Low-Carbohydrate Training Increases Protein Requirements of Endurance Athletes

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ABSTRACT

GILLEN, J. B., D. W. D. WEST, E. P. WILLIAMSON, H. J. W. FUNG, and D. R. MOORE. Low-Carbohydrate Training Increases Protein Requirements of Endurance Athletes. *Med. Sci. Sports Exerc.*, Vol. 51, No. 11, pp. 2294–2301, 2019. **Introduction:** Training with low-carbohydrate (CHO) availability enhances markers of aerobic adaptation and has become popular to periodize throughout an endurance-training program. However, exercise-induced amino acid oxidation is increased with low muscle glycogen, which may limit substrate availability for postexercise protein synthesis. We aimed to determine the impact of training with low-CHO availability on estimates of dietary protein requirements. **Methods:** Eight endurance-trained males (27 ± 4 yr, 75 ± 10 kg, 67 ± 10 mL·kg body mass^{-1}·min^{-1}) completed two trials matched for energy and macronutrient composition but with differing CHO periodization. In the low-CHO availability trial (LOW), participants consumed 7.8 g CHO^{-1}·kg^{-1} before evening high-intensity interval training (10 × 5 min at 10-km race pace, 1 min rest) and subsequently withheld CHO postexercise (0.2 g·kg^{-1}). In the high-CHO availability trial (HIGH), participants consumed 3 g CHO·kg^{-1} during the day before high-intensity interval training, and consumed 5 g CHO·kg^{-1} that evening to promote muscle glycogen resynthesis. A 10-km run (~80% HRmax) was performed the following morning, fasted (LOW) or 1 h after consuming 1.2 g CHO·kg^{-1} (HIGH). Whole-body phenylalanine flux and oxidation were determined over 8 h of recovery via oral [^{13}C]phenylalanine ingestion, according to standard indicator amino acid oxidation methodology, while consuming sufficient energy, 7.8 g CHO·kg^{-1}·d^{-1}, and suboptimal protein (0.93 g·kg^{-1}·d^{-1}). **Results:** Fat oxidation (indirect calorimetry) during the 10-km run was higher in LOW compared with HIGH (0.99 ± 0.35 g·min^{-1} vs 0.60 ± 0.26 g·min^{-1}, $P < 0.05$). phenylalanine flux during recovery was not different between trials ($P > 0.05$) whereas phenylalanine oxidation (reciprocal of protein synthesis) was higher in LOW compared with HIGH (8.8 ± 2.7 µmol·kg^{-1}·h^{-1} vs 7.9 ± 2.4 µmol·kg^{-1}·h^{-1}, $P < 0.05$), suggesting a greater amino acid requirement to support rates of whole-body protein synthesis. **Conclusions:** Our findings suggest that performing endurance exercise with low-CHO availability increases protein requirements of endurance athletes.
Key Words: AEROBIC EXERCISE, DIETARY PROTEIN, CARBOHYDRATE AVAILABILITY, EXERCISE RECOVERY

Current sport science consensus statements recommend that endurance athletes should consume protein intakes in the range of 1.2–2.0 g·kg^{-1}·d^{-1} (1–3), which is 50% to 150% higher than the recommended daily allowance (0.8 g·kg^{-1}·d^{-1}) for healthy adults. Our recent estimates using the indicator amino acid oxidation (IAAO) technique suggest endurance athletes require approximately 1.83 g protein·kg^{-1}·d^{-1} on a training day (4) in part to replace amino acid oxidative losses incurred during exercise (5) and to provide the necessary substrates for postexercise repair (e.g., protein synthesis) of muscle and other body proteins (6), which collectively contribute to the maintenance of exercise performance during training (7). Branched chain amino acids (BCAA) have been reported to be the rate limiting amino acids for maximizing whole-body protein synthesis in endurance athletes (8), which is generally consistent with these amino acids representing the preferential amino acid substrate for oxidative catabolism within working skeletal muscle during exercise (5). These findings suggest an important contribution of exercise-induced oxidative losses to the elevated protein requirements of endurance athletes (5,8). Therefore, manipulations to training and/or diet that influence fuel use and amino acid oxidation during exercise may ultimately impact nutritional recommendations for optimal postexercise recovery.

