trials ($P < 0.05$) but remained unchanged (from rest) in the glucose trials (Fig. 5C). There was a main effect of drink on FFA concentrations throughout 100SS before the intervention (Fig. 5C). No differences in this response were observed between the High and Low groups.

Blood lactate concentrations increased above fasting values in response to the carbohydrate-rich preexercise meal and remained relatively constant throughout 100SS before rising substantially after the TT (Fig. 5D). No differences were observed in this response between groups (High vs. Low groups) or drink trials (glucose vs. water trials).

**Exercise performance (T Ts).** Figure 6 displays the total time for the cycling performance TT before and after the 28-day diet and training intervention period for the Low and High groups in the water and glucose trials. When the results of the experimental
trials were pooled (for group and drink), TT performance was significantly faster after training (6.2 ± 6.3%, P < 0.01). No differences were observed between groups before or after the intervention. There was no main effect of drink (glucose vs. water) on TT performance before or after the intervention period.

**DISCUSSION**

The novel findings of the present study were that, in contrast to a matched group who exercised with lower carbohydrate availability, well-trained athletes who trained under conditions of high-carbohydrate availability (a high daily carbohydrate intake scheduled during daily training) for a 28-day training program achieved 1) a greater increase in maximal muscle citrate synthase activity and 2) an increase in the oxidation of glucose consumed during submaximal exercise. The first of these findings contradicts our original research hypothesis: that subjects exercising under conditions of low-carbohydrate availability would have an amplification of the metabolic adaptations to endurance training. Nevertheless, despite enzymatic changes suggestive of an enhanced adaptation in the group training with high-carbohydrate availability, we found no clear benefit to endurance performance.

The practical value of our study is that we manipulated carbohydrate availability during a period of high-volume training to reflect real-life dietary practices of endurance athletes. The protocols used in previous studies have maintained high daily carbohydrate intake while altering carbohydrate availability for exercise by rescheduling training times (19, 46) or by providing or withholding carbohydrate before and during the exercise session (2, 15, 31). However, dietary surveys have reported that endurance-trained athletes are both more likely to consume carbohydrate during and after daily training (8) and consume higher carbohydrate intakes than athletes in nonendurance sports (6). Therefore, the design of the present study assessed the combined effect of manipulating daily carbohydrate intake and rescheduling carbohydrate intake during daily training to better reflect these practices.

**Effects on training adaptations.** To determine whether training adaptations would be influenced by high-carbohydrate availability during daily exercise, we measured the maximal activities of citrate synthase and β-HAD along with resting muscle glycogen content. Although maximal β-HAD activity and muscle glycogen content did not change over the training period, there was a significant increase in citrate synthase activity in both the Low and High groups. Surprisingly, the magnitude of the increase in the group training with high-carbohydrate availability was greater than that observed in the group with low-carbohydrate availability. Therefore, the present study suggests that a high daily carbohydrate intake with carbohydrate consumed during daily exercise does not blunt training-induced metabolic adaptations in well-trained subjects. Although others (10, 11) have reported a reduction in the expression of genes with putative roles in fat oxidation when carbohydrate is consumed during an acute exercise bout, there appears to be no chronic metabolic implications of such a feeding regimen (2, 15). In contrast, studies (19, 46) in which carbohydrate availability was manipulated by rescheduling daily training times have found that low-carbohydrate avail-

ability augments the increase in selected markers of training adaptation.

It is difficult to explain why we did not observe superior benefits in metabolic adaptations in the Low training group in our study. It is possible that starting muscle glycogen stores before daily training were not sufficiently compromised in the Low group to promote these benefits, as observed in previous studies (19, 46). We did not measure muscle glycogen content during the intervention period to demonstrate that subjects in the Low group (who consumed a moderate daily carbohydrate intake of 5.3 g/kg body wt) commenced daily training with reduced muscle glycogen stores compared with subjects in the High group (who consumed a high daily carbohydrate intake of 8.5 g/kg body wt). It is possible that the spacing of the daily training sessions and the inclusion of a rest day each week provided sufficient time for muscle glycogen stores to normalize in both the Low and High groups (41). However, several previous studies (12, 13, 24) using similar diet and exercise protocols to those in the present study have demonstrated higher resting muscle glycogen stores after the intake of a high daily carbohydrate intake compared with a moderate carbohydrate intake. Furthermore, we suggest that the volume and intensity of training [−16 h/wk including high-intensity interval sessions previously shown to reduce resting muscle glycogen stores by 50% (43)] would place high demands on muscle glycogen stores.

