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RESEARCH ARTICLE

Heat stress impairs exogenous carbohydrate oxidation during prolonged running when maintaining euhydration

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Abstract

This study investigated the effect of running in a hot environment compared with a temperate environment on exogenous carbohydrate oxidation, while maintaining a state of euhydration. Ten trained runners (24 ± 6 yr; 72.7 ± 8.3 kg; $\dot{V}O_{2peak}$: 63 ± 6 mL/kg/min) completed two trials [100 min of steady state running at ~65% $\dot{V}O_{2peak}$ in either a temperate (19°C; TEMP) or a hot environment (34°C; HOT)]. Water was provided every 20 min to replace ~90% of body mass losses (TEMP: 0.8 ± 0.2 L; HOT: 1.7 ± 0.4 L). In each trial, participants consumed 60 g/h (bolus every 20 min) of a 35% dextrose solution enriched with [U-^{13}C] glucose (145 ± 2 δ‰ vs. PDB). Expired breath (analyzed for ^{13}C:^{12}C) and blood samples were collected every 20 min during exercise. Average (40–100 min) and peak exogenous carbohydrate oxidation rates were 20% (HOT: 0.43 ± 0.09 vs. TEMP: 0.54 ± 0.12 g/min; $P = 0.006$) and 18% (HOT: 0.67 ± 0.10 vs. TEMP: 0.81 ± 0.11 g/min; $P = 0.002$) lower in HOT than in TEMP, respectively. Total carbohydrate oxidation ($P = 0.111$) was not significantly different between trials, resulting in a greater contribution from endogenous sources in HOT versus TEMP (2.10 ± 0.35 vs. 1.86 ± 0.30 g/min; $P = 0.020$). Gastrointestinal temperature and heart rate ($P < 0.001$) were greater in HOT. Even with adequate hydration, running in a hot environment reduced exogenous carbohydrate.

NEW & NOTEWORTHY This study showed that exogenous carbohydrate oxidation is reduced by ~20% during running in the heat, even while controlling fluid intake to maintain euhydration, highlighting that heat stress alone impairs exogenous carbohydrate use. These findings suggest a lower exogenous carbohydrate oxidation and a greater reliance on endogenous stores when exercising in the heat, independently of the effects of dehydration.

glucose; nutrition; stable isotopes; substrate utilization; temperature

INTRODUCTION

Exercising in hot environments (>30°C) is becoming increasingly common due to global warming (1). During exercise, ambient heat exposure, combined with metabolic heat production, triggers thermoregulatory responses, such as increased sweat production to prevent/mitigate excessive elevations in body core temperature (2). The increase in evaporative heat loss is facilitated by increased skin blood flow (3), leading to competing cardiovascular system demands: *1*) supplying active skeletal muscles with oxygen to meet metabolic needs and *2*) promoting heat dissipation by increasing cutaneous blood flow (3). These concurrent demands increase cardiovascular strain, resulting in cardiovascular drift overtime (4) and can alter substrate metabolism, potentially contributing to the performance impairment observed during prolonged exercise in hot compared with temperate environments [0.3–0.4% for every degree wet-bulb globe temperature (WBGT) > 15°C WBGT (5)].

During prolonged exercise in the heat, there is an increase in carbohydrate oxidation and muscle glycogen use [standardized mean difference: 0.3–0.8 higher in the heat (6)] due to limited oxygen delivery and increased epinephrine concentrations (6–8). To compensate for this earlier glycogen depletion and limit fatigue, carbohydrate, primarily in the form of drinks, gels, and bars, can be ingested. However, despite extensive research on substrate use in extreme environmental conditions, only two studies (9, 10) have investigated the oxidation of exogenous carbohydrates (i.e., from drinks, gels, or bars) in the heat. Unlike whole body carbohydrate oxidation, exogenous carbohydrate oxidation is reduced by ~10%–15% in the heat compared with temperate conditions (9, 10). These effects could be explained by impairments in glucose uptake/release, gastric emptying, and intestinal absorption (11–13). This may adversely impact performance, and contribute to the higher incidence and severity of gastrointestinal distress observed in hot environments (14), especially in running,

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where greater mechanical stresses in the abdominal region are observed (15).

Heat exposure is often accompanied by dehydration due to increased evaporative heat loss and inadequate fluid replacement (16, 17). Dehydration amplifies cardiovascular drift observed in hot conditions by reducing plasma and blood volume and decreasing blood flow redistribution [i.e., reduced blood flow at the muscle level and possibly around the gastrointestinal (GI) tract] (17, 18). These responses have been shown to increase muscle glycogen use (19–21) and muscle lactate concentrations (22) and may affect exogenous carbohydrate oxidation. Moreover, reduced/restricted fluid intake, regardless of hydration status, can influence gastric emptying (23), which may affect exogenous carbohydrate delivery (24).

Previous studies in hot conditions measuring exogenous carbohydrate oxidation (9, 10) did not control for hydration status. Therefore, it remains unclear whether the reduction in exogenous carbohydrate oxidation is a direct effect of heat and increased core temperature, a difference in fluid intake pattern, or a consequence of dehydration resulting from prolonged exposure to high temperatures. For example, a reduction in splanchnic blood flow during exercise in hot conditions may be attributed to a direct effect of heat exposure and a blood flow redistribution to the body's extremities to facilitate heat dissipation through evaporative cooling (25), and/or direct effects of dehydration with a decrease in plasma volume resulting from dehydration (18). Moreover, the two studies investigating exogenous carbohydrate oxidation in hot conditions were performed in cycling (9, 10), where hydration is more easily maintained than in activities such as running.

Therefore, this study aimed to investigate the effect of running in a hot versus temperate environment on exogenous carbohydrate oxidation, while maintaining euhydration. It was hypothesized that exogenous carbohydrate oxidation would decrease in hot conditions even when maintaining euhydration.

