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RESEARCH ARTICLE

Effect of carbohydrate ingestion on central fatigue during prolonged running exercise in moderate hypoxia

Hunter L. Paris,[1] Timothy J. Fulton,[1] Robert F. Chapman,[1] Alyce D. Fly,[2] David M. Koceja,[1] and Timothy D. Mickleborough[1]

[1]*Department of Kinesiology, School of Public Health-Bloomington, Indiana University, Bloomington, Indiana; and*
[2]*Department of Applied Health Science, School of Public Health-Bloomington, Indiana University, Bloomington, Indiana*

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Paris HL, Fulton TJ, Chapman RF, Fly AD, Koceja DM, Mickleborough TD. Effect of carbohydrate ingestion on central fatigue during prolonged running exercise in moderate hypoxia. *J Appl Physiol* 126: 141–151, 2019. First published November 9, 2018; doi:10.1152/japplphysiol.00684.2018.—To determine whether acute exposure to moderate hypoxia alters central and peripheral fatigue and to test whether carbohydrate ingestion impacts fatigue characteristics, 12 trained runners completed three running trials lasting 1 h each at 65% of normoxic maximum oxygen uptake. The first trial was performed in normoxia [inspired O_2 fraction ($F_{I_{O_2}}$) = 0.21], and the last two trials were completed in hypoxia ($F_{I_{O_2}}$ = 0.15). Participants ingested a placebo drink in normoxia (NORM-PLA), a placebo drink in hypoxia (HYP-PLA), or a carbohydrate solution in hypoxia (HYP-CHO). HYP conditions were randomized. Peripheral [change in potentiated quadriceps twitch force ($\Delta Q_{tw,pot}$)] and central [change in voluntary activation (ΔVA)] fatigue were assessed via preexercise-to-postexercise changes in magnetically evoked quadriceps twitch. In HYP, blood was drawn to determine the ratio of free-tryptophan (f-TRP) to branched-chain amino acids (BCAA). After exercise, peripheral fatigue was reduced to a similar degree in normoxia and hypoxia ($\Delta Q_{tw,pot}$ = −4.5 ± 1.3% and −4.0 ± 1.5% in NORM-PLA and HYP-PLA, respectively; P = 0.61). Central fatigue was present after normoxic and hypoxic exercise but to a greater degree in HYP-PLA compared with NORM-PLA (ΔVA: −4.7 ± 0.9% vs. −1.9 ± 0.7%; $P < 0.01$). Carbohydrate ingestion did not influence central fatigue (ΔVA in HYP-CHO: −5.7 ± 1.2%; P = 0.51 vs. HYP-PLA). After exercise, no differences were observed in the ratio of f-TRP to BCAA between HYP-PLA and HYP-CHO (P = 0.67). Central fatigue increased during prolonged running exercise in moderate hypoxia although the ratio of f-TRP to BCAA remained unchanged. Ingesting carbohydrates while running in hypoxia did not influence fatigue development.

NEW & NOTEWORTHY Hypoxic exposure influences the origin of exercise-induced fatigue and the rate of fatigue development depending on the severity of hypoxia. Our data suggest that moderate hypoxia increases central, but not peripheral, fatigue in trained runners exercising at 65% of normoxic maximum oxygen uptake. The increase in central fatigue was unaffected by carbohydrate intake and occurred although the ratio of free tryptophan to branched-chain amino acids remained unchanged.

altitude; peripheral fatigue; quadriceps twitch, sports nutrition

INTRODUCTION

Endurance exercise capacity is limited by the onset and development of fatigue, which can originate from both peripheral (muscle) and central (central nervous system) locations within the body. Whereas peripheral fatigue is often associated with short-duration, high-intensity exercise, central fatigue is more commonly observed during prolonged exercise of moderate or light intensity (32, 37, 54). Exercise modality also influences the etiology of fatigue, with weight-bearing, full-body exercise (e.g., running) incurring a greater degree of central fatigue compared with modalities that recruit less muscle mass (e.g., cycling or single-limb exercise) (32). In conjunction with intensity, duration, and modality, fatigue origin can also vary with environmental conditions such as hypoxia.

The influence of hypoxia on fatigue development depends on the severity of hypoxia, such that moderate hypoxia (3,000 m) amplifies the rate of peripheral fatigue development but severe hypoxia (5,500 m) diminishes work capacity via central inhibition (33, 50). Indeed, central fatigue predominates over peripheral fatigue only when hypoxia is severe (3). Reliance upon single-leg knee extension or cycling exercise, however, may prioritize peripheral factors and limit the impact that moderate hypoxia can have on central fatigue. Therefore, the possibility exists that moderate hypoxia may limit exercise capacity via central mechanisms, but only when the type of exercise performed is susceptible to central fatigue (e.g., prolonged, weight-bearing exercise).

One theory put forth to explain the relationship between prolonged exercise and central fatigue is the serotonin-fatigue hypothesis. This model posits that the ratio of free tryptophan (f-TRP) to branched-chain amino acids (BCAA) found in the blood alters the rate of serotonin-induced fatigue development and that this ratio is partially controlled by circulating concentrations of free fatty acids (FFA) (1, 36, 59). Specifically, the serotonin-fatigue hypothesis suggests that prolonged exercise mobilizes FFA, which releases TRP from albumin and allows the nascent f-TRP to cross the blood-brain barrier, where it is converted to serotonin. Serotonin is associated with feelings of drowsiness and lethargy (59), and when administered to cyclists a serotonin reuptake inhibitor significantly reduced exercising time to exhaustion compared with a placebo (57). To achieve this cascade of events, f-TRP must outcompete BCAA for transport across the blood-brain barrier. Therefore, the ratio of f-TRP to BCAA is a stronger marker of fatigue than individual amino acid concentration alone.

Address for reprint requests and other correspondence: T. D. Mickleborough, Dept. of Kinesiology, School of Public Health-Bloomington, Indiana Univ., Suite 112, 1025 East 7th St., Bloomington, IN 47405 (e-mail: tmickleb@indiana.edu).

Hypoxia upregulates the mobilization of FFA (20, 24, 53, 58), and thus the serotonin-fatigue hypothesis represents a potential explanation for hypoxia-induced increases in central fatigue. According to the serotonin-fatigue postulate, interventions that reduce the ratio of f-TRP to BCAA can attenuate the development of central fatigue (10, 11). Carbohydrate ingestion may represent one such intervention. Several studies (37, 51) have examined the influence of carbohydrate intake on measures of central fatigue, but findings have been inconsistent, likely because of differences in exercise modality. Similar to studies using hypoxia, the use of cycling exercise may limit the magnitude of central fatigue and therefore limit the impact that carbohydrate ingestion can have on central fatigue development. For example, a recent systematic review (22) examining the influence of carbohydrate ingestion on central fatigue utilized seven studies for analysis, all of which employed cycling exercise.

Whereas the impact of carbohydrate ingestion on central fatigue during cycling exercise in normoxia has produced conflicting results, the potential for carbohydrate ingestion to attenuate central fatigue may be most apparent when using a modality and environment where central fatigue is upregulated (e.g., running exercise in hypoxia). Therefore, the purpose of our study was *1*) to characterize the fatigue development elicited by prolonged running exercise performed at a moderate hypoxia and *2*) to assess whether carbohydrate intake alters fatigue development in hypoxia. We hypothesized that moderate hypoxia would significantly increase both peripheral and central fatigue during running exercise and that ingesting carbohydrates during prolonged running in hypoxia would attenuate the development of central and peripheral fatigue.

METHODS

Participants

Participant characteristics are provided in Table 1. Twelve endurance-trained men participated in the study, all of whom were competitive runners or triathletes involved in endurance activities on a regular basis (≥5 sessions/wk). Women were not included in this study since it has been shown that women differ from men in regard to fatigue resistance (2) and also to limit any possible confounding hormonal factors (e.g., progesterone and estrogen) that could influence our primary dependent variables (26, 47, 48). Participants were excluded if they had a current or recent (≤2 mo) injury, were not currently engaged in endurance-type exercise at least 5 times/wk, or were reluctant to adhere to dietary requirements before exercise. Participants were tested at the same time of day for each of the experimental visits and were instructed to arrive at the laboratory on a 12-h fast having abstained from alcohol for the previous 24 h and from caffeine for the previous 12 h. All testing procedures and the informed consent statement were approved by the Indiana University Human Subjects Committee (protocol no. 1701018961). Before giving written informed consent, all participants completed a health and medical questionnaire to screen for cardiovascular, metabolic, and pulmonary disease and were informed of possible risks.

Table 1. *Participant characteristics*

Variable	Mean ± SE
Age, yr	25 ± 1
Body mass, kg	70.5 ± 2.0
Height, cm	179.5 ± 1.1
$\dot{V}O_{2max}$, l/min	5.0 ± 0.1
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	71.0 ± 1.4
Training history, days/wk	6 ± 1
Training history, min/days	57.1 ± 6.1

n = 12 participants. $\dot{V}O_{2max}$, maximum oxygen uptake.