Amino acids typically contribute up to approximately 5% of energy provision during endurance exercise (5), although this may be increased up to twofold under conditions of low-carbohydrate (CHO) availability (9,10). Early studies using indirect estimates of protein turnover through the measurement of blood urea nitrogen (9) and arterial-venous amino acid balance (11) suggested that amino acid oxidation may increase when exercise is commenced with low endogenous CHO availability (i.e., low preexercise muscle glycogen). This was supported by Howarth et al. (10) who demonstrated using stable isotope tracer methodology that a low-CHO diet for 2 d after glycogen-depleting exercise augmented rates of whole-body leucine oxidation during a subsequent exercise

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bout. This greater leucine oxidation was also concomitant with an efflux of amino acids from skeletal muscle (10), suggesting low-CHO availability also accelerates muscle protein breakdown as a means to provide substrates for oxidative metabolism. Given that exercise-induced amino acid oxidation includes the essential and, especially, branched-chain amino acids, these important substrates must ultimately be replaced through dietary sources to support postexercise rates of protein synthesis during recovery (8). Thus, although it is established that exercising with low-CHO availability increases amino acid oxidation during exercise (10), there is limited information on whether these oxidative losses ultimately translate into a heightened dietary protein requirement.

Commencing select endurance training sessions with low-CHO availability has become popular to periodize throughout an endurance-training program as a means to enhance markers of aerobic adaptation (12–14). Recently, a "sleep-low" paradigm has been employed whereby athletes withhold CHO after intense evening training sessions before performing a morning exercise session under low-CHO availability (15,16). Compared with performing all training sessions with high-CHO availability, "sleep low" has been shown to augment several exercise responsive oxidative genes (16) and improve endurance performance after 3 wk (15). However, given the reciprocal relationship between CHO availability and amino acid oxidation during exercise (9,10), and the importance of dietary protein to support muscle protein synthesis (17) and postexercise recovery (6,7), it remains to be determined if low-CHO availability training influences dietary protein requirements. Therefore, the purpose of the present study was to determine the impact of contemporary low-CHO availability training on estimates of protein requirements of endurance athletes. Using the IAAO technique, we hypothesized that commencing endurance exercise with low CHO availability would increase phenylalanine oxidation (the reciprocal of protein synthesis) during exercise recovery, which would reflect a heightened protein requirement after training.

MATERIALS AND METHODS

Subjects and Ethics Approval

Eight endurance-trained males who regularly ran 56 ± 16 km·wk^{-1} participated in the study. Participant characteristics are shown in Table 1. The experimental protocol was approved by the University of Toronto Health Sciences Research Ethics Board, and all procedures were conducted in accordance with the Declaration of Helsinki. All participants were informed of the study purpose, experimental procedures, and potential risks before obtaining written informed consent.

TABLE 1. Participant characteristics.

Age (yr)	27 ± 4
Body mass (kg)	75 ± 10
Height (cm)	180 ± 7
Fat-free mass (kg)	66 ± 7
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	67 ± 10
Resting energy expenditure (kcal·d^{-1})	1614 ± 267
Habitual training distance (km·wk^{-1})	56 ± 16

Values are means ± SD.

Experimental Protocol

Baseline testing. After an 8-h overnight fast and minimum 7 h of sleep, body composition was assessed using air-displacement plethysmography (BodPod; Cosmed USA Inc., Chicago, IL). Subsequently, participants sat comfortably for 20 min for determination of resting energy expenditure (REE) using indirect calorimetry (iWorx GA-300, Dover, NH), with an average of the final 15 min used for analyses. Participants consumed a CHO beverage (1.2 g·kg^{-1}) before performing an incremental peak oxygen uptake ($\dot{V}O_{2peak}$) test on a powered treadmill (LifeFitness 9500HR, Mettawa, IL) as previously described (4). Briefly, participants ran for 2 min at a constant self-selected speed at 0% grade, after which the treadmill on the incline increased by 2% every 2 min until volitional fatigue (11.8 ± 1.4 min). Rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production (VCO_2) were acquired through a metabolic cart with an online gas collection system (iWorx GA-300), and HR was measured using a chest-worn strap (HR; Polar A3, Lake Success, NY). After a brief rest, participants performed a 10-km familiarization run on a treadmill. Participants began running at 8.9 km·h^{-1}, after which, the speed was increased by investigators in an effort to find the pace eliciting 80% of maximal HR (HRmax), which was recorded for subsequent metabolic trials.

