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Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise

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Jentjens, Roy L. P. G., Anton J. M. Wagenmakers, and Asker E. Jeukendrup. Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise. *J Appl Physiol* 92: 1562–1572, 2002. First published December 21, 2001; 10.1152/japplphysiol.00482.2001.—The aim of the present study was to test the hypothesis that the oxidation rate of ingested carbohydrate (CHO) is impaired during exercise in the heat compared with a cool environment. Nine trained cyclists (maximal oxygen consumption 65 ± 1 ml·kg body wt^{-1}·min^{-1}) exercised on two different occasions for 90 min at 55% maximum power ouptput at an ambient temperature of either 16.4 ± 0.2°C (cool trial) or 35.4 ± 0.1°C (heat trial). Subjects received 8% glucose solutions that were enriched with [U-^{13}C]glucose for measurements of exogenous glucose, plasma glucose, liver-derived glucose and muscle glycogen oxidation. Exogenous glucose oxidation during the final 30 min of exercise was significantly ($P < 0.05$) lower in the heat compared with the cool trial (0.76 ± 0.06 vs. 0.84 ± 0.05 g/min). Muscle glycogen oxidation during the final 30 min of exercise was increased by 25% in the heat (2.07 ± 0.16 vs. 1.66 ± 0.09 g/min; $P < 0.05$), and liver-derived glucose oxidation was not different. There was a trend toward a higher total CHO oxidation and a lower plasma glucose oxidation in the heat although this did not reach statistical significance ($P = 0.087$ and $P = 0.082$, respectively). These results demonstrate that the oxidation rate of ingested CHO is reduced and muscle glycogen utilization is increased during exercise in the heat compared with a cool environment.

exogenous glucose; metabolism; stable isotopes; substrate utilization; cycling exercise

IT IS GENERALLY ACCEPTED THAT carbohydrate (CHO) ingestion during exercise can postpone fatigue and improve performance when the exercise duration is ~45 min or longer (2, 23). The possible mechanisms underlying the ergogenic effect of CHO ingestion are a better maintenance of blood glucose levels (6) and increased ability to maintain high CHO oxidation rates during prolonged exercise (4, 6). The effect of CHO feedings on muscle glycogen utilization is, however, less clear. Although exogenous CHO ingestion may spare muscle glycogen during running (47), this does not seem to be the case during cycling (4, 6, 25).

There are several factors that affect the oxidation rate of ingested CHO such as type and intensity of exercise; amount, type, and timing of CHO ingestion; preexercise muscle glycogen concentration; and diet (24). Interestingly, even when large amounts of CHO were ingested at rates up to 2.4–3.0 g/min during prolonged exercise at 50–65% maximal oxygen consumption uptake ($\dot{V}O_{2\,max}$) (27, 48), exogenous CHO oxidation rates did not exceed 1.1 g/min. According to the studies above, it appears that exogenous CHO oxidation is limited to rates of 1.0–1.1 g/min or 60–70 g/h for exercise durations up to 180 min (4, 19, 24, 27, 48). This finding has resulted in clear guidelines for athletes in terms of CHO ingestion during exercise (24). However, these guidelines are based on studies performed in cool and thermoneutral environments, and it is possible that these guidelines are not suitable for exercise in hot environments.

CHO availability has also been shown to be important during exercise in the heat because both CHO feeding during exercise (8, 31) and a high-CHO diet (38) have been shown to improve exercise performance in hot conditions. It must be noted, however, that such improvements may only be observed if the heat stress is compensable (10).

The combination of exercise and heat stress results in major alterations in CHO metabolism. Increased ambient temperature leads to increased CHO oxidation during exercise caused by increased muscle glycogen use (14, 18) with no change in glucose uptake by the muscle (18). Furthermore, it has been suggested that there is an increased hepatic glucose production with no alteration in glucose uptake, leading to hyperglycemia (18).

To our knowledge, there are no studies available in the literature that have investigated simultaneous estimates of exogenous CHO oxidation and muscle glycogen oxidation in hot environments.

A recent study suggests that exogenous CHO oxidation during exercise in a thermoneutral environment might be limited at the level of intestinal absorption or disposal by the liver (27). It is likely that intestinal

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CHO absorption is reduced during exercise in the heat. Exercise in hot environments leads to increased blood flow to the skin to allow for evaporative cooling (28). As a consequence, blood flow in other tissues and organs, like the liver (44), kidney (39), and splanchnic region (43, 45), is reduced during exercise in the heat. A reduced blood flow to the intestine may impair absorption of CHO (and other nutrients) (51), which may subsequently lead to a reduced oxidation rate of ingested CHO. Furthermore, an accelerated muscle glycogenolysis during exercise in the heat may result in elevated muscle glucose 6-phosphate concentrations (18). An increase in glucose 6-phosphate concentration has been associated with a reduced muscle glucose uptake (50), and this may contribute to a decreased exogenous CHO oxidation rate during exercise in the heat. The aim of the present study was, therefore, to examine exogenous CHO oxidation in the heat compared with a cool environment.

We hypothesize that the oxidation rate of the ingested CHO is impaired in the heat compared with a cool environment. The findings of this study may have impact on the recommendations for CHO intake in athletes exercising in the heat.

METHODS

Subjects. Nine trained nonacclimated male cyclists or triathletes, aged 24.4 ± 2.6 yr and with a body weight of 72.4 ± 1.3 kg, took part in this study. Subjects trained at least three times a week for >2 h/day and had been involved in endurance training for at least 2–4 yr. All subjects were told the purpose, practical details, and risks associated with the procedures before giving their written informed consent to participate. All subjects were healthy as assessed by a general health questionnaire. The study was approved by the Ethics Committee of the School of Sport and Exercise Sciences of the University of Birmingham (Birmingham, UK).