METHODS

Participants

Ten trained nonacclimated runners were recruited based on their training status (24 ± 6 yr; 1.85 ± 0.06 m; 72.7 ± 8.3 kg; 63.4 ± 5.8 mL/min/kg $\dot{V}O_{2peak}$). Trained runners participated in running sessions at least three times per week and were classified (at least) as "Tier 2" according to the Participant Classification Framework of McKay et al. (26). All participants were informed of the purpose, practical details, and risks associated with the procedures before giving their verbal and written informed consent to participate. All participants were healthy as assessed by a health screening questionnaire. The study received ethical approval from the Loughborough University Ethics Approvals (human participants) Sub-Committee (LEON17245). Using the data from a previous experiment comparing exogenous glucose oxidation in hot compared with temperate conditions (9), with an α of 0.05 and a statistical power of 0.8, it was estimated that 10 participants would be required to detect differences in exogenous carbohydrate oxidation between trials. The study used a repeated-measures design with trials completed in a randomized order.

Participants completed a preliminary trial and two experimental trials at the same time of day (standardized within participants) in a randomized order and separated by ≥72 h. One experimental trial was conducted in a temperate environment (TEMP: 18.9 ± 0.2°C and 57 ± 11% relative humidity), and the other was performed in a hot environment (HOT: 34.5 ± 0.6°C and 44 ± 2% relative humidity). For both sessions, water (at room temperature; TEMP: 18.9 ± 1.0°C; HOT: 31.7 ± 2.0°C) was provided to replace 90% of estimated body mass loss (i.e., estimated from the preliminary trial).

Preliminary Visit

During the first visit, nude body mass (AFW-120K, Adam Equipment Co., Milton Keynes, UK) and height (Seca 216, Hamburg, Germany) were measured. Participants completed four 4-min stages at progressively increasing speeds on a motorized treadmill (h/p/cosmos, Nussdorf, Germany) until a heart rate of >160 beats/min was elicited (measured using Polar M400, Polar, Kemple, Finland). Expired gas was collected into Douglas bags during the final minute of each stage for oxygen and carbon dioxide concentrations (Servomex 1400 Oxygen and Carbon Dioxide Gas Analyzer; Servomex, Crowborough, UK), volume (Harvard dry gas meter; Harvard Apparatus Ltd., Edenbridge, UK), and temperature (RS PRO Digital Thermometer; RS Components, Corby, UK). To correct $\dot{V}O_2$ and $\dot{V}CO_2$ values, ambient air was measured simultaneously with expired gas samples (27). After a short period of rest (~10–15 min), participants ran to volitional exhaustion using a ramp test at the speed of the final submaximal stage, with the incline increased by 1% each minute. An expired gas sample was collected during the final minute of exercise to determine peak oxygen uptake ($\dot{V}O_{2peak}$). Data from the submaximal exercise were then extrapolated using linear regression to determine the speed equivalent to 65% of $\dot{V}O_{2peak}$ for use in subsequent trials.

Following a rest period (~15 min), participants completed a 20-min exercise at a speed corresponding to 65% of $\dot{V}O_{2peak}$ in temperate conditions (18°C). After a ~30-min rest period, participants sat in a hot environment for 10 min before completing a 20-min exercise at a speed corresponding to 65% of $\dot{V}O_{2peak}$ in hot conditions (34°C). Nude body mass was measured before (after passive heating in the hot condition) and after each exercise bout to estimate body mass loss, and therefore required fluid intake, for subsequent trials.

Pretrial Standardization

In the 3–4 days before each experimental trial, participants were asked to follow a specific exercise and diet regimen designed to reduce the background shift in $^{13}CO_2$ (28). At least 3–4 days before each experimental trial, participants completed an exhaustive bout of exercise (60–90 min continuous run at moderate to high intensity) to deplete glycogen stores and oxidize ^{13}C-enriched glycogen. This protocol was replicated at the same time before subsequent trials. Following this run, participants were asked to avoid foods naturally high in ^{13}C, including commercially available sports drinks and carbohydrate derived from C4 plants (e.g.,

Table 1. *Whole body oxygen consumption, carbon dioxide production, respiratory exchange ratio, and substrate utilization during the 40- to 100-min period of exercise in TEMP and HOT trials*

	TEMP	HOT	*P*	% Difference
$\dot{V}O_2$, L/min	2.82 ± 0.42	2.70 ± 0.38	**0.001**	−4.5
$\dot{V}CO_2$, L/min	2.56 ± 0.34	2.50 ± 0.35	**0.012**	−2.3
RER	0.91 ± 0.02	0.93 ± 0.02	**0.015**	+2.2
Fat oxidation, g/min	0.44 ± 0.16	0.32 ± 0.09	**0.015**	−26.7
Total carbohydrate oxidation, g/min	2.40 ± 0.31	2.53 ± 0.37	0.113	+5.5
Peak exogenous carbohydrate oxidation, g/min	0.81 ± 0.11	0.67 ± 0.10	**0.002**	−18.1
Exogenous carbohydrate oxidation, g/min	0.54 ± 0.12	0.43 ± 0.09	**0.006**	−20.1
Endogenous carbohydrate oxidation, g/min	1.86 ± 0.30	2.10 ± 0.35	**0.018**	+13.0
Blood glucose concentrations, mmol/L	5.4 ± 0.2	6.3 ± 1.0	**0.013**	+17.5
Blood lactate concentrations, mmol/L	1.06 ± 0.19	1.62 ± 0.55	**0.011**	+53.1

Values are means ± SD. %difference between TEMP and HOT. Bold values are significant. HOT, hot environment; RER, respiratory exchange ratio; TEMP, temperate environment.

cane sugar and maize). Each participant received a detailed list of restricted foods and confirmed adherence to all pretrial standardization requirements before beginning each trial. In the 24 h before the first trial, participants were asked to record their dietary intake (food and fluid) and physical activity and were asked to replicate this before the second experimental trial, with adherence verbally checked. They were also asked to consume ≥40 mL/kg body mass from beverages to ensure euhydration and to refrain from alcohol intake and strenuous exercise.