Metabolic Trial Overview

The design of the present study was modeled from that of a previous acute "sleep-low" training protocol (16) (Fig. 1). Participants completed two metabolic trials in a randomized crossover design, with each trial separated by a minimum of 5 d. In each trial, participants performed an evening session of high-intensity interval training (HIIT) on day 1, followed by a moderate-intensity 10 km run on the morning of day 2. In the low-CHO availability trial (LOW), participants consumed the majority of their daily CHO intake before the evening HIIT session (7.8 g·kg^{-1}), and subsequently withheld CHO postexercise and overnight (0.2 g·kg^{-1}). In the high-CHO availability trial (HIGH), participants consumed less than half of their daily CHO intake before the evening HIIT session (3 g·kg^{-1}), with the majority of CHO consumed postexercise (5 g·kg^{-1}). In both trials, participants left the laboratory overnight before returning in the morning of day 2 to perform the 10-km run in either the fasted- (LOW) or CHO-fed (1.2 g CHO·kg^{-1}; HIGH) state. Immediately after the 10-km run, participants received a postexercise meal (LOW: 1.8 g·kg^{-1}; HIGH: 0.6 g·kg^{-1}) to ensure groups were energy-matched before commencing the 8-h IAAO protocol (described below).

Pretrial Dietary and Exercise Control

Participants performed a 5-km run on their own supervision and consumed a controlled diet consisting of commercially

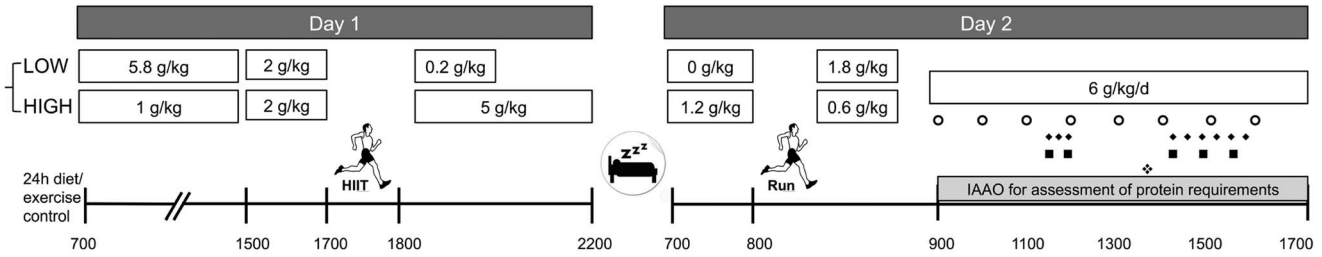

FIGURE 1—CHO periodization in LOW and HIGH before determination of postexercise protein requirements. 10 × 5 min run intervals at 10 km race pace, 1 min recovery; Run, 10 km run at ~80% HRmax.

available prepackaged foods 24 h before the metabolic trial. The standardized exercise ensured participants performed physical activity broadly similar to their habitual training, which was verified by accelerometer (Actigraph; GT3X-BT, Pensacola, FL). The 24-h controlled diet supplied sufficient energy calculated as 1.6× REE plus the exercise-induced energy expenditure of the 5-km run estimated as 1 kcal·kg^{-1}·km^{-1} (18). In accordance with CHO and protein recommendations for endurance athletes (1) the diet supplied 8 g CHO·kg^{-1}·d^{-1} and 1.4 g protein·kg^{-1}·d^{-1}, with the remaining calories provided as dietary fat (1.15 ± 0.22 g·kg^{-1}). Dietary checklists were used to verify adherence to the controlled diet.