Preliminary testing. At least 1 wk before the start of the experimental trials, an incremental cycle exercise test to volitional exhaustion was performed to determine the individual maximum power output ($\dot{W}max$) and $\dot{V}O_{2\,max}$. This test was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), modified to the configuration of a racing bicycle with adjustable saddle height and handlebar position. Subjects started with a 3-min warm-up at 95 W, followed by incremental steps of 35 W every 3 min until exhaustion. $\dot{W}max$ was determined by the following formula [adapted from Kuipers et al. (29)]

$$\dot{W}max = \dot{W}_{out} + [(t/180) \cdot 35]$$

where $\dot{W}out$ is the power output (W) during the last completed stage, and t is the time (s) in the final stage. Heart rate (HR) was recorded continuously by a radiotelemetry HR monitor (Polar Vantage, Kempele, Finland). $\dot{W}max$ values were used to determine the 55% $\dot{W}max$ workload, which was later employed in the experimental trials. Breath-by-breath measurements were performed throughout exercise by using an Oxycon Alpha automated gas-analysis system (Jaeger, Wuerzberg, Germany). The volume sensor was calibrated by using a 3-liter calibration syringe, and the gas analyzers were calibrated by using a 4.11% CO_2-16.48% O_2-79.41% N_2 gas mixture. Average inspired and expired ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were determined over eight breaths. $\dot{V}O_2$ was considered to be maximal ($\dot{V}O_{2\,max}$) when at least two of the three following criteria were met: *1*) a leveling off of $\dot{V}O_2$ with increasing workload (increase of no more than 2 ml·kg body $wt^{-1}\cdot min^{-1}$), *2*) a HR within 10 beats/min of predicted maximum (HR of 220 beats/min − age), and *3*) a respiratory exchange ratio (RER) >1.05. $\dot{V}O_{2\,max}$ was calculated as the average $\dot{V}O_2$ over the last 60 s of the test. The $\dot{V}O_{2\,max}$ and $\dot{W}max$ achieved during the incremental exercise test were 65 ± 1 ml·kg body $wt^{-1}\cdot min^{-1}$ and 374 ± 10 W, respectively.

Experimental design. All subjects completed two exercise trials, which were randomly assigned and separated by at least 1 wk. Each trial consisted of 90 min of cycling at 55% $\dot{W}max$ at an ambient temperature and relative humidity of either 16.4 ± 0.2°C and 60 ± 1% (cool trial) or 35.4 ± 0.1°C and 27 ± 1% (heat trial), respectively, while the subjects were ingesting an 8% glucose solution. During both trials, subjects ingested a glucose solution that was enriched with a [U-^{13}C]tracer. All experiments took place in the Human Performance Laboratory of the University of Birmingham.

Diet and activity before testing. Subjects were asked to record their food intake and activity pattern 3 days before the first exercise trial and were then instructed to follow the same diet and activities before the second trial. In addition, 5–7 days before each experimental testing day, they were asked to perform an intense training session ("glycogen-depleting" exercise bout) in an attempt to empty any ^{13}C-enriched glycogen stores. Subjects were further instructed not to consume any food products with a high natural abundance of ^{13}C (CHO derived from C4 plants: maize, cane sugar) at least 1 wk before and during the entire experimental period to reduce the background shift (change in $^{13}CO_2$) from endogenous substrate stores.

Protocol. The subjects arrived at the Human Performance Laboratory in the morning (between 7:30 and 9:00 AM) after an overnight fast (10–12 h) and having refrained from any strenuous activity or drinking any alcohol in the previous 24 h. On arrival, a 21-gauge Teflon catheter (Quickcath, Baxter, Norfolk, UK) was inserted in an antecubital vein for blood sampling. The catheter was kept patent by flushing with 1.0–1.5 ml of isotonic saline (0.9%, Baxter) after each sample collection. After voiding, the subject was weighed in cycling shorts to the nearest 0.1 kg by using a platform scale (Seca Alpha, Hamburg, Germany). Thereafter, a rectal thermistor (Grant Instruments, Cambridge, UK) was positioned 10–15 cm beyond the anal sphincter, and rectal temperature (T_{re}) was monitored continuously.

The subjects then mounted the cycle ergometer and sat quietly on the bike for 5–7 min while thermistors (Grant Instruments) were attached to the skin of the forehead, lower back, dorsal surface of the right hand, and calf muscle of the right leg for measurements of skin temperature. Weighted mean skin temperature (T_{sk}) was calculated by using the equation of Nielsen and Nielsen (34). Next, a resting breath sample was collected in 10-ml vacutainer tubes (Becton Dickinson, Plymouth, UK), which were filled, directly from a mixing chamber in duplicate to determine the $^{13}C/^{12}C$ ratio in the expired air. A resting blood sample (10 ml) was collected and stored on ice and later centrifuged. Additional blood samples were drawn at 15-min intervals during exercise. Expiratory breath samples were collected every 15 min until the end of exercise. $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were measured every 15 min for periods of 5 min by using an Oxycon Alpha automated gas-analysis system (Jaeger). HR was recorded in 30-s intervals using a Polar HR monitor (Polar Vantage), and averages were taken of the final 5 min

of each 15-min interval. T_{re} and T_{sk} were recorded at rest and every 2.5-min interval during exercise by using an automatic data logger (Squirrel meter/logger, 1000 series, Grant Instruments). T_{re} and T_{sk} data were later averaged for 5-min periods.

Subjects were asked to rate their perceived exertion every 15 min on a scale from 6 to 20 by using the Borg category scale (3). In addition, subjects were asked every 30 min to fill in a questionnaire to rate (possible) stomach and/or gut problems. Approximately 30 min after catherization, subjects started a warm-up of 2.5 min at 150 W immediately followed by 90 min of exercise at a workload equivalent to 55% $\dot{W}$max. During the 2.5-min warm-up period, subjects drank an initial bolus (8 ml/kg body wt) of an 8% (80 g/l) glucose drink. Thereafter, every 15 min a beverage volume of 3 ml/kg body wt was provided. This feeding schedule was chosen to minimize dehydration and has been shown in earlier studies (22, 25) to result in tracer steady states after 60 min of exercise. The average amount of glucose and fluid consumed during the 90-min exercise bout was 133 ± 2 g and 1.67 ± 0.03 liters, respectively. A standing floor fan was placed in front of the subject to circulate air during all trials. Immediately after exercise, subjects voided and were towel dried before they were weighed again wearing cycling shorts only (accurate to 0.1 kg; Seca Alpha).