Experimental Visits

Participants attended the laboratory after swallowing a GI temperature pill (eCelsius Performance Capsule, BodyCAP, Hérouville Saint-Clair, France) before going to bed [∼10–14 h before arrival at the laboratory (29)]. For each trial, participants arrived at the laboratory at the same time of day (standardized for each subject) after an overnight fast and 90 min after ingesting 8 mL of water/kg body mass. On arrival, participants provided a urine sample and had nude body mass measured. Skin thermistors (iButtons, DS1922L, iButtonLink Technology) were positioned on the upper arm, chest, thigh, and calf (right side). Weighted mean skin temperature (T_{skin}) was subsequently calculated using the weighted average of the four sites (30). A sweat patch (Tegaderm + Pad; 3M Healthcare, Loughborough, UK) was put on the right forearm for the whole exercise period. A flexible 20-gauge Teflon cannula was then inserted into an antecubital vein of one arm to allow repeated blood sampling during the trial. After sitting for 15 min, expired breath samples were collected into 12-mL evacuated glass tubes (Exetainers; Labco, Ceredigion, UK) for determination of $^{13}CO_2$ enrichment, followed by a 5-min collection of expired gas (analyzed for oxygen and carbon dioxide content and gas volume and temperature as described for the preliminary trial). An 11-mL venous blood sample was then collected, and the heart rate was recorded. Participants were asked to rate the rating of perceived exertion [RPE; 6–20 scale (31)], thermal sensation [RTS; −10 to +10 scale (32)], feelings of thirst, urge to urinate, GI comfort, and stomach bloatedness using scales (0, no feeling; 10, extreme feeling). As used in similar studies (33), responses ≥5 were classed as severe, with values <5 classed as nonsevere. Urine was collected, and nude body mass was measured before the start of exercise.

Immediately before the start of exercise, participants consumed the first drink. They then completed 100-min running at 65% $\dot{V}O_{2peak}$. A sample period occurred every 20 min: at 18 min, expired breath samples were collected into exetainers, a 60-s expired gas collection occurred at 19 min, with heart rate, core (T_{GI}) and skin temperature, ambient temperature, and relative humidity (Kestrel 4400; Nielsen-Kellerman Co., Philadelphia), and perceptual responses recorded during this collection. At 20 min, participants straddled the treadmill for 90 s, and an 11-mL venous blood sample was collected. Once running had recommenced, the next drink was ingested. This was repeated every 20 min. Final drinks were consumed at 80 min. Appropriate convective cooling, depending on treadmill velocity (∼2.5–3.5 m/s and standardized within participants), was applied to participants using two fans (one aimed at the torso, one aimed at the lower body) positioned 1 m in front of participants. After the completion of the exercise, participants towel-dried to remove unevaporated sweat, had nude body mass measured, and urine collected. Body mass was corrected for urine weight to avoid the confounding influence of bladder contents when determining hydration status.

Drink Composition

In each trial, participants consumed 60 g/h (20 g bolus every 20 min) of a 35% dextrose (dextrose powder, MyProtein, Northwich, UK) solution enriched with [U-^{13}C] glucose (∼0.208 g ingested in total; TEMP: 144.8 ± 1.8 δ‰ vs. Pee Dee Belemnite (PDB); HOT: 145.5 ± 1.3 δ‰ vs. PDB; Cambridge Isotope Laboratory Inc., Andover). Each bolus was followed by ingestion of a 20-mL water rinse to ensure complete tracer delivery. To compensate 90% of the estimated body mass losses, 1,665 ± 437 mL of water (in addition to the carbohydrate solution) was provided in HOT, and 795 ± 213 mL in TEMP.

Analysis

Urine specific gravity (USG) was measured on the trial day using a handheld analyzer (PAL10S, Digital Urine Specific Gravity Refractometer, ATAGO CO. LTD., Tokyo, Japan), before samples were aliquoted and stored at −80°C. Sweat was extracted from the patch via a 10-mL syringe, aliquoted, and stored at −80°C until analysis of sweat sodium and potassium by flame photometry (M410C Flame Photometer,

Sherwood Ltd., Cambridge, UK; coefficients of variation 2.4% and 1.7%, respectively). Ventral forearm sweat sodium and potassium concentrations were corrected to estimate whole body sweat concentrations (34).

For each 11 mL of venous blood, 1 mL was aliquoted into a K_2EDTA tube, 2.5 mL into a prechilled lithium heparin tube, 2.5 mL into a prechilled K_2EDTA tube, and 100 μL was directly analyzed using a blood gas analyzer (RAPIDPoint 500e System, Siemens, Erlangen, Germany) to determine electrolyte and metabolite concentrations. Both lithium heparin and EDTA tubes were immediately centrifuged (2,200 *g*, 20 min, 4°C) before being stored at −80°C until analysis. The remaining whole blood was added to a tube containing a clotting catalyst and was allowed to clot at room temperature for at least 20 min before centrifugation at 2,200 *g* for 20 min at 4°C. The serum was removed and frozen at −80°C until later analysis. The 1-mL tube of whole blood was used for analysis of hemoglobin (cyanmethemoglobin method; Sigma, St. Louis, MO) and hematocrit (microcentrifugation; Hawksley, Worthing, UK). Plasma volume changes from baseline were calculated from hemoglobin and hematocrit values (35). Breath (gas chromatography isotope ratio mass spectrometry, Hydra 20-20 IRMS; Europa Scientific, Crewe, UK) and drink (elemental analyzer isotope ratio mass spectrometry, 20-20 IRMS; Europa Scientific, Crewe, UK) samples were analyzed for $^{13}C/^{12}C$ ratio (both Elemental Microanalysis Ltd., Okehampton, UK). Plasma osmolality was calculated using measured concentrations of glucose, urea, and sodium (36). Enzyme-linked immunosorbent assays (ELISAs) were used to measure plasma adrenaline [adrenalin (epinephrine) ELISA, DRG; CV, 2.8%] and cortisol (Cortisol ELISA, DiaSource; CV, 3.7%) concentrations.