Experimental Diet

In both trials, participants consumed diets for the preceding 24 h before the 10-km run that were energy (1.6× REE plus 1 kcal·kg^{-1}·km^{-1}; 14,954 kJ ± 1895 kJ [3574 ± 453 kcal]) and macronutrient-matched (8 g CHO·kg^{-1}, 1.4 g protein·kg^{-1}) with only the timing of CHO consumption differing, as summarized in Fig. 1. After accounting for CHO and protein intake, dietary fat was provided to meet individual energy needs and thus was consistent within—but not between—participants (1.15 ± 0.22 g·kg^{-1}). On day 1, participants were provided with prepackaged foods to be consumed before reporting to the laboratory at 3:00 PM, which consisted of 5.8 g CHO·kg^{-1}, 0.85 g protein·kg^{-1} and ~0.80 g fat·kg^{-1} in LOW and 1 g CHO·kg^{-1}, 0.68 g protein·kg^{-1} and ~0.52 g fat·kg^{-1} in HIGH. At 3:00 PM, participants in LOW and HIGH consumed a standardized preexercise meal in the laboratory (rice, mixed vegetables, butter, deli meat, apple sauce, and juice) providing 2 g CHO·kg^{-1}, 0.25 g protein·kg^{-1}, and 0.25 g fat·kg^{-1} (both conditions). Immediately after HIIT, LOW received a beverage and cookie providing 0.2 g CHO·kg^{-1}, 0.3 g protein·kg^{-1}, and 0.1 g fat·kg^{-1}, and consumed nothing thereafter for the remainder of the evening. In HIGH, participants consumed the same postexercise beverage and cookie, with an additional 1 g·kg^{-1} CHO added to the drink (1.2 g CHO·kg^{-1}, 0.3 g protein·kg^{-1} and 0.1 g fat·kg^{-1}). Participants in HIGH also consumed a further 3.8 g CHO·kg^{-1}, 0.17 g protein·kg^{-1} and 0.28 g fat·kg^{-1} throughout the remainder of the evening, with CHO provided at a rate of 1.2–1.4 g·kg^{-1}·h^{-1} for 3 h to maximize rates of muscle glycogen replenishment (19). Therefore, both groups consumed 0.3 g·kg^{-1} of BCAA-enriched whey protein immediately after exercise to help replenish any amino acid oxidative losses and support postexercise muscle protein synthesis with the additional small amount of protein (i.e., 0.17 g·kg^{-1}) consumed by the HIGH group being lower quality (i.e., relatively BCAA- and EAA-deficient) plant-based proteins associated with the CHO-dense foods (e.g., rice, mixed vegetables) (20).

On the morning of day 2, participants in LOW remained fasted, whereas HIGH consumed 1.2 g CHO·kg^{-1} 1 h before performing a 10-km treadmill run. Immediately after exercise, LOW and HIGH consumed a protein-free meal (drink and cookies), providing sufficient energy to replace the exercise-induced energy expenditure (1 kcal·kg^{-1}·km^{-1}) and ensure groups were in energy and macronutrient balance prior postexercise measurements. Thus, LOW received 1.8 g CHO·kg^{-1} and 0.31 g fat·kg^{-1} and HIGH received 0.6 g CHO·kg^{-1} and 0.31 g fat·kg^{-1} postexercise.

High-Intensity Evening Exercise Session

On the evening of day 1, participants performed 10 × 5 min run intervals at 10-km race pace, interspersed with 1 min of recovery. We used running as opposed to cycling protocol to ensure activation and glycogen utilization of the gastrocnemius and soleus muscles, in addition to the vastus lateralis (21) as well as to be consistent with our previous IAAO studies (4,8). The protocol was modified from the 6 × 5 min run intervals at 10-km race pace used previously in a "sleep-low" training study (15). Although Marquet and colleagues (15) did not quantify muscle glycogen utilization, 5 to 6 × 3 min run intervals at 90% $\dot{V}O_{2peak}$, involving 15 to 18 min of intermittent running (compared with 50 min in the present study) has been reported to lower glycogen content of the vastus lateralis and gastrocnemius by 35% (22,23) and 45% (23), respectively. During HIIT, HR was recorded at the end of each interval.

Moderate-Intensity Morning Exercise Session

On the morning of day 2, participants performed a 10-km run at ~80% HRmax using speeds that were predetermined during baseline testing. This intensity and duration of exercise is similar to previous sleep-low studies (15,24). HR was monitored continuously whereas gas exchange was measured at 2 and 8 km for 7 min to quantify oxygen consumption and fuel utilization. Whole-body rates of CHO and fat oxidation (g·min^{-1}) were calculated from the final 5 min of respiratory data at each collection point using standardized

equations (25) and reported as an average. HR was averaged over the full exercise session.