Glucose drinks. To quantify exogenous glucose oxidation, the 8% glucose solutions provided were prepared from corn-derived glucose (Sigma Aldrich, Dorset, UK), which has a high natural abundance of ^{13}C [−11.2 δ‰ vs. Pee Dee Bellemnitella (PDB)]. To increase the ^{13}C content of the glucose solution even further, a trace amount of uniformly labeled [^{13}C]glucose was added (~0.034 g [U-^{13}C]glucose/l; Cambridge Isotope Laboratories, Cambridge, MA). The glucose solution provided to the subjects had a ^{13}C enrichment of 25.6 δ‰ vs. PDB. The ^{13}C enrichments of the corn-derived glucose and experimental glucose drink were determined by elemental analyzer-isotope ratio mass spectrometry (IRMS; Carlo Erba-Finnigan MAT 252, Bremen, Germany).

Questionnaires. Subjects were asked to fill out a questionnaire every 30 min during the exercise trials (after the first drink was received). The questionnaire contained questions regarding the presence of gastrointestinal (GI) problems at that moment and addressed the following complaints: stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate/defecate. While subjects were on the bike and continued their exercise, each question was answered by simply ticking a box on the questionnaire that corresponded to the severity of the GI problem addressed. The items were scored on a 10-point scale (1 = not at all, 10 = very, very much). The severity of the GI symptoms was divided into two categories: severe and nonsevere symptoms, as was previously described by Jeukendrup et al. (26). Severe complaints included nausea, stomach problems, bloated feeling, diarrhea, urge to vomit, and stomach and intestinal cramps because these are symptoms that commonly impair performance and may bring with them health risks. The above symptoms were only registered as severe symptoms when a score of 5 or higher out of 10 was reported. When a score below 5 was given, they were registered as nonsevere. All other symptoms were registered as nonsevere regardless of the score reported.

Analyses. Blood (10 ml) was collected into prechilled EDTA-containing tubes containing 200 μl of 0.2 M EDTA and centrifuged at 3,500 rpm for 10 min at 4°C. Aliquots of plasma were stored at −70°C until further analyses of glucose and lactate. Approximately 1 ml of the EDTA-treated blood was used for measurements of hematocrit and hemoglobin so that changes in plasma volume from rest could be calculated as described by Dill and Costill (9). Hematocrit was determined in triplicate by microcentrifugation, and hemoglobin (duplicate) was analyzed by the cyanmethemoglobin method (Drabkin's reagent, 525, Sigma Aldrich) by using a spectrophotometer (Cecil Instruments, Cambridge, UK). Glucose (Glucose K kit, 17-UV, Sigma Aldrich), lactate (Lactate kit, 735, Sigma Aldrich), and free fatty acids (FFA; NEFA-C Wako Chemicals, Neuss, Germany) were analyzed on COBAS BIO semiautomatic analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Ultrasensitive human insulin kit, Linco Research, St. Charles, MO). Breath samples were analyzed for $^{13}C/^{12}C$ ratios by gas chromatography-isotope ratio mass spectrometry (IRMS; Finnigan MAT 252, Bremen, Germany).

To determine $^{13}C/^{12}C$ ratios in plasma glucose, glucose was first extracted with chloroform-methanol-water and derivatization was performed with butyl-boronic acid and acetic anhydride (36). Thereafter, the derivative was measured by gas chromatography-IRMS (Finnigan MAT 252). The measured $^{13}C/^{12}C$ ratios in the derivative were corrected for isotopic carbon dilution. This was done by measuring a series of glucose standards both in the derivatized form (by combustion-IRMS; Carlo Erba-Finnigan MAT 252, Bremen, Germany) and by direct combustion of underivatized glucose (elemental analyzer-IRMS). The standard curve thus constructed was linear over a range from 0 to 500‰ vs. PDB. From indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope measurements (breath $^{13}CO_2/^{12}CO_2$ ratios and plasma [^{13}C]glucose enrichments), oxidation rates of total fat, total CHO, muscle glycogen, liver-derived glucose, plasma glucose and exogenous glucose were calculated.

Calculations. From the volume of CO_2 production per unit time (l/min; $\dot{V}CO_2$) and $\dot{V}O_2$, total CHO and fat oxidation rates (g/min) were calculated by using stoichiometric equations of Frayn (16), with the assumption that the nitrogen excretion rate during exercise was negligible

$$\text{Glucose oxidation} = 4.55\ \dot{V}CO_2 - 3.21\ \dot{V}O_2 \quad (1)$$

$$\text{Fat oxidation} = 1.67\ \dot{V}O_2 - 1.67\ \dot{V}CO_2 \quad (2)$$

The isotopic enrichment was expressed as δ‰ difference between the $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard according to the formula of Craig (7)

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

The $\delta^{13}C$ was then related to an international standard (PDB).