Calculations

The respiratory exchange ratio (RER) was calculated by dividing carbon dioxide production by oxygen consumption. Carbohydrate and fat oxidation rates were assessed using *Eq. 1* and *Eq. 2* (respectively) proposed by Jeukendrup and Wallis (37):

$$\text{Carbohydrate oxidation (g/min)} = 4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2, \quad (1)$$

$$\text{Fat oxidation (g/min)} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2. \quad (2)$$

Substrate partitioning via nonprotein respiratory quotient (NPRQ) was calculated using equations proposed by Zarins et al. (38):

$$\text{NPRQ (\%)} = \frac{\dot{V}CO_2 - (0.01 \times 4.89)}{\dot{V}O_2 - (0.01 \times 6.04)}, \quad (3)$$

$$\text{\%Carbohydrate oxidation (\%)} = \frac{\text{NPRQ} - 0.707}{0.293} \times 100, \quad (4)$$

$$\text{\%Fat oxidation (\%)} = (100 - \text{\%Carbohydrate oxidation}). \quad (5)$$

For each expired breath sample, the isotopic enrichment was expressed as δ per mille difference between $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard. The formula (39) used was as follows:

$$\delta^{13}C = \left[\left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}}\right) - 1\right] \times 10^3 \text{ per mil}. \quad (6)$$

Following this, $\delta^{13}C$ was then related to the international standard Vienna Pee Dee Bellemnitella (PDB). Exogenous carbohydrate oxidation rates were calculated as follows:

$$\text{Exogenous carbohydrate oxidation (g/min)} = \dot{V}CO_2\left(\frac{\delta\text{Exp} - \text{Exp}_{bkg}}{\delta\text{Ing} - \text{Exp}_{bkg}}\right)\left(\frac{1}{k}\right), \quad (7)$$

where δExp is the ^{13}C expired breath enrichment, δIng is the^{13}C enrichment of the ingested beverage, δExp_{bkg} is the ^{13}C expired breath enrichment at baseline (0 min; before any drink ingestion), and k is the CO_2 volume that is produced by oxidation of 1 g of glucose ($k = 0.7467$ L).

The enrichment of each drink was measured and used for each individual participant's calculations in that trial. There was no evidence for a difference in drink enrichment between TEMP and HOT (TEMP: 144.8 ± 1.8 δ‰ vs. PDB; HOT: 145.5 ± 1.3 δ‰ vs. PDB: $P = 0.401$).

When calculating exogenous carbohydrate oxidation rates using $^{13}CO_2$ from expired gas, a methodological consideration is the $^{13}CO_2$ trapped in the bicarbonate pool. During the onset of exercise, some CO_2 arising from the oxidation of glucose will be retained (40); however, $\dot{V}CO_2$ will increase during exercise until a condition of physiological steady state occurs (~30 min), resulting in equilibration between the $^{13}CO_2$ in expired gas and the CO_2/HCO_3 pool. Therefore, there is likely to be some underestimation of exogenous carbohydrate oxidation rates during the first 30 min of exercise (41, 42). Therefore, data should be interpreted as the minimum estimates at time points before 30 min.

T_{GI} was recorded every 20 min, and T_{Skin} was averaged over the final minute of each 20-min interval.

Statistical Analysis

Data are presented as means ± SD. Statistical analyses were conducted using RStudio (v. 2023.03.0, RStudio, PBC, Boston). Shapiro–Wilk tests were used to check the normality of the dependent variables. No violation of normality was observed. Linear mixed models (LMMs) were performed to examine the differences between conditions and timepoints. Fixed (Conditions, Timepoints) and random (Participants) effects for LMM were fitted for each dependent variable. The most appropriate model was chosen using the smallest Hurvich and Tsai's criterion (AICc) in accordance with the principle of parsimony. Pairwise comparisons (a *t* test for normally distributed data and a Wilcoxon test for nonnormal distribution) of estimated marginal means were performed using the Holm–Bonferroni adjustment to control for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

A total of 12 participants were recruited for this study. One experienced vomiting during the HOT condition, and another reached a gastrointestinal temperature of 39.9°C

and was withdrawn for safety reasons. Data from the remaining 10 participants are presented.

Baseline Measures and Pretrial Standardization

Body mass (TEMP: 72.9 ± 8.3; HOT: 72.9 ± 8.4 kg; P = 0.971), USG (TEMP: 1.0092 ± 0.0039; HOT: 1.0118 ± 0.0047; P = 0.100), T_{GI} (TEMP: 36.9 ± 0.1; HOT: 36.9 ± 0.2°C; P = 0.640), and heart rate (HR) (TEMP: 55 ± 11; HOT: 59 ± 16 beats/min; P = 0.145) were not different between trials, providing a good indication that participants arrived at the laboratory in a similar state. Resting values of ^{13}C breath enrichment were also not different between trials (TEMP: −27.13 ± 0.50; HOT: −27.38 ± 0.30 δ‰ vs. PDB; P = 0.112).

Thermoregulatory Responses

A time × condition interaction effect for T_{GI} was observed ($F_{5,99}$ = 15.23; P < 0.001) with higher T_{GI} observed at each timepoint in HOT compared with TEMP from 40 min to 100 min ($P \leq 0.001$; Fig. 1*A*) with T_{GI} reaching 39.2 ± 0.4°C in HOT and 37.9 ± 0.3°C in TEMP at the end of the exercise. A time × condition interaction effect for T_{skin} was observed ($F_{5,99}$ = 27.59; P < 0.001). T_{skin} was higher (P < 0.001) at each time point during exercise in HOT than in TEMP. Average (20–100 min) T_{skin} was 35.4 ± 1.1°C in HOT and 30.7 ± 1.1°C in TEMP (P < 0.001). A time × condition interaction effect for heart rate was observed ($F_{5,99}$ = 9.59; P < 0.001), with a greater heart rate observed in HOT compared with TEMP from 20 min to the end of the exercise (P < 0.001 for all; Fig. 1*B*). Heart rate reached 137 ± 16 beats/min in TEMP and 165 ± 14 beats/min in HOT at 100 min.