Study Design

Participants completed four visits to the laboratory. A repeated-measures design was implemented in which hypoxic conditions (*visits 3* and *4*) were counterbalanced to avoid any order effect. Participants were blinded to the inspired O_2 fraction ($F_{I_{O_2}}$) they were breathing as well as to the composition of drinks they were consuming, whereas the investigators were blinded only to the composition of the drinks. *Visits 2–4* occurred 1 wk apart, at the same time of day. All exercise sessions took place on a motorized treadmill. *Visit 1* consisted of an incremental running exercise test to exhaustion in normoxia to determine maximum oxygen uptake ($\dot{V}O_{2max}$). Participants with the qualifying $\dot{V}O_{2max}$ ($\dot{V}O_{2max}$ > 55 ml·kg^{-1}·min^{-1}) were familiarized with all testing procedures, including maximal voluntary contractions (MVCs) and magnetic stimulation techniques, which were used to quantify central and peripheral fatigue. *Visit 2* consisted of a 1-h run in normoxia ($F_{I_{O_2}}$ = 0.21, ~230 m) at a speed eliciting 65% of $\dot{V}O_{2max}$. At *minutes 15*, *30*, and *45* participants ingested a noncaloric sweetener (NORM-PLA). For *visits 3* and *4*, participants ran for 1 h in hypoxia ($F_{I_{O_2}}$ = 0.15, simulating ~3,000 m) at a speed eliciting 65% of normoxic $\dot{V}O_{2max}$. At *minutes 15*, *30*, and *45*, participants ingested either a carbohydrate drink (HYP-CHO) or a placebo drink (HYP-PLA) that was matched for sweetness. During *visits 3* and *4*, blood was drawn from an antecubital vein before exercise, at *minute 30* of exercise, and ~5 min after exercise for measurement of glucose, FFA, f-TRP, and BCAA. During *visits 2–4*, central and peripheral fatigue were assessed before exercise as well as immediately upon exercise termination.

Visit 1: $\dot{V}O_{2max}$ test. During *visit 1*, participants completed a running test to exhaustion on a motorized treadmill (model 18-72; Quinton, Bothell, WA). The running test began with 3 min of quiet standing for collection of resting ventilatory and metabolic data. After the resting period, participants ran for 3 min at each of four constant submaximal speeds of 12.1, 12.9, 13.8, and 14.9 km/h (8:00, 7:30, 7:00, and 6:30 min/mile, respectively) at 0% grade. After the third minute of running at 14.9 km/h the incline of the treadmill was raised to 2% for the next 2 min and continued to increase 2% every 2 min until volitional fatigue (i.e., participants voluntarily terminated the test by stepping off the treadmill). Criteria for the determination of $\dot{V}O_{2max}$ followed those used in published studies from our laboratory (12). During *visit 1*, participants were familiarized with MVC procedures as well as femoral magnetic stimulation techniques.

Visits 2–4: prolonged running exercise. During *visits 2–4*, participants ran for 1 h on a treadmill at a fixed speed while breathing either normoxic or hypoxic gas. Upon arrival at the laboratory, participants were weighed and adequate hydration was confirmed with a urine refractometer (PEN S.G., Atago). If participants arrived at the laboratory with urine specific gravity > 1.02 (38), they were provided with 300 ml of water to consume immediately. After the assessment of hydration, participants completed a training log and 24-h dietary recall (Nutrition Data System for Research 2017 version; University of Minnesota, Minneapolis, MN). Since muscle glycogen depletion can influence fatigue development (29), dietary recalls were used to ensure that macronutrient intake in the 24 h before exercise did not differ between visits and that participants complied with 12-h fasting requirements.

The velocity of the treadmill (average velocity = 13.0 ± 0.2 km/h) was determined according to the speed that would produce 65% of normoxic $\dot{V}O_{2max}$ and was maintained throughout the 1-h run. The rationale behind choosing this intensity (65% of $\dot{V}O_{2max}$) was based on *1*) a pace that allowed comparison with previous studies (27, 39, 45) and *2*) unpublished pilot data from our laboratory indicating the

pace that trained runners could consistently achieve while breathing 15% O_2. Before testing, participants completed a 5-min warm-up on the treadmill at the same speed as the exercise trial. Environmental conditions of the laboratory were kept consistent between visits (relative humidity ~30%, barometric pressure ~740 mmHg, temperature ~19.3°C).

Hypoxic Delivery System

Two 1,000-liter balloon reservoirs were placed on the inspired breathing line, distal to the inspired pneumotachograph. Before participant arrival, the balloons were filled to capacity with a gas composition of 15% O_2, balance N_2 with a nitrogen generator (CAT 12; Colorado Altitude Training, Boulder, CO). As participants breathed from one balloon, the other was filled with hypoxic gas, and thus participants were kept in hypoxia for the duration of exercise. Balloons were filled during all test days (NORM and HYP conditions) in order to blind participants to the inspirate. The balloon valves stayed closed during NORM tests so that participants breathed only room air. A secondary O_2 analyzer was used to determine the $F_{I_{O_2}}$ of inspired air in real time, and this value was continuously used for calculation of oxygen uptake.

Carbohydrate Ingestion

A 6% CHO or PLA solution was consumed at an ingestion rate of 15 $ml \cdot kg^{-1} \cdot h^{-1}$. For a 70-kg person, this equates to a consumption rate of ~16 g of carbohydrate mixed into 250 ml of water consumed every 15 min. This rate of ingestion and dosage of carbohydrate conform to the recommended ingestion procedure outlined in a 2017 review (22) examining carbohydrate consumption and central fatigue and are similar to what have previously been shown to effectively maintain euglycemia during endurance exercise (37). Additionally, this ingestion procedure provides carbohydrate at ~1.1 g/min, the maximum rate at which muscle can oxidize exogenous glucose (17). The intake rate of our study is similar to past procedures that resulted in minimal gastrointestinal discomfort (41). Glucose solutions were prepared by mixing a commercial dextrose powder (NOW Foods, Bloomingdale, IL) with tap water. Carbohydrate and placebo drinks were matched for sweetness, color, and flavor with a noncaloric artificial sweetener free of caffeine or added electrolytes (MiO Liquid Water Enhancer; Kraft Foods; 1 ml Mio added to CHO, 4 ml Mio added to PLA). To keep participants breathing the required normoxic or hypoxic gas during carbohydrate and placebo intake, solutions were ingested with the use of a straw that was threaded through the spit-trap port of the face mask worn throughout exercise.

Metabolic and Ventilatory Responses to Exercise

Metabolic and ventilatory variables were continuously measured during rest and exercise by open-circuit, indirect calorimetry with a customized metabolic cart. Participants wore an oronasal face mask (7450; Hans Rudolph, Shawnee, KS) and breathed through a low-resistance, two-way nonrebreathing valve (2700; Hans Rudolph) from which expired gases were collected in a 5-liter mixing chamber. Dried samples from this mixing chamber were used to determine fractional concentrations of O_2 and CO_2 by separate O_2 and CO_2 gas analyzers (S-3A and CD-3A; Ametek Thermox Instruments, Pittsburgh, PA). A pneumotachograph (series 3818/4813; Hans Rudolph) on the inspired line was used to measure minute ventilation ($\dot{V}_E$). Participants breathed either normoxic or hypoxic gas for the duration of exercise. During postexercise fatigue measurements, the participants continued to breathe the appropriate inspired gas by being temporarily connected to a Douglas bag filled with either normoxic or hypoxic air. Heart rate was measured with a telemetry transmitter (FT7; Polar Electro, Lake Success, NY). Arterial O_2 saturation (Sp_{O_2}) was estimated with a pulse oximeter (OxiMax N-600x; Nellcor, Minneapolis, MN). Ratings of perceived exertion (RPE; Borg original 6–20 scale) and dyspnea (modified 0–10 scale) (5) were obtained every 15 min.

Blood Measurements

During hypoxia visits (*visits 3* and *4*), 15 ml of blood was collected in EDTA-coated tubes and silicone-coated tubes with a clot activator for the analysis of plasma and serum, respectively. Blood samples were collected from participants upon arrival at the laboratory, after 30 min of exercise, and immediately after postexercise fatigue assessment. Samples were centrifuged at 1,300 *g* and 4°C for 10 min (model X-22R; Beckman, St. Louis, MO). After centrifugation, the supernatant was pipetted from the blood collection tube, transferred to microfuge tubes for storage at −80°C, and later measured in duplicate for glucose, FFA, and amino acids. Glucose was determined with a colorimetric assay [lower limit of detection: 0.23 mg/dl; intra-assay precision: coefficient of variation (CV) $< 4.6\%$; interassay precision: CV $< 1.7\%$] per the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI). The assay produced a pink hue, and absorption was read at 514 nm. FFA were also measured with a colorimetric assay (intra-assay precision: CV $< 10\%$; interassay precision: CV $< 15\%$) per the manufacturer's protocol (Cell Biolabs, San Diego, CA). Absorption was read at 540 nm. Both glucose and FFA were analyzed with a Powerwave XS spectrophotometer (Bio-Tek Instruments, Winooski, VT).