Exogenous glucose oxidation was calculated by using the formula (37)

$$\text{Exogenous glucose oxidation} = \dot{V}CO_2 \cdot \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta Ing - \delta Exp_{bkg}}\right)\left(\frac{1}{k}\right) \quad (4)$$

in which δExp is the ^{13}C enrichment of expired air during exercise at different time points, δIng is the ^{13}C enrichment of the ingested glucose, δExp_{bkg} is the ^{13}C enrichment of expired air before exercise (background), and k is the amount of CO_2 (in liters) produced by the oxidation of 1 g of glucose (k = 0.7467 l CO_2/g glucose). Because glycogen stores are also ^{13}C enriched, shifts in substrate utilization (for instance in the transition from rest to exercise) may result in a change in background enrichment (49). Previous studies have shown

that the dietary intervention performed in the present study is effective in reducing the background shift from endogenous substrate stores in European subjects (42, 48, 49). Furthermore, the ^{13}C enrichment of the CHO ingested was artificially increased by adding [U-^{13}C]glucose to the CHO beverage. It is therefore not necessary to correct for the relatively small shift in the background ^{13}C enrichment (27).

Plasma glucose enrichment was measured, and the following formula was used to calculate plasma glucose oxidation

$$\text{Plasma glucose oxidation} = \dot{V}_{CO_2} \cdot \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta PG - \delta PG_{bkg}}\right)\left(\frac{1}{k}\right) \quad (5)$$

in which, δPG is the plasma glucose ^{13}C enrichment and δPG_{bkg} is the plasma glucose ^{13}C enrichment before exercise (background).

Because plasma glucose oxidation represents the oxidation of both glucose coming from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis and gluconeogenesis), liver-derived glucose oxidation could be calculated by the following formula

$$\text{Liver-derived glucose oxidation} = \text{plasma glucose oxidation} - \text{exogenous glucose oxidation} \quad (6)$$

Muscle glycogen oxidation was estimated by using the formula

$$\text{Muscle glycogen oxidation} = \text{total CHO oxidation} - \text{plasma glucose oxidation} \quad (7)$$

A methodological consideration when using $^{13}CO_2$ in expired air to calculate exogenous substrate oxidation is the trapping of $^{13}CO_2$ in the bicarbonate pool, in which an amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped. However, during exercise, the CO_2 production increases severalfold so that a physiological steady-state condition will occur relatively rapidly, and $^{13}CO_2$ in the expired air will be equilibrated with the $^{13}CO_2$/$H^{13}CO_3^-$ pool. Recovery of $^{13}CO_2$ from [U-^{13}C]glucose oxidation will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (25). Therefore, data from the initial 60 min were not used for calculation of exogenous glucose oxidation. As a consequence of this, all calculations on substrate oxidation were performed over last 30 min of exercise (60–90 min).

Statistics. ANOVA for repeated measures was used to compare differences in substrate utilization and in blood related parameters over time between the trials. A Tukey's post hoc test was applied in the event of a significant *F*-ratio. Where appropriate, the comparison of variables between the two conditions was conducted by using a Student's *t*-test for paired samples. All values are expressed as means ± SE. Statistical significance was set at $P < 0.05$.

RESULTS

T_{re} and T_{sk}. No difference was found in resting T_{re} between the two trials (Fig. 1*A*). In the cool trial, T_{re} gradually increased ($P < 0.01$) from 36.9 ± 0.1°C at rest to a steady-state level of 38.0 ± 0.1°C by 40 min of exercise and finally reached a T_{re} of 38.3 ± 0.1°C by the end of exercise. In the heat trial, T_{re} rose significantly ($P < 0.01$) during the first 60 min of exercise (from 37.1 ± 0.1 to 38.7 ± 0.2°C) and was 39.1 ± 0.2°C by the end of exercise (not significantly different from 60 min). No difference in T_{re} was found between the heat and cool trials during the first 45 min of exercise. Thereafter, T_{re} in the heat trial was significantly higher ($P < 0.05$) than in the cool trial until the end of exercise (Fig. 1*A*, Table 1).

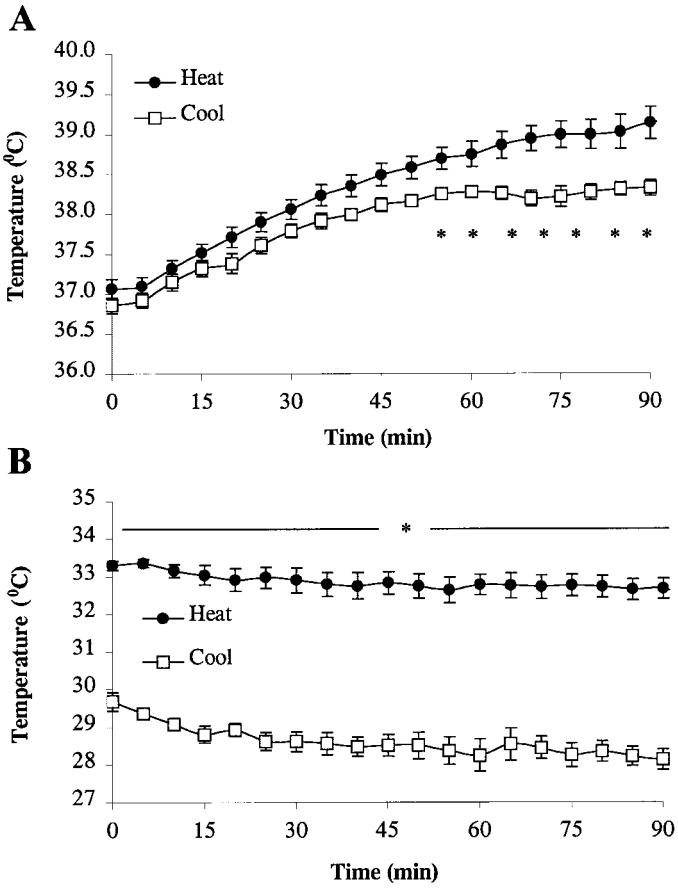

Fig. 1. Rectal temperature (*A*; $n = 8$ subjects) and weighted mean skin temperature (*B*; $n = 9$ subjects) during exercise in heat and cool trials while subjects were ingesting a carbohydrate solution. Values are means ± SE. *Significant difference between heat and cool trials, $P < 0.05$.