Assessment of Protein Required to Maximize Whole Body Protein Synthesis

After the 10-km run and postexercise meal, we used the IAAO technique to assess differences in postexercise phenylalanine metabolism as an estimate for protein requirements, as previously described (8). Participants consumed eight hourly isoenergetic meals that included a beverage and protein-free cookie (4). Each meal provided 1/12th of the participants total daily energy requirement and a protein intake of 0.93 $g \cdot kg^{-1}$. Protein was provided as crystalline amino acids modeled on the basis of egg protein with the exception of phenylalanine (the indicator amino acid; 30.5 $mg \cdot kg^{-1} \cdot d^{-1}$) and tyrosine (40 $mg \cdot kg^{-1} \cdot d^{-1}$), which were provided in excess to ensure the indicator amino acid was directed toward oxidation (26). The amino acid profile of egg protein was selected because it is the current reference standard complete protein (27) and is consistent with previous IAAO studies (4,8,28). A protein intake of 0.93 $g \cdot kg^{-1} \cdot d^{-1}$ was selected as an intake estimated to be insufficient to maximize whole-body protein synthesis based on previous research from our laboratory in endurance-trained athletes (4). When an indispensible amino acid is deficient for protein synthesis, all other amino acids, including the indicator amino acid, cannot be directed toward synthesis, and as a result are oxidized (28). Thus, a greater deficiency of one or more indispensible amino acids (and hence a greater protein requirement to maximize protein synthesis) would result in higher oxidation of the indicator amino acid. As protein intake was controlled for on the metabolic trials (0.93 $g \cdot kg^{-1}$ in LOW and HIGH), differences in the oxidation of the indicator amino acid can be attributed to a difference in protein requirement that is the result of the periodized CHO/exercise intervention. The remaining macronutrients in the study meals provided sufficient energy (1.6× REE) and 6 g $CHO \cdot kg^{-1} \cdot d^{-1}$. At meal 5, a priming dose of $NaH^{13}CO_3$ (0.176 $mg \cdot kg^{-1}$; CIL Canada, Inc.) and L-[^{13}C]phenylalanine (1.86 $mg \cdot kg^{-1}$; CIL Canada, Inc.) was ingested (4,28). The remaining hourly meals provided 1.20 mg L-[^{13}C]phenylalanine$\cdot kg^{-1}$ to maintain isotopic steady state until the end of the metabolic trial (4).

Sample Collection and Analyses

Three baseline breath samples and two baseline urine samples were collected at 15-min intervals during the hour before consuming meal 5. Six plateau breath samples were collected every 15 min and three plateau urine samples were collected every 30 min, 2.5 h after consuming meal 5. Steady-state CO_2 production (VCO_2) was measured for 20 min at 30 min after meal 5 using indirect calorimetry (iWorx GA-300). Breath samples were analyzed for $^{13}CO_2$ enrichment by continuous-flow isotope ratio mass spectrometry (IDmicro Breath; Compact Science Systems, Staffordshire, UK). Urine samples were stored at −20°C before measurement of [1-^{13}C]phenylalanine enrichment by liquid chromatography tandem mass spectrometry (28) to determine whole-body phenylalanine flux (PheRa, $\mu mol \cdot kg^{-1} \cdot h^{-1}$) and oxidation (PheOX; $\mu mol \cdot kg^{-1} \cdot h^{-1}$) as previously described (4).

Tracer Kinetics

[^{13}C]phenylalanine excretion ($F^{13}CO_2$) was determined with a bicarbonate retention factor of 0.82 and standard equations, as previously described (4). The PheRa and PheOX were calculated according to traditional IAAO methodology (29) and as described previously (4). Net protein balance was calculated as the algebraic difference between nonoxidative phenylalanine disposal and PheRa and assuming a phenylalanine content of 4% in body protein (30). To determine the difference in protein recommendations between LOW and HIGH, PheOX (y) of this suboptimal protein intake was fitted to the following slope (m) and y-intercept (b), as estimated from our previous study: m = 7.2 $\mu mol \cdot h^{-1} \cdot (g \cdot d^{-1})$ and b = 16.13 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (4). The difference in protein recommendations (x) between LOW and HIGH were calculated using $y = mx + b$.

Statistical Analyses

A paired Student's t test was used to detect differences in variables between LOW and HIGH. GraphPad Prism (v6.00; GraphPad Software, San Diego, CA) was used for statistical analyses and significance was set at $P < 0.05$.

RESULTS

Descriptive characteristics of exercise. Participants completed the evening 10 × 5 min run intervals at 15.6 ± 1.1 $km \cdot h^{-1}$, which elicited 93 ± 3 and 93 ± 1 $\%HR_{max}$ in LOW and HIGH, respectively (data not shown, P = 0.46). The morning 10-km run was performed at 13.0 ± 1.1 $km \cdot h^{-1}$, which corresponded to 62 ± 4 and 62 ± 8 $\%\dot{V}O_{2peak}$ and elicited 81 ± 3 and 81 ± 2 $\%HR_{max}$ in LOW and HIGH, respectively (Table 2, $P > 0.05$ for both variables). RER during the 10-km run was lower in LOW compared with HIGH (0.81 ± 0.05 vs 0.88 ± 0.05; Table 2, P = 0.008), which corresponded with an increased rate of fat oxidation and decreased rate of CHO oxidation during exercise in LOW (Table 2, P = 0.01 and P = 0.004, respectively).