Serum amino acids were determined with the Phenomenex EZ: faast for Free (Physiological) Amino Acid Analysis by GC-MS Kit (Phenomenex, Torrance, CA). In brief, the procedure consisted of a solid-phase extraction, cleanup, derivatization of the amino acids, and liquid/liquid extraction. The resulting organic layer containing the derivatized amino acids was analyzed with a 10-m × 0.25-mm Zebron ZB-AAA chromatography column fitted to an Agilent 7890B/G7250 gas chromatograph (GC)-mass spectrometer (MS) (Santa Clara, CA) equipped with a Gerstel MPS Robotic Pro autosampler. The GC was operated in the constant-flow mode (He, 1.5 ml/min, 38.7 cm/s); the separation column fed into a purged union to which a 1.5-m length of 0.15-mm-internal diameter deactivated fused silica tubing was attached to transfer the analytes into the MS interface (total flow from union to MS = 1.6 ml/min). A split injection ratio of 1:100 was used, and the injector was held at 250°C and the MS transfer line at 300°C. The column oven temperature was programmed from 110°C to 320°C at 30°C/min and held at 320°C for 2 min. The overall run time was 9 min. The MS scanned 45–450 m/z at 6 scans/s. MassHunter Quan 9.00 (Agilent, Santa Clara, CA) was used to record and manage the chromatographic data, and all extracted ion chromatograms were extracted with a ±100-ppm mass window around the preferred fragment. The concentrations were found with internal standardization (norvaline added during extraction), and all final concentrations are expressed as nanomoles per milliliter of serum.

Force and Compound Muscle Action Potentials

Knee extensor force during voluntary and magnetically evoked contractions was used to quantify fatigue development and was measured with a calibrated load cell (model Z Tension Load Cell; Dillon, Fairmont, MN). The load cell was fixed to a table and connected to a noncompliant cuff attached just superior to the ankle malleoli of the participant's right leg. The height of the load cell was adjusted to each individual to maintain a direct line with applied force. Participants lay supine on the table with the right knee joint angle set at 90° of flexion. Compound muscle action potentials (M waves) were recorded from surface electrodes placed 4 cm apart over the muscle belly of the vastus lateralis. A reference electrode was placed over the patella. Evoked signals were amplified, band-pass filtered (EMG only: 60–500 Hz), digitized, and analyzed (AcqKnowledge Software v 5.0; BIOPAC Systems, Goleta, CA) for peak-to-peak amplitude.

Magnetic Stimulation

Magnetic stimulation (Magstim 200–2; Jali Medical, Newton, MA) of the femoral nerve was used to elicit a quadriceps twitch. The area of stimulation associated with the largest quadriceps twitch (Q_{tw}) and M-wave amplitudes was located by placing the magnetic coil high in the femoral triangle (40). This position was marked with indelible ink to ensure similar placement for subsequent stimulations. To confirm supramaximal nerve stimulation, three single twitches were obtained every 30 s at 50%, 60%, 70%, 80%, 85%, 90%, 95%, and 100% of the magnetic stimulator's maximal power output. A near plateau in Q_{tw} and M-wave amplitudes was observed in every participant, indicating maximal depolarization of the femoral nerve (Fig. 1). Twitch force at 100% of power output measured at the beginning of the progressive increase in power output was the same as that obtained at the end, indicating that the protocol did not elicit twitch potentiation. After confirmation of supramaximal stimulation, participants performed three repetitions of 5-s MVCs with 1-min rest between contractions. These MVCs were used to familiarize participants with performing a maximal contraction, although without the anticipation of receiving magnetic stimulation. A computer monitor provided participants with visual feedback after each MVC. These procedures occurred before warm-up exercise.

Fatigue Assessment

Peripheral and central fatigue were evaluated before and after exercise (<2 min after warm-up and exercise cessation) with magnetic stimulation of the femoral nerve. Preexercise measurements were obtained with participants breathing normal room air. For postexercise measurements, after the 1-h run participants were immediately attached to a Douglas bag containing either normoxic or hypoxic gas, depending on the condition of the visit. Q_{tw} was measured 5 s after a 5-s MVC. The potentiated quadriceps twitch ($Q_{tw,pot}$) has been shown to be more sensitive than the nonpotentiated twitch for detecting fatigue, especially when the degree of fatigue is small (25). Similar to previous studies (25, 43), the degree of potentiation was less after the first MVC, and occasionally the second MVC as well. Therefore, the first measurement in the fatigue assessment was not used, and the second measurement was used only when potentiation resembled that of the subsequent twitches. The MVC was used as a global index of fatigue (56). Activation of the quadriceps during the MVC was assessed with a superimposed twitch (SIT) technique (31, 52). The force produced during a SIT at the peak of the MVC was compared with the force produced by the potentiated twitch delivered 5 s afterward (3, 52): voluntary activation (VA, %) = [1 – (SIT/$Q_{tw,pot}$)] × 100. As has been done previously (52), a correction was applied to the SIT because it did not always occur at the MVC plateau. Even when the correction was applied, if superimposed twitches did not occur within 0.5 s of the plateau they were excluded from analysis. A decline in VA after exercise was used to indicate a reduction in central drive to the muscle (i.e., central fatigue) (42).

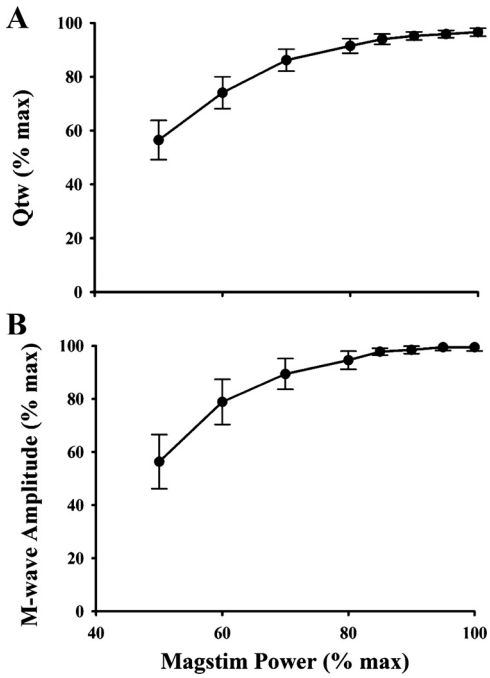

Fig. 1. Quadriceps twitch force (Q_{tw}; *A*) and M-wave amplitude (*B*) of the vastus lateralis muscle during magnetic stimulation of the femoral nerve at different power outputs of the magnetic stimulator. Data were collected before preexercise fatigue assessment. All preexercise data were collected with participants breathing room air (inspired O_2 fraction = 0.21). Values are means ± SE.

Reliability Measurements

Between-day CV was determined by the logarithmic method and preexercise measurements of fatigue. To test within-day reproducibility, three participants dismounted the fatigue assessment apparatus after baseline measurements of muscle function had been obtained and rested in a chair for 30 min. Participants then remounted the fatigue assessment apparatus, and measurements of quadriceps muscle function were repeated. Additionally, to examine whether carbohydrate ingestion resulted in fatigue characteristics that were consistent between days, two participants repeated the HYP-CHO trial.

Statistical Analysis

Statistical analyses were performed with SPSS statistical software (version 24; IBM, Chicago, IL), and significance was established when $P < 0.05$. Data were assessed for sphericity with Mauchly's test. Two-way repeated-measures ANOVA tests with Bonferroni post hoc for simple main effects were used to test for differences in metabolic, ventilatory, and plasma variables across time and between conditions. One-way repeated-measures ANOVA tests were performed to evaluate differences in preexercise macronutrient intake as well as differences in quadriceps function among trials. Effect sizes (ESs) were assessed with Cohen's *d* and reported for peripheral ($Q_{tw,pot}$) and central (VA) fatigue outcomes only. Power analysis (G*Power 3.1; Franz Faul) showed that a sample size of 9 would allow detection of significant differences in fatigue (40), 11 would demonstrate reductions in FFA (10), 8 for glucose (41), and 9 for amino acids (10) with statistical power of $1 - \beta = 0.80$ and $\alpha = 0.05$. Therefore, we recruited 12 participants. Data are expressed as means ± SE.

RESULTS

M Waves

Preexercise M-wave amplitudes did not differ between conditions (NORM-PLA = 6.2 ± 0.7 mV, HYP-PLA = 5.5 ± 0.7 mV, and HYP-CHO = 7.3 ± 0.3 mV; $P = 0.41$). Postexercise M-wave amplitudes were unchanged from preexercise baseline values in NORM-PLA (6.0 ± 0.7 mV; $P = 0.55$), HYP-PLA (5.1 ± 0.6 mV; $P = 0.13$), and HYP-CHO (7.1 ± 0.5 mV; $P = 0.19$).

MVC Force

Baseline MVC values were the same between NORM-PLA (570 ± 21 N), HYP-PLA (597 ± 21 N), and HYP-CHO (583 ± 21 N) ($P = 0.13$). In NORM-PLA, postexercise MVC (537 ± 20 N) was significantly reduced compared with preex-

ercise values ($P = 0.01$). Similarly, in HYP-CHO, postexercise MVC (538 ± 25 N) was significantly less than preexercise measurements ($P = 0.02$). In the HYP-PLA condition, postexercise MVC (576 ± 19 N) was not statistically different from preexercise values ($P = 0.12$).