T_{sk} was significantly higher ($P < 0.01$) during exercise in the heat than in the cool trial (Fig. 1*B*). There was a decrease ($P < 0.01$) in T_{sk} over time in the cool trial (from 29.7 ± 0.3°C at rest to 28.1 ± 0.3°C at 90 min), whereas T_{sk} during exercise in the heat trial was maintained around 33°C.

Stable-isotope measurements. Glucose ingestion resulted in a rise of the $^{13}CO_2/^{12}CO_2$ ratios from values around −27 δ‰ vs. PDB at rest to values ranging from −14 and −16 δ‰ vs. PDB by the end of exercise in the cool and heat trials, respectively (Fig. 2*A*). From the 30-min time point onward, breath $^{13}CO_2$ enrichment was significantly higher ($P < 0.05$) in the cool compared with the heat trial. Plasma [^{13}C]glucose enrichments are shown in Fig. 2*B*. Both breath $^{13}CO_2$ enrichments and plasma [^{13}C]glucose enrichments leveled off after 60 min of exercise (Fig. 2, *A* and *B*).

Substrate utilization during 60- to 90-min exercise period. Substrate oxidation is summarized in Table 2 and in Fig. 3. Exogenous glucose oxidation was signif-

Table 1. *Oxygen uptake, ventilation rate, respiratory exchange ratio, heart rate, rectal temperature, and weighed mean skin temperature, glucose and lactate concentrations during the 60- to 90-min period of exercise in heat and cool trials*

	Heat Trial	Cool Trial	*P* Value
$\dot{V}O_2$, ml/min	3,108 ± 82	3,113 ± 91	0.960
$\dot{V}E$, l/min	78 ± 3†	73 ± 2	0.029
RER	0.931 ± 0.009	0.908 ± 0.008	0.164
Glucose, mmol/l	5.0 ± 0.2†	4.7 ± 0.2	0.034
Lactate, mmol/l	1.5 ± 0.3†	1.0 ± 0.1	0.047
HR, beats/min	164 ± 5†	140 ± 4	0.000
T_{re}, °C	38.90 ± 0.19*†	38.26 ± 0.08*	0.003
T_{sk}, °C	32.71 ± 0.29†	28.31 ± 0.29	0.000

Values are means ± SD for 9 subjects except as noted by *. $\dot{V}O_2$, oxygen uptake; $\dot{V}E$, ventilation rate; RER, respiratory exchange ratio; HR, heart rate; T_{re}, rectal temperature; T_{sk}, weighted mean skin temperature. †Significantly different from cool trial, $P < 0.05$.

icantly lower ($P < 0.01$) in the heat compared with the cool trial (0.76 ± 0.06 vs. 0.84 ± 0.05 g/min, respectively). Liver-derived glucose oxidation was not different between the two trials. There was a trend for a higher total CHO oxidation (3.2 ± 0.1 vs. 2.9 ± 0.1 g/min, respectively; $P = 0.087$) and a lower plasma glucose oxidation (1.12 ± 0.06 vs. 1.19 ± 0.04 g/min, respectively; $P = 0.082$) in the heat compared with the cool trial (Table 2). Muscle glycogen oxidation was increased by 25% in the heat trial (2.07 ± 0.16 vs. 1.66 ± 0.09 g/min, respectively; $P < 0.05$). Fat oxidation tended to be lower in the heat compared with the cool trial, but this did not reach statistical significance (Table 2).

Data for $\dot{V}O_2$, $\dot{V}E$, and RER during the final 30 min of exercise in both trials are shown in Table 1. RER tended to be higher in the heat, but this difference did not reach statistical significance. There was no difference in $\dot{V}O_2$ between the heat and the cool trials. $\dot{V}E$ was higher during exercise in the heat than in the cool trial (78 ± 3 vs. 73 ± 2 l/min; $P < 0.05$).

Blood metabolites. Resting concentrations of plasma glucose and lactate were not different between the heat and cool trials (Fig. 4, *A* and *B*). There was no significant interaction between treatment (ambient temperature) and time for glucose, although there was a main effect for time ($P < 0.001$) and treatment ($P < 0.05$). At rest, plasma glucose concentrations were in the range of 4.6–4.9 mmol/l. The ingestion of a large glucose bolus at the start of exercise resulted in significantly ($P < 0.01$) elevated plasma glucose concentrations (~6 mmol/l) after 15 min of exercise compared with resting values. Plasma glucose then fell to resting values and remained at this concentration for the duration of exercise. The plasma glucose concentration was significantly higher in the heat compared with the cool trial (main effect for ambient temperature; $P < 0.05$). In addition, the average plasma glucose concentration for the final 30 min of exercise was 5.0 ± 0.2 and 4.7 ± 0.2 mmol/l in the heat and cool trials, respectively ($P < 0.05$; Table 1). Plasma lactate levels increased ($P < 0.05$) during the first 15 min of exercise in the heat