Stable-Isotope Measurements

Glucose ingestion resulted in a rise in the $^{13}CO_2/^{12}CO_2$ ratios from rest in both conditions. A time × condition interaction effect for breath $^{13}CO_2$ enrichment versus PDB was observed ($F_{5,99}$ = 2.58; P = 0.031), post hoc analysis detected greater enrichment in TEMP at 60 min (P = 0.040), 80 min (P = 0.010), and 100 min (P = 0.003; Fig. 2*A*).

Substrate Use

Average (40–100 min) $\dot{V}O_2$and $\dot{V}CO_2$ during exercise were reduced in HOT (Table 1). Average exogenous carbohydrate oxidation rates between 40 and 100 min were 20% lower in HOT than in TEMP (P = 0.006; Table 1), and peak exogenous carbohydrate oxidation rates were 18% lower in HOT (P = 0.002; Table 1). Exogenous carbohydrate oxidation was lower at 60 min (P = 0.026), 80 min (P = 0.003), and 100 min (P < 0.001; Fig. 2*B*). Average total carbohydrate oxidation was not different between conditions (P = 0.113; Table 1) and there was no time × condition interaction effect ($F_{5,99}$ = 0.82; P = 0.536; Fig. 2*C*). Average endogenous carbohydrate oxidation (between 40 and 100 min) was increased in HOT (P = 0.018; Table 1) but there was no time × condition interaction effect ($F_{5,99}$ = 1.64; P = 0.156; Fig. 2*D*). Fat oxidation between 40 and 100 min was lower in HOT compared with TEMP (P = 0.015; Table 1), with no time × condition interaction effect ($F_{5,99}$ = 1.28; P = 0.278). Blood glucose concentrations between 40 and 100 min were higher in HOT compared with TEMP (P = 0.013; Table 1), with no time × condition interaction effect ($F_{5,99}$ = 1.99; P = 0.087; Fig. 3*A*). Blood lactate concentrations between 40 and 100 min were higher in HOT compared with TEMP (P = 0.011; Table 1), with a time × condition interaction effect ($F_{5,99}$ = 2.74; P = 0.024; higher in HOT compared with temp at 80 and 100 min; Fig. 3*B*).

Plasma adrenaline concentrations were not different between HOT and TEMP, with no time × condition interaction effect ($F_{1,27}$ = 1.35; P = 0.256; Fig. 3*C*). A time × condition interaction effect ($F_{1,27}$ = 7.07; P = 0.013; Fig. 3*D*) was observed for plasma cortisol concentrations, with higher concentrations in HOT compared with TEMP at 100 min.

Fluid Balance

At the end of exercise, percentage body mass loss was greater in HOT than in TEMP (TEMP: +0.38 ± 0.54%; HOT: −0.34 ± 0.53%; P = 0.003; Fig. 4*A*). After the onset of exercise, plasma volume decreased from resting values in both trials with no evidence for a difference between conditions (Fig. 4*B*). Sweat losses were greater in HOT (2.39 ± 0.64 L) than in TEMP (1.21 ± 0.38 L; $P \leq 0.001$). Whole body sweat sodium concentrations were greater in HOT (57.9 ± 19.7 mmol/L) than in TEMP (33.6 ± 10.3 mmol/L; P = 0.002), whereas whole body sweat potassium concentrations were not different between trials (TEMP: 6.42 ± 1.63 mmol/L; HOT: 7.41 ± 3.09 mmol/L; P = 0.144). Plasma osmolality was not different between trials at the end of the exercise (TEMP: 293 ± 5 mOsmol/kgH_2O; HOT: 296 ± 3 mOsmol/kgH_2O; P = 0.124), and there was no time × condition interaction effect during the exercise ($F_{5,99}$ = 0.70; P = 0.652).

Perceptual Responses

Table 2 shows the RPE, RTS, and gastrointestinal and related complaints at 100 min of exercise.

DISCUSSION

The aim of this study was to investigate the effect of running in a hot versus temperate environment on exogenous

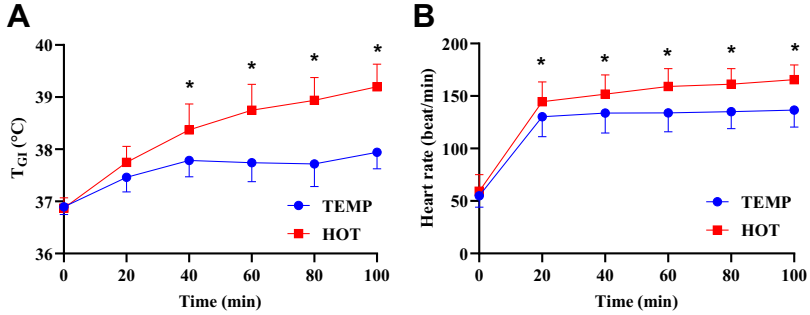

Figure 1. Gastrointestinal temperature (*A*) and heart rate (*B*) during exercise in TEMP and HOT. *Difference between trials. HOT, hot environment; TEMP, temperate environment.