Quadriceps Twitch Force

Preexercise $Q_{tw,pot}$ was similar between all three conditions (NORM-PLA = 218 ± 11 N, HYP-PLA = 222 ± 11 N, and HYP-CHO = 217 ± 12 N; $P = 0.34$). Postexercise $Q_{tw,pot}$ was significantly reduced compared with preexercise values in all three conditions [NORM-PLA = 207 ± 9 N ($P < 0.01$), HYP-PLA = 213 ± 10 N ($P = 0.02$), HYP-CHO = 201 ± 10 N ($P < 0.01$)]. The percent changes in $Q_{tw,pot}$ ($\Delta Q_{tw,pot}$) for NORM-PLA (−4.5 ± 1.3%), HYP-PLA (−4.0 ± 1.5%), and HYP-CHO (−6.9 ± 1.7%) were not significantly different between any of the three conditions (NORM-PLA vs. HYP-PLA: $P = 0.61$, ES = 0.12; NORM-PLA vs. HYP-CHO: $P = 0.09$, ES = 0.52; HYP-PLA vs. HYP-CHO: $P = 0.07$, ES = 0.60) (Fig. 2*A*). To determine whether an order effect was present, we compared $\Delta Q_{tw,pot}$ in *visit 3* to *visit 4* (regardless of condition). No differences in $\Delta Q_{tw,pot}$ were observed between visits ($P = 0.39$).

Voluntary Activation

Preexercise measurements of VA were similar between the two placebo conditions (NORM-PLA = 83.3 ± 2.0% vs. HYP-PLA = 86.9 ± 2.4%; $P = 0.12$). Likewise, preexercise measurements of VA were similar between hypoxia conditions (HYP-CHO = 86.3 ± 1.6%; $P = 0.72$ compared with HYP-PLA). Preexercise measurements of VA differed between NORM-PLA and HYP-CHO ($P = 0.02$). After 1 h of running exercise, VA was significantly reduced in NORM-PLA compared with preexercise values within the same condition (postexercise VA = 82.0 ± 1.9%; $P = 0.02$). Similarly, postexercise VA was significantly reduced compared with preexercise levels in HYP-PLA (82.7 ± 2.5%; $P < 0.01$) and HYP-CHO (80.9 ± 1.8%; $P < 0.01$). The change in VA (ΔVA) from before to after exercise was significantly greater in HYP-PLA (−4.7 ± 0.9%) compared with NORM-PLA (−1.9 ± 0.7%; $P < 0.01$, ES = 1.14). ΔVA was not statistically different between hypoxia visits ($P = 0.51$, ES = 0.31) (Fig. 2*B*). To determine whether an order effect was present, we compared ΔVA in *visit 3* to *visit 4* (regardless of condition). No differences in ΔVA were observed between visits ($P = 0.53$).

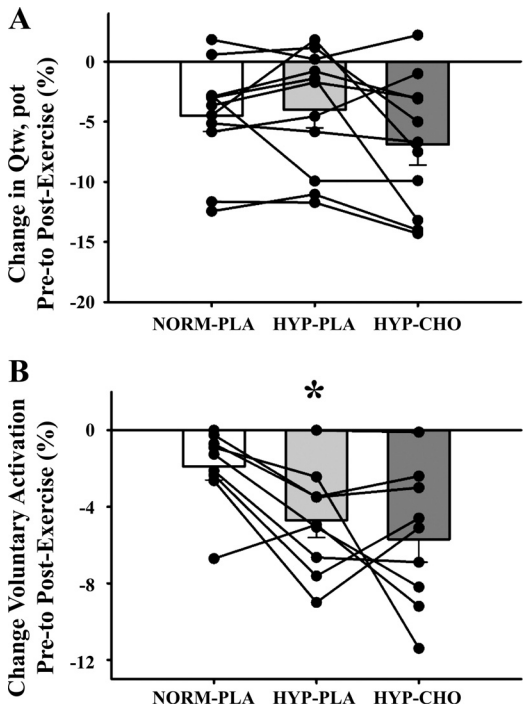

Fig. 2. Group (bars) and individual (line) data displaying the change (Δ) in potentiated quadriceps twitch ($Q_{tw,pot}$; *A*) and voluntary activation (VA; *B*) when running in normoxia and ingesting a placebo (NORM-PLA) or running in hypoxia and ingesting either a placebo (HYP-PLA) or carbohydrate (HYP-CHO). *Significantly different ($P < 0.05$) from NORM-PLA. [ΔVA was statistically different ($P < 0.05$) between HYP-CHO and NORM-PLA; however, preexercise VA values were also significantly different ($P < 0.05$) between these 2 groups].

Reliability Measurements

No systemic bias was observed in baseline measurements between days. Between-day CVs were 3.3% (2.3–5.9) [CV (95% confidence limits)] for $Q_{tw,pot}$, 3.3% (2.3–5.7) for MVC, and 4.3% (3.0–7.6) for VA. These values compare well to published data on reliability measurements using similar techniques (43). To test within-day reliability, three participants repeated preexercise measurements of fatigue after a 30-min rest period. The average within-day percent change in MVC was 1.6 ± 0.2%. Average change was 5.1 ± 1.9% for $Q_{tw,pot}$ and 2.9 ± 1.5% for VA. Additionally, two participants repeated the HYP-CHO trial. The average difference in MVC between HYP-CHO visits was 3.8%. The average difference in $Q_{tw,pot}$ and VA between HYP-CHO visits was 3.8% and 1.7%, respectively.

Metabolic and Ventilatory Responses to Exercise

The effects of hypoxia and carbohydrate ingestion on the physiological responses to 1 h of running exercise are summarized in Table 2. Oxygen uptake during exercise was 67 ± 1% of $\dot{V}O_{2max}$ in NORM-PLA, 65 ± 1% of normoxic $\dot{V}O_{2max}$ in HYP-PLA, and 65 ± 1% of normoxic $\dot{V}O_{2max}$ in HYP-CHO ($P > 0.05$ between all 3 conditions). Exercising $\dot{V}E$ was significantly greater in hypoxia conditions compared with NORM-PLA (89 ± 3 l/min; $P < 0.01$). $\dot{V}E$ was similar between HYP-PLA (102 ± 3 l/min) and HYP-CHO (106 ± 2 l/min; $P > 0.05$). RPE during exercise was significantly greater in the hypoxia conditions compared with NORM-PLA (11 ± 1; $P < 0.01$). RPE in HYP-PLA (12 ± 1) was similar to RPE in HYP-CHO (12 ± 1; $P = 0.65$). Likewise, dyspnea was significantly greater in the hypoxia conditions compared with NORM-PLA (2 ± 0; $P < 0.01$), but dyspnea was similar between HYP-PLA (3 ± 0) and HYP-CHO (3 ± 0; $P = 0.54$).

Plasma Glucose, Free Fatty Acids, Free Tryptophan, and Branched-Chain Amino Acids

Blood samples were obtained only during hypoxic conditions (*visits 3* and *4*). Plasma glucose concentrations were similar between conditions before exercise (80.7 ± 2.9 mg/dl and 80.4 ± 3.7 mg/dl for HYP-PLA and HYP-CHO, respectively; $P = 0.94$) as well as 30 min into exercise (103.2 ± 6.9 mg/dl and 107.6 ± 6.4 mg/dl for HYP-PLA and HYP-CHO,

Table 2. *Physiological responses to 1 h of running exercise when running in normoxia and ingesting a placebo or running in hypoxia and ingesting a placebo or carbohydrate beverage*