**Effects on exogenous glucose oxidation.** In agreement with our original hypothesis, during the exercise trial in which subjects consumed glucose, exogenous carbohydrate oxidation increased in the High group after the intervention. In the present study, a high rate of glucose was provided during the glucose exercise trials to challenge the maximal rates of exogenous glucose oxidation, previously reported as 1.1 g/min (45). Although after training both groups showed a decreased reliance on total carbohydrate utilization during the exercise trial in which they consumed glucose, subjects in the High group also showed a substantial increase in total exogenous glucose oxidation, which was not observed in the Low group. To the best of our knowledge, this is the first study in humans to show an increase in whole body oxidation rates of exogenous carbohydrate during exercise in response to the chronic ingestion of carbohydrate (predominantly glucose) during daily training. Exogenous carbohydrate oxidation is more likely to be limited by the rate of absorption and subsequent transport of the ingested carbohydrate into the systemic circulation than the rate of uptake from the blood and subsequent oxidation by the working muscle (21, 40). Indeed, the increase in the whole body oxidation of ingested carbohydrate in the High group occurred in the absence of a difference in muscle GLUT4 concentrations. Several studies (22, 29) have supported the role of the gut and its possible trainability in determining oxidation rates of ingested carbohydrate. Further work is warranted to directly test whether a chronic high-carbohydrate diet with carbohydrate ingestion during exercise upregulates intestinal GLUTs and increases intestinal glucose absorption, as previously shown in animal studies (22, 29). In the meantime, we propose that ingesting carbohydrate during daily training may offer an advantage to athletes who compete in endurance events where exogenous carbohydrate is an important fuel source and there is ample opportunity to ingest carbohydrate during the competition (23, 35).
Performance outcomes. There was an overall increase in performance for both groups as a result of the 28-day diet and training intervention. We and others (42, 46) have previously shown that the supplementation of high-intensity interval sessions to endurance training programs improves performance in already well-trained athletes. In contrast to our original hypothesis, subjects undertaking daily training with high-carbohydrate availability improved cycling performance similar to subjects training with low-carbohydrate availability. Previous studies (2, 15, 30, 31, 46) that altered carbohydrate availability by rescheduling training bouts or providing/withholding carbohydrate during training have also failed to demonstrate a clear difference in endurance exercise performance. The present data extend these findings to trained subjects undertaking nutritional strategies characterized by those of endurance athletes in competitive sporting events (7, 26).

While several chronic diet and training studies (1, 12, 24, 41) investigating different daily carbohydrate intakes have found enhanced performance of a prolonged exercise task after a period of training on a high daily carbohydrate intake compared with moderate carbohydrate intake, others (25, 39, 44) have failed to find any differences. To overcome methodological differences of previous studies that may account for the disparity in research findings, we (1) provided additional carbohydrate in the high-carbohydrate treatment during daily training to maximize carbohydrate availability during exercise and (2) normalized muscle glycogen stores before exercise testing to differentiate between the acute and chronic effects of consuming a moderate- versus high-carbohydrate diet. This has practical significance as endurance athletes typically undertake strategies to normalize muscle glycogen stores before competition (7). Despite addressing these dietary methodological issues in the present study, we were unable to detect a difference in the magnitude of improvement in endurance cycling performance between the low- and high-carbohydrate availability groups.

The beneficial effect of ingesting carbohydrate throughout endurance exercise (>90-min duration) on exercise performance is consistent and reproducible finding in studies using a performance exercise model (i.e., T Ts) (3, 17, 27). In our study, we found no such benefit of ingesting a 10% glucose solution during endurance exercise compared with water, despite plasma glucose being better maintained throughout exercise. A possible explanation for this is that subjects were fed a standard carbohydrate-rich breakfast (2.1 g/kg body wt) before undertaking the water and glucose exercise cycle trials (16). This practice has been shown to alter substrate utilization during subsequent exercise toward a greater reliance on carbohydrate utilization and may represent an unfavorable condition in some situations or individuals (14) but nevertheless is an observed dietary practice among athletes in shorter, high-intensity endurance events (7, 26).

Conclusions. In conclusion, the results of this investigation demonstrate that adaptations to endurance training with high-carbohydrate availability (a high-carbohydrate diet, with additional carbohydrate scheduled during exercise) are similar to training with lower-carbohydrate availability (a moderate-carbohydrate diet, with a minimum 2-h fast before exercise) in well-trained athletes undertaking a high weekly training volume. A significant difference was that training with high-carbohydrate availability increased exogenous glucose oxidation when subjects consumed glucose throughout a performance exercise trial. Nevertheless, no clear advantage was apparent to performance during a ~2-h cycling protocol preceded by a carbohydrate-rich meal compared with training with lower carbohydrate availability.

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REFERENCES