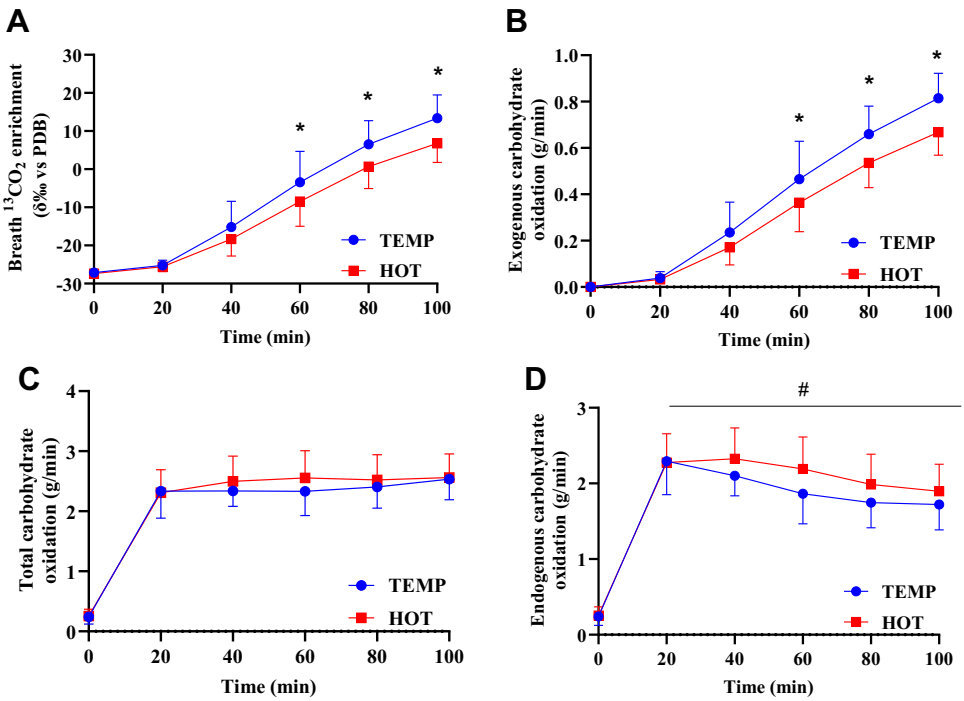

Figure 2. Breath $^{13}CO_2$ [U-^{13}C] glucose enrichment (*A*), exogenous (*B*), total (*C*), and endogenous (*D*) carbohydrate oxidation rates during exercise in TEMP and HOT. *Difference between trials. #Main condition effect. HOT, hot environment; TEMP, temperate environment.

carbohydrate oxidation while controlling fluid intake to maintain euhydration, as hypothesized. Despite maintaining euhydration [defined by ±1% body mass loss and no difference in plasma volume and osmolality (43)], prolonged running exercise in the heat led to a ∼20% reduction in exogenous carbohydrate oxidation. This suggests that heat stress independently impairs exogenous carbohydrate metabolism by a substantial amount, even without the contributing effect of dehydration. In addition, a greater use of endogenous carbohydrate stores was observed in the heat, whereas total carbohydrate oxidation remained similar across conditions.

The reduction (∼20%) in exogenous carbohydrate oxidation here exceeded those previously reported during cycling exercise in hot conditions [∼10% (9); ∼15% (10)], even though hydration was not controlled in those studies. This greater reduction, compared with previous studies, may be attributed to differences in mode of exercise (running vs. cycling) and the muscle mass engaged. Running, as opposed to cycling, involves lower mechanical efficiency and greater muscle recruitment (44), potentially exacerbating blood flow redistribution away from the gut. This intensified redistribution could further impair nutrient delivery and absorption, amplifying reductions in exogenous carbohydrate oxidation. In addition, runners demonstrate a strong correlation between elevated core temperature and gastrointestinal permeability (45, 46), even in the absence of overt gastrointestinal symptoms, which may have contributed here.

A smaller reduction in exogenous carbohydrate oxidation might have been expected under euhydrated conditions in this study, as dehydration would likely contribute additionally to impaired exogenous carbohydrate oxidation. Indeed, dehydration amplifies cardiovascular drift observed in hot conditions by reducing plasma and blood volume, which restricts blood flow redistribution and impairs oxygen delivery to working muscles (17, 18). Moreover, dehydration can exacerbate the rise in core temperature during exercise (47), further impairing metabolic and thermoregulatory function. The reduction, observed in this study, may reflect impairments across multiple steps involved in the pathway from carbohydrate ingestion to utilization in the muscle, directly explained by heat exposure (9).

The reduction in exogenous carbohydrate oxidation during exercise in the heat may be primarily driven by reduced blood flow to the muscle and around the gastrointestinal tract. In response to elevated core temperature, blood flow is redistributed toward the skin to facilitate heat dissipation via evaporative cooling (2). At the muscle level, this can reduce oxygen and nutrient delivery, whereas decreased splanchnic circulation reduces gastrointestinal blood flow (11, 48), potentially impairing gastric emptying and nutrient absorption. This hypothesis is supported by the negative correlation between core temperature and gastric emptying rate (12). However, although gastric emptying rates might be reduced during heat exposure [∼15–18 mL/min (12)], previous studies have shown that the rate of carbohydrate delivery to the intestine remains well above the oxidation rate observed in the heat (9). Consequently, impaired gastric emptying alone cannot fully account for the reduction in exogenous carbohydrate oxidation in hot conditions.