		Rest	15 min	30 min	45 min	60 min
Sp_{O_2}, %	NORM-PLA	99 ± 0	97 ± 1	96 ± 1	96 ± 1	96 ± 1
	HYP-PLA	92 ± 1#	83 ± 1#	82 ± 1#	82 ± 1#	81 ± 1#
	HYP-CHO	93 ± 1#	82 ± 1#	82 ± 1#	82 ± 1#	82 ± 1#
HR, beats/min	NORM-PLA	70 ± 3	148 ± 4	154 ± 2	154 ± 2	154 ± 2
	HYP-PLA	82 ± 5#	162 ± 2	162 ± 1	165 ± 1#	164 ± 1#
	HYP-CHO	82 ± 5	161 ± 1#	164 ± 1#	165 ± 1#	165 ± 2#
$\dot{V}_E$, l/min	NORM-PLA	14 ± 1	87 ± 3	88 ± 3	89 ± 3	88 ± 3
	HYP-PLA	15 ± 1	101 ± 3#	103 ± 3#	103 ± 3#	104 ± 3#
	HYP-CHO	14 ± 1	102 ± 2#	107 ± 2#*	107 ± 2#	109 ± 3#
$\dot{V}_{O_2}$, l/min	NORM-PLA	0.41 ± 0.02	3.33 ± 0.06	3.38 ± 0.08	3.38 ± 0.08	3.37 ± 0.09
	HYP-PLA	0.39 ± 0.01	3.28 ± 0.07	3.29 ± 0.09	3.28 ± 0.07	3.26 ± 0.08
	HYP-CHO	0.37 ± 0.02	3.20 ± 0.07#	3.27 ± 0.08#	3.28 ± 0.08	3.28 ± 0.09
$\dot{V}_{O_2}$, % max	NORM-PLA	8.4 ± 0.4	66.7 ± 1.1	67.5 ± 1.3	67.5 ± 1.3	67.4 ± 1.3
	HYP-PLA	7.8 ± 0.3	65.5 ± 1.2	65.6 ± 1.3	65.3 ± 0.8	64.9 ± 0.9
	HYP-CHO	7.3 ± 0.4	64.0 ± 1.1#	65.4 ± 1.1#	65.4 ± 1.3	65.4 ± 1.5
$\dot{V}_{CO_2}$, l/min	NORM-PLA	0.32 ± 0.02	2.83 ± 0.07	2.80 ± 0.08	2.81 ± 0.07	2.73 ± 0.08
	HYP-PLA	0.34 ± 0.01	2.83 ± 0.10	2.75 ± 0.09	2.81 ± 0.09	2.77 ± 0.10
	HYP-CHO	0.32 ± 0.02	2.79 ± 0.07	2.82 ± 0.09	2.86 ± 0.07	2.88 ± 0.09#
$\dot{V}_E/\dot{V}_{O_2}$	NORM-PLA	35.1 ± 1.4	26.1 ± 0.8	26.1 ± 0.8	26.4 ± 0.8	26.2 ± 0.8
	HYP-PLA	38.2 ± 1.1	31.0 ± 0.6#	31.2 ± 0.6#	31.4 ± 0.6#	31.7 ± 0.5#
	HYP-CHO	39.6 ± 1.4	32.1 ± 0.6#	32.9 ± 0.5#*	32.6 ± 0.6#	33.4 ± 0.5#
$\dot{V}_E/\dot{V}_{CO_2}$	NORM-PLA	44.9 ± 1.8	30.9 ± 1.0	31.8 ± 0.9	32.1 ± 1.0	32.5 ± 0.9
	HYP-PLA	44.0 ± 1.5	35.9 ± 0.8#	37.3 ± 0.8#	36.6 ± 1.0#	37.2 ± 0.9#
	HYP-CHO	45.0 ± 1.9	36.7 ± 0.7#	38.0 ± 0.8#	37.2 ± 0.9#	37.9 ± 0.9#
RER	NORM-PLA	0.78 ± 0.02	0.84 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	0.81 ± 0.01
	HYP-PLA	0.87 ± 0.01#	0.86 ± 0.01	0.84 ± 0.02	0.86 ± 0.01#	0.86 ± 0.01#
	HYP-CHO	0.88 ± 0.01#	0.88 ± 0.01#	0.87 ± 0.01#	0.88 ± 0.01#	0.88 ± 0.01#

Values are mean ± SE physiological responses to 1 h of running exercise when running in normoxia and ingesting a placebo (NORM-PLA) or running in hypoxia and ingesting either a placebo (HYP-PLA) or carbohydrate (HYP-CHO) beverage at *minutes 15*, *30*, and *45*. HR, heart rate; RER, respiratory exchange ratio; Sp_{O_2}, arterial oxyhemoglobin saturation; $\dot{V}_{CO_2}$, carbon dioxide production; $\dot{V}_E$, minute ventilation; $\dot{V}_{O_2}$, oxygen uptake; %max, % of normoxic maximum $\dot{V}_{O_2}$. *Significantly different ($P < 0.05$) from HYP-PLA at same time point.#Significantly different ($P < 0.05$) from NORM-PLA at same time point.

respectively; $P = 0.24$). After exercise, plasma glucose was significantly elevated in the HYP-CHO (116.1 ± 7.6 mg/dl) compared with HYP-PLA (96.2 ± 5.3 mg/dl) condition ($P = 0.01$). FFA concentrations were similar between HYP-PLA and HYP-CHO before exercise, during exercise, and after exercise ($P > 0.05$). A main effect of time was observed for FFA concentration, where levels of FFA in the blood were greater at the end of exercise compared with *minute 30* ($P < 0.01$).

From before to after exercise, f-TRP concentration remained the same in HYP-PLA but significantly decreased ($P = 0.01$) in the HYP-CHO condition (Fig. 3*A*). No differences in f-TRP concentrations were observed between conditions, however, at any time point. From before to after exercise, BCAA concentration decreased significantly in both HYP-PLA ($P < 0.01$) and HYP-CHO ($P < 0.01$) conditions (Fig. 3*B*). A main effect of time was observed for BCAA, where valine, leucine, and isoleucine concentrations all decreased throughout exercise ($P < 0.02$). A main effect of condition was observed for leucine, where concentrations were lower in the HYP-CHO condition (104 ± 4 nmol/ml) compared with HYP-PLA (111 ± 5 nmol/ml; $P = 0.02$). No other differences in BCAA concentrations between conditions were observed (Table 3). In both HYP-PLA and HYP-CHO conditions, the ratio of f-TRP to BCAA was unchanged by prolonged running exercise (Fig. 3*C*). The preexercise ratio of f-TRP to BCAA was similar between conditions ($P = 0.07$). Likewise, the postexercise ratio did not differ between conditions ($P = 0.67$).

Preexercise Macronutrient Ingestion

No differences were observed in macronutrient ingestion between conditions on the day preceding exercise. Participants ingested 2,556 ± 255 kcal before NORM-PLA, 2,468 ± 201 kcal before HYP-PLA, and 2,649 ± 158 kcal before HYP-CHO ($P = 0.76$). Macronutrient intake was similar between conditions [carbohydrate: 302 ± 41 g, 311 ± 33 g, 343 ± 30 g ($P = 0.42$); fat: 108 ± 13 g, 94 ± 11 g, 105 ± 11 g ($P = 0.59$); protein: 104 ± 11 g, 110 ± 15 g, 96 ± 8 g ($P = 0.42$) for NORM-PLA, HYP-PLA, and HYP-CHO, respectively].

DISCUSSION

The novel finding of our study was that central, but not peripheral, fatigue was increased beyond normoxic levels in trained endurance runners exercising on a treadmill for 1 h at 65% $\dot{V}_{O_{2max}}$ in a moderate hypoxia. Whereas running exercise alone caused both central and peripheral fatigue in normoxia (as seen by reductions in VA and $Q_{tw,pot}$), performing this same bout of exercise in hypoxia further reduced VA without altering $Q_{tw,pot}$. Additionally, our data indicate that carbohydrate ingestion did not alter central fatigue in moderate hypoxia.

Central Fatigue and Hypoxia

Hypoxia influences the origin and development of fatigue such that a threshold may exist where central fatigue dominates over peripheral fatigue once altitude reaches ~5,500 m ($F_{I_{O_2}}$ ~0.11) (3, 33). The etiology of fatigue is known, however, to

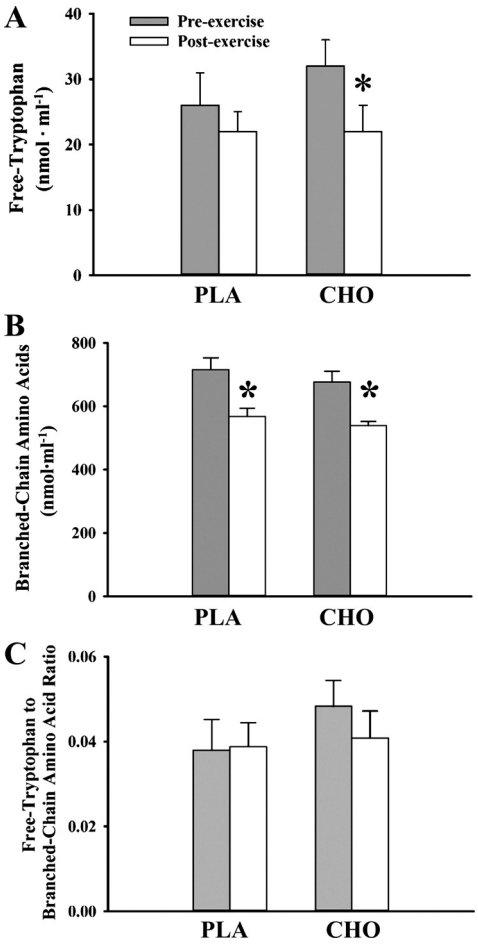

Fig. 3. Serum free tryptophan (*A*), branched-chain amino acids (*B*), and free tryptophan-to-branched-chain amino acid ratio (*C*) before exercise and after 60 min of running in hypoxia. Participants ingested either placebo (PLA) or carbohydrate (CHO) beverage intermittently during exercise. *Significantly different ($P < 0.02$) from preexercise values within the same condition. No significant differences were observed between conditions.

vary with the amount of muscle mass being recruited and the type of contractions being elicited. Studies assessing the influence of hypoxia on fatigue have traditionally used severe hypoxia or cycling exercise (3, 21, 33, 43), with only a few studies using running exercise in moderate hypoxia (34, 46). These few studies have followed ultraendurance runners during mountain races, and although reductions in VA have been observed, the role of hypoxia in fatigue development is difficult to determine because the mountain races are associated with continual fluctuations in elevation and changes in percent incline and decline that runners are experiencing. Because studies examining fatigue have often used cycling or single-limb exercise, we sought to determine the impact of running exercise, which uses more muscle mass than cycling, on fatigue development in moderate hypoxia. Our data demonstrate that moderate hypoxia alone may have accounted for some of the reductions in VA observed in these ultramarathon studies.