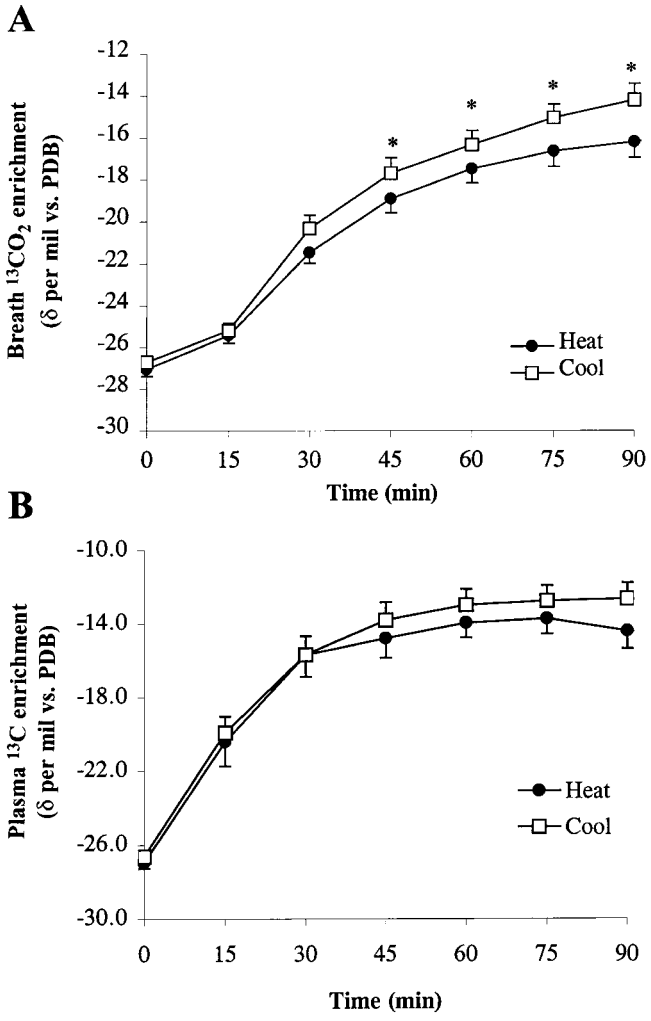

Fig. 2. Breath $^{13}CO_2$ (*A*) and plasma [U-^{13}C]glucose (*B*) enrichment during exercise in heat and cool trials while subjects were ingesting a carbohydrate solution. Values are means ± SE; $n = 9$. PDB, Pee Dee Bellemnitella. *Significant difference between heat and cool trials, $P < 0.05$.

trial, and the highest values were observed at the end of exercise (1.5 ± 0.3 mmol/l; Fig. 4*B*). In the cool trial, plasma lactate concentration rose slightly ($P > 0.05$) during the first 15 min of exercise and was maintained around 1 mmol/l throughout exercise. Plasma lactate concentration was higher in the heat compared with the cool trial, but this only reached statistical signifi-

Table 2. *Substrate utilization calculated during the 60- to 90-min period of exercise in heat and cool trials*

	Heat Trial	Cool Trial	*P* Value
CHO total	3.18 ± 0.15	2.85 ± 0.10	0.087
Fat total	0.36 ± 0.05	0.48 ± 0.05	0.197
Exogenous glucose	0.76 ± 0.06*	0.84 ± 0.05	0.017
Muscle glycogen	2.07 ± 0.16*	1.66 ± 0.09	0.042
Plasma glucose	1.12 ± 0.06	1.19 ± 0.04	0.082
Liver-derived glucose	0.36 ± 0.04	0.36 ± 0.04	0.783

Values are presented as means ± SE given in g/min for 9 subjects. CHO, carbohydrate. *Significantly different from cool trial, $P < 0.05$.

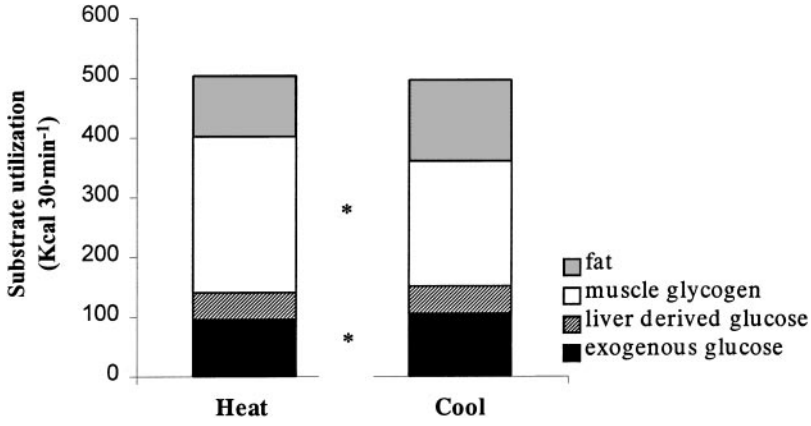

Fig. 3. Energetic contribution (kcal/30 min) of substrates during the 60- to 90-min period of exercise in heat and cool trials. Values are means ± SE; $n = 9$ subjects. *Significant difference between heat and cool trials, $P < 0.05$.

cance after 75 min of exercise ($P < 0.05$) and just failed to reach significance at 60 min ($P = 0.06$; Fig. 4*B*). The average plasma lactate concentration during the final 30 min of exercise in the heat trial was significantly higher ($P < 0.05$) than in the cool trial (Table 1).

Plasma FFA and insulin concentrations are depicted in Fig. 5, *A* and *B*, respectively. Because of collection problems, analyses were only performed on eight subjects. Plasma insulin concentrations in the heat and cool trials increased during the first 15–30 min of exercise, and this was accompanied by a decrease in plasma FFA levels. Plasma insulin then returned to resting values and remained at this concentration for the duration of exercise. After the drop in plasma FFA during the early part of exercise, plasma FFA then gradually rose until the end of the exercise. No differ-