Other mechanisms, such as reduced intestinal absorption, might also explain the reduced exogenous carbohydrate oxidation in hot conditions (9). Elevated core temperatures (>39°C) have been shown to increase gastrointestinal permeability and compromise barrier function (45, 46), which could affect carbohydrate absorption. However, exercising in hot conditions is often accompanied by higher catecholamine levels (in this study, there was no evidence for differences in adrenaline concentrations between HOT and

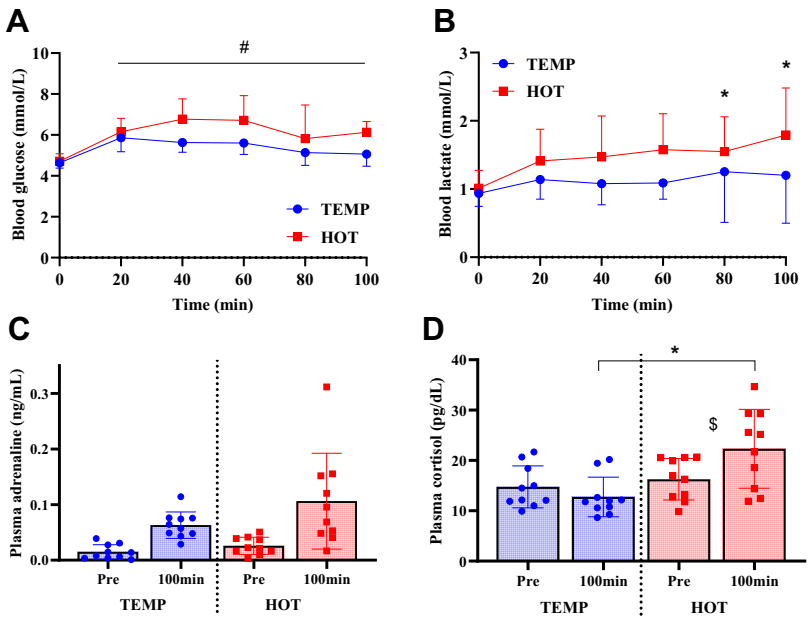

Figure 3. Blood glucose (*A*) and lactate (*B*) concentrations during exercise in TEMP and HOT. Plasma adrenaline (*C*) and cortisol (*D*) at baseline and at 100 min of exercise in TEMP and HOT. *Difference between trials. #Main condition effect. $Difference between pre and 100 min. HOT, hot environment; TEMP, temperate environment.

TEMP, but higher cortisol concentrations were observed in HOT), which typically upregulate intestinal glucose absorption via enhanced sodium glucose cotransporter 1 (SGLT1) activity (49). However, heat-induced splanchnic hypoperfusion/hypoxia (48) may override this effect, especially at "higher" exercise intensities. Given that SGLT1-mediated glucose absorption relies on the sodium gradient maintained by ATP-dependent Na^+/K^+-ATPase activity, reduced oxygen delivery and intestinal ischemia, resulting from blood flow redistribution, may impair its function, whereas GLUT5 mediated fructose transport may be less affected or even preserved (50). Consequently, heat stress-induced intestinal ischemia could reduce glucose absorption, limiting exogenous carbohydrate availability for use/oxidation.

At the muscle level, increased sympathetic activation may also augment muscle glycogenolysis via β-adrenergic stimulation (51). This can lead to intracellular glucose-6-phosphate accumulation that inhibits further glucose uptake into muscle cells, potentially explaining the observed reduction in exogenous glucose oxidation in the heat. Interestingly, in this study, total carbohydrate oxidation was not different between conditions, in contrast to many studies comparing carbohydrate oxidation in hot versus temperate conditions (6). This discrepancy is possibly explained by the exogenous carbohydrate intake during exercise in this study. However, the reduced exogenous carbohydrate oxidation in hot conditions was concomitant with an increased endogenous carbohydrate oxidation. The elevated circulating adrenaline concentrations during exercise in the heat, observed in previous studies (52, 53), likely stimulated intramuscular glycogen phosphorylase activity, enhancing glycogenolysis and intramuscular carbohydrate oxidation (51, 54), although no differences in adrenaline concentrations were observed in this study (although this may be due to the study being underpowered for adrenaline comparison). This metabolic shift may be further driven by reduced oxygen delivery due to compromised muscle perfusion, favoring glycolytic over oxidative metabolism and contributing to the increased blood lactate accumulation observed in the HOT trials, as in previous studies (3, 55). Moreover, elevated lactate concentrations can inhibit lipolysis (56) and impair fatty acid transport into mitochondria by downregulating carnitine palmitoyl transferase 1, which may also explain the concurrent reduction in fat oxidation. Although insulin also inhibits lipolysis, previous studies have shown no significant insulin differences during exercise in the heat versus temperate conditions when glucose is ingested (9, 10), indicating other factors are likely responsible.

Taken together, these findings confirm that heat stress impairs exogenous carbohydrate oxidation during prolonged exercise, independently of differences in hydration status. This impairment is likely explained by compromised intestinal glucose absorption and muscle glucose uptake rather than gastric emptying and is accompanied by increased reliance on endogenous carbohydrate stores.

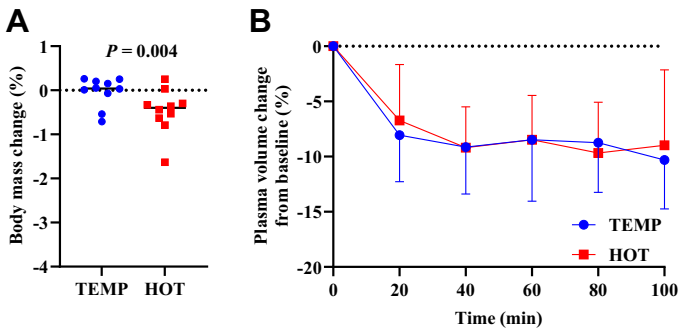

Figure 4. Body mass loss (*A*) and percentage plasma volume change from baseline (*B*) in TEMP and HOT. HOT, hot environment; TEMP, temperate environment.

Applications

Heat exposure (and associated increased thermal strain) can significantly impair exogenous carbohydrate oxidation

Table 2. *RPE, RTS, and gastrointestinal and related complaints at 100 min of exercise*

	TEMP	HOT	*P*
Rate of perceived exertion	11 ± 1	14 ± 1	**0.001**
Rate of thermal sensation	1 ± 2	5 ± 1	**0.001**
Thirst	1 ± 1	1 ± 1	0.735
Urge to urinate	5 ± 3	4 ± 3	**0.030**
Gastrointestinal comfort	2 ± 2	3 ± 3	0.497
Stomach bloatedness	2 ± 2	3 ± 2	0.186

Values are means ± SD. Bold values are significant. HOT, hot environment; RPE, rating of perceived exertion; RTS, rating of thermal sensation; TEMP, temperate environment.

by 20% compared with temperate conditions. These findings underscore the need to adopt adapted nutritional strategies in hot environments, to optimize exogenous carbohydrate oxidation (and performance), and/or to limit gastrointestinal distress.