After exercise in hypoxia, we observed an ~5% decrease in VA. How relevant a 5% reduction in VA is to overall performance is difficult to determine. Previously, cyclists who breathed a gas mixture with a $F_{I_{O_2}}$ of 0.11 reached exhaustion when VA was reduced by ~8% (2). Similarly, cyclists exercising for 5 h experienced high subjective levels of fatigue alongside an 8% decrease in VA (27). When compared with our study, a 5% reduction in VA would appear to be an appreciable decline. When VA was measured at the conclusion of an ultramarathon race (34), however, VA was reduced by nearly 20%, indicating that our 5% reduction may have contributed minimally to performance impairment. Ultimately, the level at which VA begins to impair performance is unclear, and we are not able to speculate on how VA would have changed if runners had continued to exercise longer than 1 h.

Central Fatigue and Carbohydrate Ingestion

Although the impact of carbohydrate ingestion on exercise performance is well known, the direct influence of carbohydrate intake on central pathways is less understood. Although our data showed no clear connection between carbohydrate intake and the development of central fatigue, carbohydrate intake has been shown to preserve central drive when supplementation prevents hypoglycemia (37). Regardless of changes in glycemia, however, carbohydrates have been shown to influence the central nervous system, which is perhaps most evident when considering the performance improvements elicited by a carbohydrate mouth rinse (6, 18, 19, 35). Therefore, we thought it reasonable to assume that even though runners would not experience hypoglycemia during a 1-h run, carbohydrate intake could still influence fatigue via central mechanisms. The fact that carbohydrate ingestion did not impact central fatigue in our study may indicate that the effect of carbohydrates on central fatigue is most apparent when hypoglycemia and glycogen depletion are otherwise present.

A recent review (22) on the topic concluded that methodological differences between studies, and a lack of research in this area, make it difficult to discern whether carbohydrate supplementation influences central fatigue. One of the methodological differences that we suspected might have limited previous findings was the common practice of utilizing cycling exercise instead of more full-body or weight-bearing exercise. We therefore attempted to strengthen the potential influence of carbohydrate ingestion on central fatigue by utilizing running exercise. Even using a weight-bearing, full-body exercise, however, our study observed no differences in central fatigue between the carbohydrate supplementation and placebo conditions and supports those studies that have recorded similar findings (9, 13).

Table 3. *Amino acid concentrations during 1 h of running exercise, breathing 15% O_2, after ingesting placebo or carbohydrate solution*

		Rest	30 min	Postexercise
Valine	PLA	498 ± 29	458 ± 22	413 ± 19
	CHO	466 ± 23	442 ± 17	392 ± 9
Leucine	PLA	127 ± 5	112 ± 6	94 ± 5
	CHO	121 ± 7	107 ± 4	87 ± 3*
Isoleucine	PLA	91 ± 6	75 ± 4	62 ± 4
	CHO	91 ± 7	74 ± 3	60 ± 3

Values (in nmol/ml) are mean ± SE amino acid concentrations during 1 h of running exercise, breathing 15% O_2, after ingesting either a placebo (PLA) or carbohydrate (CHO) solution at *minutes 15*, *30*, and *45*. *Significantly different ($P < 0.05$) from PLA at same time point.

Potential Explanations for Observed Central Fatigue

Proposed mechanisms for the etiology of central fatigue include reductions in arterial oxygen content (3), hypoglycemia and muscle glycogen depletion (37), as well as elevations in fatigue-inducing neurotransmitters—specifically via alterations in the ratio of f-TRP to BCAA (1, 10, 36). Since running for 1 h at a moderate intensity is insufficient to diminish muscle glycogen or result in hypoglycemia (15), plausible explanations for a hypoxia-induced upregulation in central fatigue include reductions in arterial oxygen content and elevations in neurotransmitters associated with fatigue. We anticipated that the desaturation elicited by a FI_{O_2} of 0.15 would not reduce Sp_{O_2} below 75%—the threshold suggested to link arterial oxygen content with central fatigue (3). Thus, we speculated that if moderate hypoxia did increase central fatigue, the most likely explanation was a hypoxia-induced increase in FFA, which would initiate the cascade of events described by the serotonin-fatigue hypothesis (1, 36).

The serotonin-fatigue hypothesis posits that central fatigue is the result of elevated levels of serotonin elicited by an increase in the ratio of f-TRP to BCAA (8, 36). Elevations in serotonin can therefore arise by increases in f-TRP, decreases in BCAA, or both. Because of competitive binding with albumin, increases in f-TRP are associated with elevations in FFA. Although we observed an increase in FFA, we did not observe increases in f-TRP, and the ratio of f-TRP to BCAA remained unaltered by either 1 h of running exercise or intermittent carbohydrate ingestion (Fig. 3*C*). Interestingly, preexercise to postexercise central fatigue occurred despite there being no change in the ratio of f-TRP to BCAA. Therefore, our findings support an explanation other than the serotonin-fatigue hypothesis for the central fatigue that occurred. This is not to say that f-TRP and serotonin lack the capacity to induce fatigue. Indeed, when considering that blood glucose and FFA increased toward the end of exercise, the concentrations of f-TRP and serotonin may have just started to increase when exercise was terminated. If exercise had continued beyond 60 min, it is possible that the plasma concentration of f-TRP would have risen an appreciable amount and resulted in measurable differences in VA between HYP-PLA and HYP-CHO. This idea is strengthened by a recent study demonstrating that preexercise carbohydrate ingestion attenuated increases in serotonin concentration and lowered central fatigue following 90 min of running (23).

We considered whether oxyhemoglobin desaturation may represent a viable explanation for the central fatigue observed in our study. Once saturation drops below a threshold of ~75%, central factors have been suggested to dictate exercise capacity more than peripheral factors (3). Had we used a simulated altitude of 5,000 m we suspect that the resultant increase in central fatigue would have been much more pronounced. Since few people run at such high altitudes, however, the practical application of such a finding would be limited. Although Sp_{O_2} did not reach the threshold of 75% in our study, we question whether the accumulation of a reduced Sp_{O_2} over time (i.e., experiencing a Sp_{O_2} of ~81% for $\geq$1 h) may lessen central drive in a manner similar to high-intensity exercise at severe hypoxia. In some ways, this reflects the discussion of a "hypoxic dose" for sea level adaptation—where physiological responses depend not only on the severity of hypoxia but also on the time spent at that hypoxic level (14). Whether a similar concept can be applied to central fatigue (where the development of central fatigue depends not only on the degree of desaturation but also on how long individuals experience that level of desaturation) requires further investigation.

Peripheral Fatigue

Whereas moderate hypoxia increased central fatigue, we observed no effect of hypoxia on peripheral fatigue as measured by $Q_{tw,pot}$ (Fig. 2*A*). M-wave amplitudes were unchanged preexercise to postexercise in normoxia, and therefore the reductions in $Q_{tw,pot}$ elicited by running exercise alone were likely the result of metabolic changes within the muscle itself and not due to signal propagation inhibition. We did not observe any change in M waves as a result of hypoxia, and thus our data agree with previous findings demonstrating that acute hypoxia does not influence signal propagation (2, 44). Furthermore, we recorded no change in peripheral fatigue due to moderate hypoxia, which is in agreement with some data, though not all (2, 33). It is likely that the discrepancies observed between studies examining the effect of hypoxia on peripheral fatigue may be the result of differences in study design (exercise modality, intensity, etc.) as noted above.

Global Fatigue

We used MVC as an indicator of full-body fatigue. In line with exercise-induced peripheral and central fatigue, full-body fatigue (i.e., postexercise reductions in MVC) was observed in NORM-PLA and HYP-CHO conditions. Although peripheral and central fatigue also occurred in HYP-PLA, we observed no decrease in MVC. Preservation of MVC despite peripheral and central fatigue is difficult to explain. When we looked at individual data, one participant experienced a postexercise increase in MVC in all three conditions. Interestingly, this participant was the oldest athlete in our study—nearly a decade older than the average age of our participant population. If the warm-up time for this participant was insufficient, his recorded preexercise values may have been underreported. This same participant had a 6% difference in preexercise VA between NORM-PLA and HYP-CHO, which accounted for much of the group difference in preexercise VA between these two conditions. Although this participant's data may represent some individual variation, we found no reason to justify exclusion and therefore included the data in the final analysis. Importantly, primary outcomes compared the two placebo conditions (NORM-PLA vs. HYP-PLA) and the two hypoxic conditions (HYP-PLA vs. HYP-CHO). For these comparisons, no preexercise differences were detected in M-wave amplitudes, MVC values, $Q_{tw,pot}$, or VA.