Phenylalanine oxidation and tracer kinetics. Phenylalanine flux was not different between trials (Fig. 2A, P = 0.31). Phenylalanine oxidation was 11% higher in LOW compared with HIGH (Fig. 2B, P = 0.03). This resulted in an estimated difference in daily protein requirement of

TABLE 2. 10 km run performed with high or low-CHO availability.

	High	Low
Speed ($km \cdot h^{-1}$)	13.0 ± 1.1	13.0 ± 1.1
HR (% maximum)	81 ± 2	81 ± 3
$\dot{V}O_2$ (% maximum)	62 ± 8	62 ± 4
RER	0.88 ± 0.05	0.81 ± 0.05*
Fat oxidation ($g \cdot min^{-1}$)	0.60 ± 0.26	0.99 ± 0.34*
CHO oxidation ($g \cdot min^{-1}$)	2.2 ± 0.55	1.4 ± 0.54*

Values are means ± SD.
*Significantly different vs HIGH ($P < 0.05$).

APPLIED SCIENCES

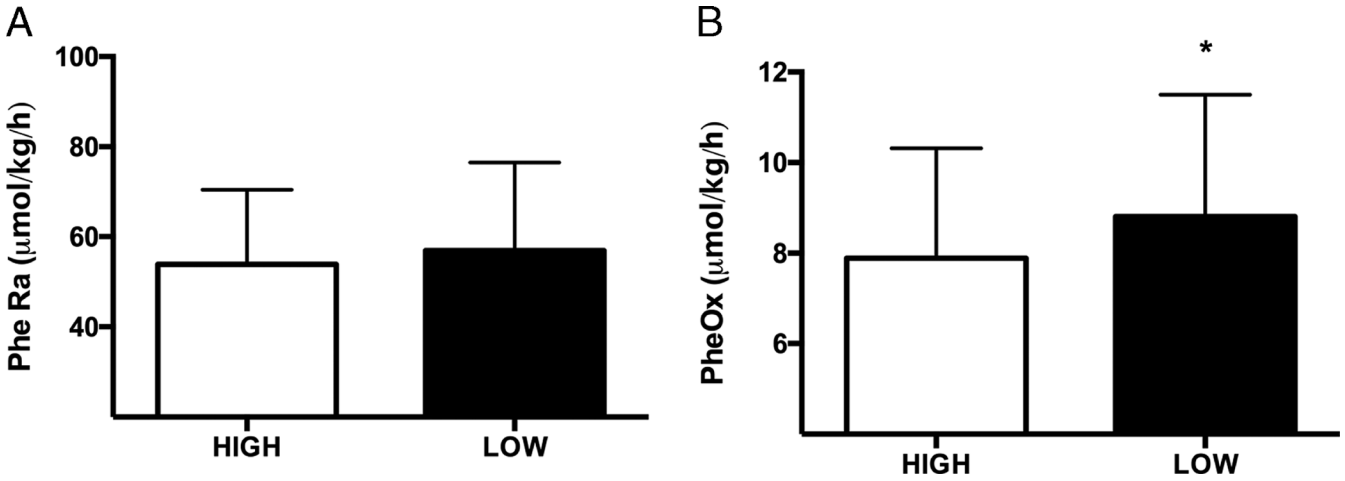

FIGURE 2—Phenylalanine oxidation and tracer kinetics. PheRa (A) and PheOx (B) after a 10-km run performed with low or high CHO availability. *Significantly different vs HIGH ($P < 0.05$).

0.12 $g \cdot kg^{-1} \cdot d^{-1}$. Net protein balance was 12% lower in LOW compared with HIGH during postexercise recovery (Fig. 3, $P = 0.03$).

DISCUSSION

Training with low-CHO availability can enhance markers of aerobic adaptation and has become a popular strategy to periodize throughout an endurance-training program (12,13,15). Using contemporary strategies for low-CHO availability training, our results demonstrate that performing endurance exercise with low-CHO availability increases the oxidation of our indicator amino acid after exercise, which suggests an increased dietary protein requirement during postexercise recovery. Our design involved withholding CHO after an intense evening training session and sleeping with low-CHO availability before performing a moderate-intensity morning run in the fasted state. This strategy—termed "sleep-low"—has previously been shown to improve athletic performance compared with performing all training sessions with high-CHO availability over a 3-wk intervention (15). Given the importance of dietary protein for postexercise remodeling of muscle proteins that provide the basis of many training-induced physiological and performance adaptations, our findings may have important implications for optimizing recovery in athletes performing endurance sessions with low-CHO availability.