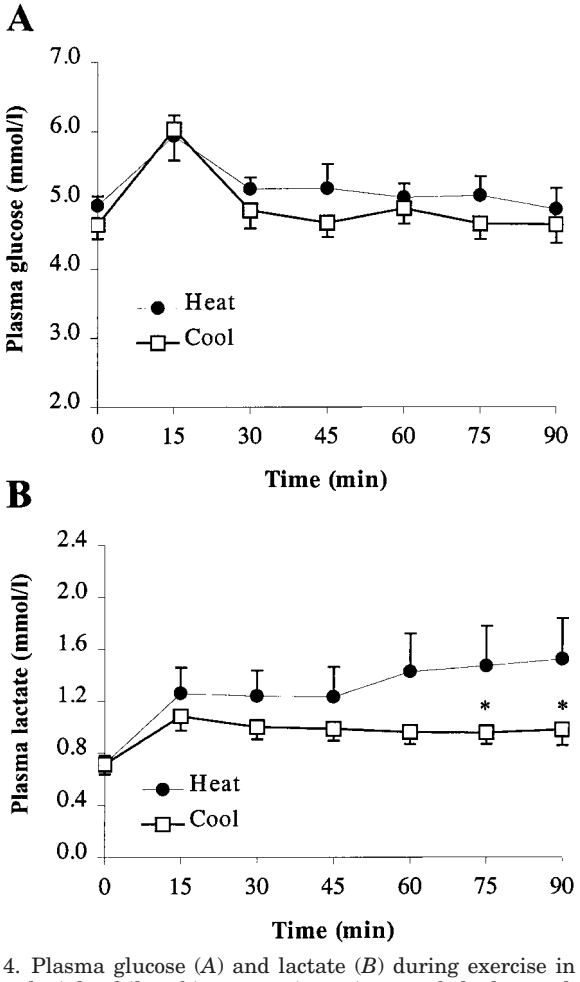

Fig. 4. Plasma glucose (*A*) and lactate (*B*) during exercise in heat and cool trials while subjects were ingesting a carbohydrate solution. Values are means ± SE; $n = 9$ subjects. *Significant difference between heat and cool trials, $P < 0.05$.

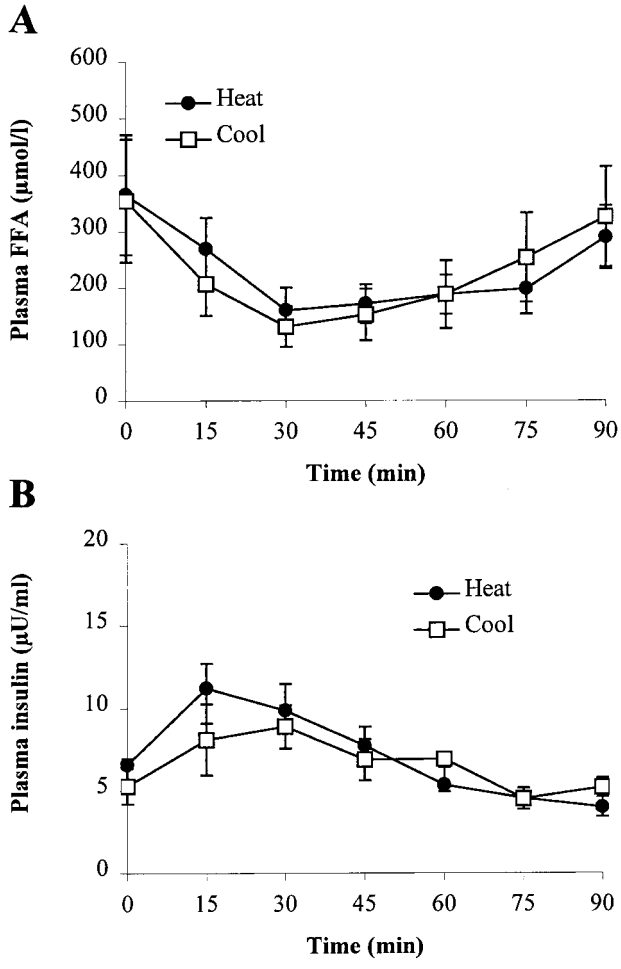

Fig. 5. Plasma free fatty acids (FFA; *A*) and insulin (*B*) during exercise in heat and cool trials while subjects were ingesting a carbohydrate solution. Values are means ± SE; $n = 8$ subjects.

ences were found in plasma insulin and FFA between the heat and cool trials.

Body mass, plasma volume changes, and HR. Subjects were more dehydrated during the heat trial compared with the cool trial, because mean body mass loss was significantly higher ($P < 0.001$) in the heat vs. cool trial (2.1 ± 0.2 vs. 1.3 ± 0.1 kg), whereas mean total fluid intake during both trials was the same (1,667 ± 31 ml). Plasma volume declined within the first 15 min of exercise and remained stable thereafter for the remaining 75 min of exercise during both treatments. The plasma volume changes were significantly greater in heat compared with cool trial and reached statistical significance from the 45-min time point onward.

There was a progressive rise in HR during exercise in the heat trial (from 145 ± 4 beats/min at 15 min to 167 ± 5 beats/min at 90 min; $P < 0.01$), whereas HR in the cool trial increased to a lesser extent (from 135 ± 4 beats/min at 15 min to 140 ± 5 beats/min at 90 min; $P < 0.01$). Mean HR was significantly higher ($P < 0.001$) during the last 30 min of exercise in the heat compared with the cool trial (Table 1).

Perceived exertion. There was a significant ($P < 0.01$) rise in rating of perceived exertion (RPE) during the 90 min of exercise in the heat trial (from 11.6 ± 0.3 units at 15 min to 15.3 ± 1.1 units at 90 min). In the cool trial, RPE was not different over time and remained around 11 units. The RPE was significantly higher ($P < 0.05$) in the heat compared with the cool trial, with the differences being observed from the 45-min time point onward.

GI discomfort. GI and related complaints are displayed in Table 3. Subjects reported more GI symptoms when glucose drinks were ingested during exercise in the heat compared with the cool trial, with most of them being classified as "nonsevere." Stomach problems, nausea, and bloated feeling were more often registered as severe during exercise in the heat than during exercise in the cool trial. Another obvious finding was that subjects reported a higher urge to urinate in the cool compared with the heat trial. None of the subjects vomited or suffered from diarrhea during the exercise trails.