Limitations

Our study design dictated that hypoxic trials were always performed after the normoxic trial. Visits were ordered in this way to ensure that participants were capable of completing the 1-h run, as well as to orient them to the procedures and requirements of the exercise protocol. As such, the possibility exists that an ordering effect influenced the observed outcome. Baseline measurements of fatigue, however, indicated no differences in preexercise fatigue between visits. Additionally, we

compared fatigue in *visit 3* vs. *visit 4* and found no differences in $Q_{tw,pot}$ or VA, indicating the absence of an order effect between these visits. Furthermore, we feel that if any order effect had occurred, it would have attenuated fatigue in the latter visits, i.e., participants would have been more comfortable with the 1-h run in HYP conditions compared with NORM-PLA and would have experienced less fatigue in hypoxia. If so, the decrease in VA observed in hypoxia vs. normoxia might have been even greater than the values we recorded.

Additionally, because differences in muscle glycogen could have influenced the development of central fatigue (29), our study utilized a 24-h dietary recall to ensure similarities between preexercise dietary intake. However, we acknowledge that the influence of diet and physical activity on muscle glycogen extends beyond the 24 h immediately before exercise (49), and thus the 24-h dietary recall is unable to establish with certainty that muscle glycogen was the same between visits. Similarly, preexercise concentrations of FFA may have differed between normoxia and hypoxia visits. Preexercise FFA concentrations were the same between HYP-PLA and HYP-CHO, however, and considering that 24-h dietary intake was consistent across all three conditions, a difference in preexercise FFA levels between the normoxic condition and the two hypoxic conditions is unlikely.

Finally, although we attempted to address central fatigue by intervening in the pathways described by the serotonin-fatigue hypothesis, we did not measure serotonin concentrations. Rat models have demonstrated an association between f-TRP in the blood and brain concentrations of serotonin (7, 8), but having a direct measurement of serotonin would have strengthened our proposition that serotonin did not impact central fatigue. In addition to having a measurement of serotonin, having a measurement of dopamine would also have strengthened our study design. A review (30) on the serotonin-fatigue hypothesis advocated an updated "revised hypothesis" and suggested that multiple neurotransmitters are involved in fatigue and an increase in the central ratio of serotonin to dopamine is a stronger indicator of fatigue than serotonin alone. In mentioning plasma concentrations of amino acids, it should be noted that we did not track changes in plasma volume, and differences in blood concentrations of glucose, FFA, and amino acids may be due to changes in the blood volume associated with sweat loss. The rate of solution ingestion used in our study (~1.1 l/h), however, is near the recommended range for fluid replacement during exercise (0.4–0.8 l/h) and was designed to address sweat loss (55). Additionally, the rate of ingestion we used was similar to previous research (10) where cyclists drank either a placebo or carbohydrate beverage throughout prolonged exercise. These studies found no differences in plasma volume between conditions; nor did plasma volume differ throughout exercise duration. Finally, when comparing our data with studies that have tracked changes in amino acids during endurance exercise, our data closely align with these studies, and thus support the validity of our values (4, 10).

Some of these limitations may be especially important when applying our findings to populations other than trained men. For example, women are more resistant to some types of fatigue than men (16, 28), which may influence the origin and rate of fatigue development.

Conclusions

In conclusion, our study demonstrated that moderate hypoxia ($F_{I_{O_2}} = 0.15$) increased central, but not peripheral, fatigue in trained endurance runners exercising on a treadmill for 1 h at 65% $\dot{V}O_{2max}$ and that ingesting carbohydrates intermittently throughout exercise did not attenuate central fatigue development. Additionally, our study showed no contribution of f-TRP and BCAA to fatigue development, and we therefore question the relevance of the serotonin-fatigue hypothesis under these conditions. The data from our treadmill running protocol imply that, for individuals traveling to altitudes of ~3,000 m, work capacity during moderate-intensity, weight-bearing exercise may be reduced by a premature fatigue stemming from central mechanisms. Furthermore, for athletes engaged in full-body exercise at a moderate intensity in hypoxia, increases in central fatigue are unaffected by intermittent carbohydrate ingestion and occur independently of changes in the ratio of f-TRP to BCAA.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

H.L.P., T.J.F., R.F.C., A.D.F., D.M.K., and T.D.M. conceived and designed research; H.L.P. and T.J.F. performed experiments; H.L.P. analyzed data; H.L.P., T.J.F., and T.D.M. interpreted results of experiments; H.L.P. prepared figures; H.L.P. drafted manuscript; H.L.P., T.J.F., R.F.C., A.D.F., D.M.K., and T.D.M. edited and revised manuscript; H.L.P., T.J.F., R.F.C., A.D.F., D.M.K., and T.D.M. approved final version of manuscript.

REFERENCES

1. **Acworth I, Nicholass J, Morgan B, Newsholme EA.** Effect of sustained exercise on concentrations of plasma aromatic and branched-chain amino acids and brain amines. *Biochem Biophys Res Commun* 137: 149–153, 1986. doi:10.1016/0006-291X(86)91188-5.
2. **Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC.** AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. *J Appl Physiol (1985)* 115: 634–642, 2013. doi:10.1152/japplphysiol.00606.2013.
3. **Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempsey JA.** Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J Physiol* 581: 389–403, 2007. doi:10.1113/jphysiol.2007.129700.
4. **Areces F, González-Millán C, Salinero JJ, Abian-Vicen J, Lara B, Gallo-Salazar C, Ruiz-Vicente D, Del Coso J.** Changes in serum free amino acids and muscle fatigue experienced during a half-ironman triathlon. *PLoS One* 10: e0138376, 2015. doi:10.1371/journal.pone.0138376.
5. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377–381, 1982. doi:10.1249/00005768-198205000-00012.
6. **Carter JM, Jeukendrup AE, Jones DA.** The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc* 36: 2107–2111, 2004. doi:10.1249/01.MSS.0000147585.65709.6F.
7. **Chaouloff F, Elghozi JL, Guezennec Y, Laude D.** Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-hydroxytryptamine metabolism of the rat. *Br J Pharmacol* 86: 33–41, 1985. doi:10.1111/j.1476-5381.1985.tb09432.x.
8. **Chaouloff F, Kennett GA, Serrurrier B, Merino D, Curzon G.** Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. *J Neurochem* 46: 1647–1650, 1986. doi:10.1111/j.1471-4159.1986.tb01789.x.
9. **Cureton KJ, Warren GL, Millard-Stafford ML, Wingo JE, Trilk J, Buyckx M.** Caffeinated sports drink: ergogenic effects and possible mechanisms. *Int J Sport Nutr Exerc Metab* 17: 35–55, 2007. doi:10.1123/ijsnem.17.1.35.
10. **Davis JM, Bailey SP, Woods JA, Galiano FJ, Hamilton MT, Bartoli WP.** Effects of carbohydrate feedings on plasma free tryptophan and