Individuals involved in regular endurance training have a greater daily protein requirement than their sedentary counterparts (2,4) with estimates using the IAAO method as high as ~1.8 $g \cdot kg^{-1} \cdot d^{-1}$ on a day in which they run 20 km (4). This increased protein requirement may reflect in part the need to replenish amino acid oxidative losses incurred during exercise (especially of the BCAA) and to provide amino acid substrates to support whole-body protein synthesis (5,6). Although amino acids have been estimated to contribute ~5% of total energy provision during endurance exercise (5), amino acid oxidation may be exacerbated under conditions of low muscle glycogen availability and, based on urea production, contribute up to ~10% of total energy expenditure during 1 h of cycling (9). In addition, activity of the branched-chain oxo-acid dehydrogenase complex, the limiting step in skeletal muscle BCAA oxidation, is increased in the presence of low muscle glycogen availability (31,32), which likely contributes to an enhanced exercise-induced whole-body leucine oxidation secondary to an increase in muscle protein catabolism (10). Collectively, these data suggest that protein catabolism is increased during exercise, including an estimated twofold increase in amino acid oxidation (9), in the glycogen-depleted state. Although we did not directly measure muscle glycogen in the present study, our high-intensity interval exercise protocol and postexercise dietary intervention was modeled after a similar "sleep low" paradigm that resulted in a ~50% lower muscle glycogen content in LOW compared with HIGH before initiating the morning exercise (16). In addition, we observed an approximately 35% lower rate of CHO oxidation during the morning run in LOW, suggesting our athletes were indeed training in a low-CHO availability state that would have presumably increased total amino acid oxidation.

The IAAO methodology has been developed to assess the amino acid and/or protein sufficiency of a diet (28), whereby

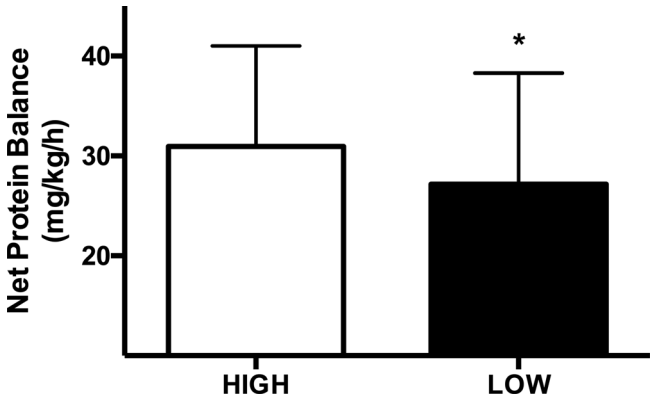

FIGURE 3—Postexercise protein balance. Net protein balance in LOW and HIGH after a 10-km run performed with low or high CHO availability. *Significantly different vs HIGH ($P < 0.05$).

any limitation in one or multiple amino acids would result in less indicator amino acid (i.e., [^{13}C]phenylalanine) being used to support whole body protein synthesis and more directed to oxidative catabolism. By testing athletes on a relatively protein-deficient diet, our study is better able to titrate the effect of different postexercise amino acid requirements (i.e., at protein intakes below optimal). Thus, our finding of a greater phenylalanine oxidation during recovery in LOW is the first to suggest that postexercise dietary protein requirements are increased when athletes perform low-CHO availability training. We speculate the elevated protein requirement is primarily related to the need to replace exercise-induced amino acid losses incurred via direct oxidation in muscle mitochondria and/or amino acids released from muscle for hepatic gluconeogenesis (5).