Repeated exposure to carbohydrate ingestion through training can enhance exogenous carbohydrate oxidation (57), potentially mitigating the reduction observed in hot conditions. Moreover, if intestinal absorption limits carbohydrate availability in the heat, using multiple transportable carbohydrates (e.g., glucose:fructose) at lower ingestion rates than typically recommended in temperate conditions may be advantageous. This approach engages both SGLT1 and GLUT5 transporters and has been shown to enhance exogenous carbohydrate oxidation in hot environments compared with glucose alone at high ingestion rates (58). For example, instead of ingesting glucose at a rate of up to 60 g/h, a glucose:fructose ratio of 1:0.8 could be considered to avoid saturating intestinal transporters. An even higher proportion of fructose may be beneficial, as its absorption via GLUT5 is neither sodium- nor ATP-dependent and may be less compromised by heat-induced splanchnic hypoperfusion. Importantly, peak oxidation rates are only ∼70%–90% of ingestion rates and should be accounted for in recommendations (59).

Given the greater impairment observed in running (present study) compared with cycling (9, 10), nutrition guidelines should account for modality-specific differences in gastrointestinal tolerance and carbohydrate metabolism. In cycling, where gastrointestinal issues are less prevalent and carbohydrate intake is often more manageable due to increased access to food and the ability to drink/eat at any time, maintaining a high carbohydrate intake does not appear problematic, particularly for athletes who have undergone gut training to enhance carbohydrate tolerance (60). However, in running, gastrointestinal distress is more common (61, 62) due to several factors such as increased biomechanical disruption (i.e., vertical motion and mechanical stress on the GI tract), limited access to fluids, and the inability to drink/eat at high speed. Although no difference in gastrointestinal (GI) symptoms was observed in this study, one participant withdrew after experiencing vomiting during exercise in the HOT trial. This highlights that consuming large fluid volumes to maintain euhydration, particularly in the heat, may exacerbate GI discomfort. Consequently, carbohydrate and hydration strategies in running should be more carefully adapted to environmental conditions. Moreover, the exercise intensity in this study was relatively low (65% $\dot{V}O_{2peak}$ and ∼11–12 km/h) to limit gastrointestinal temperature elevations above 40°C (one participant reached this threshold and was withdrawn). Higher intensities are likely to increase GI distress risk, as gastric emptying declines above ∼75% $\dot{V}O_{2max}$ (63) and mechanical stress during high-speed running further contributes to GI symptoms (15). In addition, fluid/food intake becomes more difficult at higher speeds due to elevated breathing rates and reduced drinking/eating opportunities.

Limitations

A key limitation of this study is that participants arrived at the laboratory in an overnight fasted state due to the glucose tracer methodology used. This does not fully reflect real-world athlete practices, where preexercise carbohydrate intake is common. Another limitation is the lack of direct measurements of muscle temperature, gastric emptying, transit time, or malabsorption (e.g., via breath hydrogen analysis). Although the observed differences in exogenous glucose oxidation are consistent with previous research, future studies should incorporate these measures to confirm the underlying mechanisms. Moreover, as exogenous carbohydrate oxidation was still increasing at 100 min in both conditions, we cannot confirm whether the observed differences would have been reduced, maintained, or amplified during a longer exercise bout. However, if the mechanisms responsible for the reduction in the HOT condition are primarily related to carbohydrate delivery, these differences might diminish with extended exercise. Conversely, if they are linked to carbohydrate utilization at the muscle level, the differences could be amplified with a greater heat strain due to prolonged exercise.

There may also be a direct effect of drink temperature on carbohydrate delivery by influencing the rate of gastric emptying (64). However, in the present study, beverages were provided at ambient air temperature to better reflect real-life practice. Moreover, no correction was applied for the background shift in $^{13}CO_2$ enrichment from endogenous substrate stores during exercise, which could have led to a small overestimation of exogenous glucose oxidation. To limit the effect of background, participants performed a glycogen depletion protocol 3–4 days before each trial and adhered to a diet low in naturally abundant ^{13}C sources, minimizing background shifts in tracer studies (28, 41). The glycogen depletion exercise was not directly supervised, and providing a standardized dietary intake (65) would have been preferable to relying on dietary guidance and self-replication. However, confidence in the study's results is supported by the use of well-motivated participants, pretrial interviews verifying dietary adherence and depletion protocols, and the minimal change in exhaled ^{13}C at rest.

In addition, no females were included in the studies. Despite the study being open to female participants, limited interest resulted in an exclusively male sample. Consequently, the extent to which these findings translate to female athletes remains unknown and warrants further investigation.

Conclusions

This study provides evidence that heat stress independently impairs exogenous glucose oxidation during running,

despite maintained euhydration. The observed ~20% reduction likely reflects reduced intestinal absorption and muscle glucose uptake, rather than gastric emptying limitations. This was accompanied by increased reliance on endogenous carbohydrate stores. These findings highlight the need for adapted nutritional strategies in hot environments.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

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AUTHOR CONTRIBUTIONS

L.M., L.J.J., and S.A.M. conceived and designed research; L.M., M.H., D.E., and M.N. performed experiments; L.M. analyzed data; L.M. interpreted results of experiments; L.M. prepared figures; L.M. drafted manuscript; L.M., M.H., D.E., M.N., L.T., L.J.J., and S.A.M. edited and revised manuscript; L.M., M.H., D.E., M.N., L.T., L.J.J., and S.A.M. approved final version of manuscript.

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