branched-chain amino acids during prolonged cycling. *Eur J Appl Physiol Occup Physiol* 65: 513–519, 1992. doi:10.1007/BF00602357.
11. **Fernstrom JD.** Branched-chain amino acids and brain function. *J Nutr* 135, *Suppl*: 1539S–1546S, 2005. doi:10.1093/jn/135.6.1539S.
12. **Fulton TJ, Paris HL, Stickford AS, Gruber AH, Mickleborough TD, Chapman RF.** Locomotor-respiratory coupling is maintained in simulated moderate altitude in trained distance runners. *J Appl Physiol (1985)* 125: 1–7, 2018. doi:10.1152/japplphysiol.01122.2017.
13. **Ganio MS, Klau JF, Lee EC, Yeargin SW, McDermott BP, Buyckx M, Maresh CM, Armstrong LE.** Effect of various carbohydrate-electrolyte fluids on cycling performance and maximal voluntary contraction. *Int J Sport Nutr Exerc Metab* 20: 104–114, 2010. doi:10.1123/ijsnem.20.2.104.
14. **Garvican-Lewis LA, Sharpe K, Gore CJ.** Time for a new metric for hypoxic dose? *J Appl Physiol (1985)* 121: 352–355, 2016. doi:10.1152/japplphysiol.00579.2015.
15. **Gollnick PD, Armstrong RB, Saubert CW 4th, Sembrowich WL, Shepherd RE, Saltin B.** Glycogen depletion patterns in human skeletal muscle fibers during prolonged work. *Pflugers Arch* 344: 1–12, 1973. doi:10.1007/BF00587437.
16. **Hicks AL, Kent-Braun J, Ditor DS.** Sex differences in human skeletal muscle fatigue. *Exerc Sport Sci Rev* 29: 109–112, 2001. doi:10.1097/00003677-200107000-00004.
17. **Jentjens RL, Jeukendrup AE.** High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *Br J Nutr* 93: 485–492, 2005. doi:10.1079/BJN20041368.
18. **Jeukendrup AE.** Nutrition for endurance sports: marathon, triathlon, and road cycling. *J Sports Sci* 29, *Suppl* 1: S91–S99, 2011. doi:10.1080/02640414.2011.610348.
19. **Jeukendrup AE, Chambers ES.** Oral carbohydrate sensing and exercise performance. *Curr Opin Clin Nutr Metab Care* 13: 447–451, 2010. doi:10.1097/MCO.0b013e328339de83.
20. **Jones NL, Robertson DG, Kane JW, Hart RA.** Effect of hypoxia on free fatty acid metabolism during exercise. *J Appl Physiol* 33: 733–738, 1972. doi:10.1152/jappl.1972.33.6.733.
21. **Jubeau M, Rupp T, Temesi J, Perrey S, Wuyam B, Millet GY, Verges S.** Neuromuscular fatigue during prolonged exercise in hypoxia. *Med Sci Sports Exerc* 49: 430–439, 2017. doi:10.1249/MSS.0000000000001118.
22. **Khong TK, Selvanayagam VS, Sidhu SK, Yusof A.** Role of carbohydrate in central fatigue: a systematic review. *Scand J Med Sci Sports* 27: 376–384, 2017. doi:10.1111/sms.12754.
23. **Khong TK, Selvanayagam VS, Hamzah SH, Yusof A.** Effect of quantity and quality of pre-exercise carbohydrate meals on central fatigue. *J Appl Physiol (1985)* 125: 1021–1029, 2018. doi:10.1152/japplphysiol.00221.2018.
24. **Klain GJ, Hannon JP.** Effects of high altitude on lipid components of human serum. *Proc Soc Exp Biol Med* 129: 646–649, 1968. doi:10.3181/00379727-129-33388.
25. **Kufel TJ, Pineda LA, Mador MJ.** Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle Nerve* 25: 438–444, 2002. doi:10.1002/mus.10047.
26. **Lebrun CM, McKenzie DC, Prior JC, Taunton JE.** Effects of menstrual cycle phase on athletic performance. *Med Sci Sports Exerc* 27: 437–444, 1995. doi:10.1249/00005768-199503000-00022.
27. **Lepers R, Maffiuletti NA, Rochette L, Brugniaux J, Millet GY.** Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Physiol (1985)* 92: 1487–1493, 2002. doi:10.1152/japplphysiol.00880.2001.
28. **Maughan RJ, Harmon M, Leiper JB, Sale D, Delman A.** Endurance capacity of untrained males and females in isometric and dynamic muscular contractions. *Eur J Appl Physiol Occup Physiol* 55: 395–400, 1986. doi:10.1007/BF00422739.
29. **Maughan RJ.** *The Encyclopaedia of Sports Medicine: An IOC Medical Commission Publication*. Hoboken, NJ: Wiley, 2008, vol. 7.
30. **Meeusen R, Watson P, Hasegawa H, Roelands B, Piacentini MF.** Central fatigue: the serotonin hypothesis and beyond. *Sports Med* 36: 881–909, 2006. doi:10.2165/00007256-200636100-00006.
31. **Merton PA.** Voluntary strength and fatigue. *J Physiol* 123: 553–564, 1954. doi:10.1113/jphysiol.1954.sp005070.
32. **Millet GY, Lepers R.** Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med* 34: 105–116, 2004. doi:10.2165/00007256-200434020-00004.
33. **Millet GY, Muthalib M, Jubeau M, Laursen PB, Nosaka K.** Severe hypoxia affects exercise performance independently of afferent feedback and peripheral fatigue. *J Appl Physiol (1985)* 112: 1335–1344, 2012. doi:10.1152/japplphysiol.00804.2011.
34. **Millet GY, Tomazin K, Verges S, Vincent C, Bonnefoy R, Boisson RC, Gergelé L, Féasson L, Martin V.** Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One* 6: e17059, 2011. doi:10.1371/journal.pone.0017059.
35. **Murray KO, Paris HL, Fly AD, Chapman RF, Mickleborough TD.** Carbohydrate mouth rinse improves cycling time-trial performance without altering plasma insulin concentration. *J Sports Sci Med* 17: 145–152, 2018.
36. **Newsholme E.** Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In: *Advances in Myochemistry*, edited by Benzi G. London: John Libbey Eurotext, 1987, p. 127–133.
37. **Nybo L.** CNS fatigue and prolonged exercise: effect of glucose supplementation. *Med Sci Sports Exerc* 35: 589–594, 2003. doi:10.1249/01.MSS.0000058433.85789.66.
38. **Oppliger RA, Magnes SA, Popowski LA, Gisolfi CV.** Accuracy of urine specific gravity and osmolality as indicators of hydration status. *Int J Sport Nutr Exerc Metab* 15: 236–251, 2005. doi:10.1123/ijsnem.15.3.236.
39. **Place N, Lepers R, Deley G, Millet GY.** Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc* 36: 1347–1356, 2004. doi:10.1249/01.MSS.0000135786.22996.77.
40. **Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, Moxham J.** Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. *Muscle Nerve* 19: 549–555, 1996. doi:10.1002/(SICI)1097-4598(199605)19:5<549::AID-MUS1>3.0.CO;2-B.
41. **Rollo I, Williams C.** Influence of ingesting a carbohydrate-electrolyte solution before and during a 1-hr running performance test. *Int J Sport Nutr Exerc Metab* 19: 645–658, 2009. doi:10.1123/ijsnem.19.6.645.
42. **Romer LM, Haverkamp HC, Amann M, Lovering AT, Pegelow DF, Dempsey JA.** Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 292: R598–R606, 2007. doi:10.1152/ajpregu.00269.2006.
43. **Romer LM, Haverkamp HC, Lovering AT, Pegelow DF, Dempsey JA.** Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 290: R365–R375, 2006. doi:10.1152/ajpregu.00332.2005.
44. **Sandiford SD, Green HJ, Duhamel TA, Schertzer JD, Perco JD, Ouyang J.** Muscle Na-K-pump and fatigue responses to progressive exercise in normoxia and hypoxia. *Am J Physiol Regul Integr Comp Physiol* 289: R441–R449, 2005. doi:10.1152/ajpregu.00652.2004.
45. **Sandiford SD, Green HJ, Duhamel TA, Perco JG, Schertzer JD, Ouyang J.** Inactivation of human muscle Na^+-K^+-ATPase in vitro during prolonged exercise is increased with hypoxia. *J Appl Physiol (1985)* 96: 1767–1775, 2004. doi:10.1152/japplphysiol.01273.2003.
46. **Saugy J, Place N, Millet GY, Degache F, Schena F, Millet GP.** Alterations of neuromuscular function after the world's most challenging mountain ultra-marathon. *PLoS One* 8: e65596, 2013. doi:10.1371/journal.pone.0065596.
47. **Schaeffer MR, Mendonca CT, Levangie MC, Andersen RE, Taivassalo T, Jensen D.** Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. *Exp Physiol* 99: 427–441, 2014. doi:10.1113/expphysiol.2013.074880.
48. **Schoene RB, Robertson HT, Pierson DJ, Peterson AP.** Respiratory drives and exercise in menstrual cycles of athletic and nonathletic women. *J Appl Physiol Respir Environ Exerc Physiol* 50: 1300–1305, 1981.
49. **Sherman WM, Costill DL, Fink WJ, Miller JM.** Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. *Int J Sports Med* 2: 114–118, 1981. doi:10.1055/s-2008-1034594.
50. **Sinex JA, Chapman RF.** Hypoxic training methods for improving endurance exercise performance. *J Sport Health Sci* 4: 325–332, 2015. doi:10.1016/j.jshs.2015.07.005.
51. **Stewart RD, Duhamel TA, Foley KP, Ouyang J, Smith IC, Green HJ.** Protection of muscle membrane excitability during prolonged cycle exercise with glucose supplementation. *J Appl Physiol (1985)* 103: 331–339, 2007. doi:10.1152/japplphysiol.01170.2006.
52. **Strojnik V, Komi PV.** Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol (1985)* 84: 344–350, 1998. doi:10.1152/jappl.1998.84.1.344.
53. **Sutton JR.** Effect of acute hypoxia on the hormonal response to exercise. *J Appl Physiol Respir Environ Exerc Physiol* 42: 587–592, 1977.

54. **Swart J, Lamberts RP, Lambert MI, St Clair Gibson A, Lambert EV, Skowno J, Noakes TD.** Exercising with reserve: evidence that the central nervous system regulates prolonged exercise performance. *Br J Sports Med* 43: 782–788, 2009. doi:10.1136/bjsm.2008.055889.
55. **Thomas DT, Erdman KA, Burke LM.** American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc* 48: 543–568, 2016.
56. **Thomas K, Goodall S, Stone M, Howatson G, St Clair Gibson A, Ansley L.** Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. *Med Sci Sports Exerc* 47: 537–546, 2015. doi:10.1249/MSS.0000000000000448.
57. **Wilson WM, Maughan RJ.** Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. *Exp Physiol* 77: 921–924, 1992. doi:10.1113/expphysiol.1992.sp003660.
58. **Young AJ, Evans WJ, Cymerman A, Pandolf KB, Knapik JJ, Maher JT.** Sparing effect of chronic high-altitude exposure on muscle glycogen utilization. *J Appl Physiol Respir Environ Exerc Physiol* 52: 857–862, 1982.
59. **Young S.** The clinical psychopharmacology of tryptophan. In: *Nutrition and the Brain*, edited by Wurtman RJ, Wurtman JJ. New York: Raven, 1986, vol. 7, p. 49–88.