Given the importance of dietary protein to support training-induced physiological adaptations (6), our findings suggest that athletes partaking in low-CHO availability training may benefit from an increased dietary protein intake. Based on our previous IAAO protein requirement study in endurance athletes, the difference in PheOx between LOW and HIGH consuming a suboptimal intake (i.e., below the estimated average requirement) in the present study reflects an estimated difference in protein requirement of ~0.12 g·kg^{-1}·d^{-1} (4). Given that leucine oxidation scales with energy expenditure (33) the impact of low-CHO availability training on protein requirements may be greater in athletes exercising for a longer duration and/or at a higher intensity than in the present study. Nevertheless, this small increase in protein requirements highlights the importance of considering nutritional strategies that not only augment skeletal muscle remodeling (17), but also replace whole body oxidative amino acid losses during postexercise recovery (34). Although it is possible that rates of whole body protein synthesis may not necessarily reflect changes within specific tissues (e.g., skeletal muscle), endurance exercise may be associated with physiological adaptations (e.g., red blood cell synthesis, albumin synthesis for plasma volume expansion, etc.) that are extramuscular and may require a more whole-body protein metabolic perspective. For example, we recently demonstrated that athletes consuming a protein intake that maximizes IAAO-determined rates of whole body protein synthesis (i.e., ~1.8 g·kg^{-1}·d^{-1}) (4) maintained whole body protein balance and exercise performance over a period of intensified training to a greater extent than when consuming a suboptimal (i.e., ≤1.2 g·kg^{-1}·d^{-1}) protein intake (7). This may explain in part the greater performance adaptations reported in previous low-CHO availability training studies when consuming higher (i.e., ~1.6–1.9 g·kg^{-1}·d^{-1}) (15,24) compared with lower (i.e., ~1.3 g·kg^{-1}·d^{-1}) (23,35) protein intakes, as the former may have been more effective at replacing amino acid oxidative losses and supporting postexercise protein remodeling. Therefore, in addition to recommendations for periodizing CHO and fat to enhance aerobic adaptations and endurance performance (36), our results suggest that dietary protein intake could also be an important factor in the potential success of low-CHO availability training. Although available evidence suggests that athletes generally consume protein within the recommended range of 1.2 to 2.0 g·kg^{-1} provided energy intakes are sufficient, approximately 50% may consume protein at or below approximately 1.5 g·kg^{-1}·d^{-1} with up to approximately 40% from relatively BCAA-deficient (i.e., plant) protein sources (37), which are preferentially oxidized during exercise and may be the primary limiting amino acids for endurance athletes (5,8). Based on the present data, we suggest that athletes partaking in low-CHO availability training could consume a slightly greater postexercise protein intake (especially from BCAA-enriched whole foods (38) than that previously suggested to optimize muscle protein synthesis (i.e., ~0.4 vs 0.25 g·kg^{-1} (17,39) or additional protein feedings in close proximity to exercise cessation in an effort to replace amino acid oxidative losses and facilitate skeletal muscle remodeling (34,40).

The nutritional periodization in the present study mirrors contemporary strategies for low-CHO availability training (15,16) such that energy and macronutrient composition, as well as training load and timing, were not altered to achieve the low-CHO state. Previous strategies have simultaneously increased training load (11,31) or reduced daily energy (9) and/or CHO intake (9,10) in the low-CHO condition which, in addition to having potentially limited relevance to real-life athletic practices (41), arguably preclude interpretation on the importance of glycogen availability *per se*. Moreover, the low-CHO diet that preceded the experimental trial in one study (10) resulted in a twofold greater protein intake (2.4 g·kg^{-1} vs 1.1 g·kg^{-1} in the high-CHO), which can also independently increase amino acid oxidation during exercise (42). To circumvent these limitations, the present study standardized training load and diet before the experimental exercise sessions, providing daily energy, CHO and protein intake in accordance with consensus recommendations for endurance athletes in both LOW and HIGH (1). Thus, the increased protein requirement observed in LOW was achieved by strictly modifying the timing of nutritional intake around exercise, which is a seemingly modest approach compared with earlier low-CHO training strategies. Although we cannot discount the possibility that the small amount of protein consumed by the HIGH group after the evening training bout did not impact the next day protein requirement, we speculate that the 0.3 g·kg^{-1} of BCAA-enriched protein consumed by both groups would have helped facilitate postexercise recovery in the evening and that the relative difference in estimated requirements between LOW and HIGH on the trial day was primarily influenced by differences in metabolism and fuel use during the low-CHO availability exercise bout. Nevertheless, we speculate that low-CHO availability approaches that simultaneously decrease daily CHO intake and/or increase training load may further increase amino acid oxidation during exercise and augment dietary protein requirements above that observed in the present study. It is also possible that the heightened protein requirement in the present study may be due, in part, to the need to repair/remodel damaged skeletal muscle proteins from the running-based protocol, which may

have occurred secondary to a greater exercise-induced mobilization of these endogenous amino acids in a low glycogen availability state (10).

To conclude, the present study demonstrates that performing endurance exercise with low-CHO availability increases dietary protein requirements in postexercise recovery. The increase in daily requirement is estimated to be relatively small (~0.12 g protein·kg^{-1}·d^{-1}) and may not reflect protein requirements on nontraining days. Nonetheless, our findings may be useful for the development of nutritional guidelines to enhance postexercise recovery in athletes incorporating periodized low-CHO availability exercise into their training programs. Future research should consider the impact dietary protein intake (especially from whole foods) has on postexercise recovery, skeletal muscle adaptations, and performance outcomes with contemporary low-CHO availability training strategies.

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