Table 3. *Gastrointestinal and related complaints during 90 min of exercise*

Complaint	Heat Trial	Cool Trial
Nonsevere		
Dizziness	2	0
Headache	3	1
Flatulence	5	3
Urge to urinate	3	6
Urge to defecate	3	3
Belching	7	3
Stomach burn	3	1
Urge to vomit	1	0
Side aches, left	1	0
Side aches, right	2	0
Stomach problems	3	2
Nausea	1	1
Bloated feeling	2	1
Stomach cramps	2	0
Severe		
Stomach problems	3	0
Nausea	2	1
Bloated feeling	4	2
Stomach cramps	1	0

Values are for 9 subjects. Stomach cramps, nausea, bloated feeling, and stomach problems were registered as "severe" when a score of 5 or higher (out of 10) was given.

DISCUSSION

Oxidation rates of orally ingested CHO during exercise in comfortable ambient conditions (15–23°C) have never been observed to be higher than 1.1 g/min (19, 24). Several factors may affect the oxidation rate of ingested CHO during exercise, including the type and quantity of CHO ingested, the feeding schedule, glycogen availability, and the exercise intensity (24). At present, the effect of ambient temperature on exogenous glucose oxidation is largely unknown. To our knowledge, this is the first study that compared exogenous glucose oxidation at high (35°C) and low (16°C) ambient temperatures. We have used a [U-^{13}C]glucose tracer to quantify the oxidation of ingested CHO. Furthermore, we measured plasma glucose enrichments to calculate plasma glucose oxidation, and this made it possible to determine muscle glycogen oxidation (noninvasively). The major finding of the present study is that the rate of exogenous glucose oxidation is reduced by 10% in the heat compared with a cool environment. Despite a lower exogenous glucose oxidation rate, total CHO oxidation tended to be higher in the heat as a result of an increased muscle glycogenolysis. Muscle glycogen utilization was 25% higher during exercise in the heat compared with the cool trial.

The factors that have contributed to the reduced exogenous glucose oxidation rate in the heat compared with cool trial may be the uptake and release of ingested glucose by the liver, glucose transport into the muscle, gastric emptying, and intestinal absorption of glucose. Although blood flow to the liver may be reduced during exercise in the heat (44), hepatic glucose production (HGP) has shown to be higher in the heat compared with a thermoneutral environment (1, 18). This indicates that it is unlikely that the liver has played a major role in lowering the rate of exogenous glucose oxidation during exercise in the heat.

In the present study, subjects were more dehydrated during exercise in the heat compared with the cool trial, and this may have reduced muscle blood flow (17). It could be argued that the reduced exogenous glucose oxidation rate in the heat was due to a reduced muscle blood flow and/or decreased muscle glucose uptake. In theory, an increased skin blood flow in the heat may reduce blood flow to the muscle, and, as a consequence, glucose delivery to the muscle may be impaired. Whether or not blood flow in the contracting muscle is reduced during exercise in the heat is the subject of some controversy (10, 33). Furthermore, González-Alonso et al. (17) demonstrated that dehydration during exercise in the heat reduced leg blood flow, but this did not impair glucose delivery and net

glucose uptake. However, it has recently been shown that an increase in muscle glycogen utilization after intravenous epinephrine infusion is associated with a reduction in muscle glucose uptake (50). The decreased glucose uptake was likely to be due to an increase in intracellular glucose 6-phosphate as a result of enhanced muscle glycogenolysis. Glucose 6-phosphate accumulation may inhibit hexokinase and thereby phosphorylation of glucose. Increases in intracellular glucose would reduce the gradient for glucose diffusion across the membrane, and glucose transport would be inhibited. It is therefore possible that in the present study the increased rate of muscle glycogenolysis during exercise in the heat may have resulted in a subsequent decrease in muscle glucose uptake. This may also explain the small but significantly higher glucose concentrations and the trend for plasma glucose oxidation being lower in the heat compared with cool trial (Tables 1 and 2).

It is also possible that the rate of gastric emptying was decreased in the heat compared with the cool trial. Previous studies have demonstrated that hyperthermia and dehydration can impair gastric emptying of CHO solutions and/or water during treadmill exercise performed in a cool or thermoneutral (18–25°C) environment compared with a warm environment (30–35°C) (32, 35, 40). A negative correlation was found between final exercise T_{re} and the volume emptied from the stomach (32). However, exercise in the heat (35°C) when subjects were euhydrated did not alter the gastric emptying rate of water compared with exercise in a cool environment (18°C) (32). Furthermore, even when the rate of gastric emptying in the present study was decreased during exercise in heat compared with cool trial, gastric emptying rates ranging from 15 to 18 ml/min, as were previously found during exercise in the heat (32) would have delivered 72–86 g CHO/h into the intestine during the heat trial in the present study. This estimated amount of CHO leaving the stomach is 160–190% greater than the amount of ingested CHO oxidized in our heat trial, and gastric emptying can therefore not fully account for the observed difference in exogenous glucose oxidation between the heat and cool trials.

Another explanation for the lower exogenous CHO oxidation in the heat compared with cool trial is a reduced absorptive capacity of the intestine during exercise in the heat. In the present study, HR, T_{re}, and T_{sk} were significantly higher in the heat compared with the cool trial (Fig. 1, Table 1), indicating a larger stress on thermoregulation. With the rise in T_{sk}, an almost immediate rise in HR occurs (43). The observed increase in HR is most likely due to a redistribution of central blood volume toward the skin, resulting in a decreased stroke volume (45). The increase in skin blood flow to facilitate heat dissipation during exercise in the heat (5, 28) is partly met by a reduction in splanchnic (43, 45) blood flow. Greater dehydration in the heat compared with the cool trial would have reduced splanchnic blood flow even more and absorptive capacity might have been decreased as a result of this (51). In addition, a reduced intestinal blood flow associated with malabsorption of CHO may result in an increased risk of GI complications (41). This theory fits also nicely with the present finding of higher prevalence of GI discomfort in the heat (Table 3), indicating that less CHO is leaving the GI tract.

Although exogenous glucose oxidation was significantly lower in the heat compared with the cool trial, the difference in oxidative rate appears to be only small (~10%). However, the magnitude of difference in exogenous glucose oxidation between the heat and cool trials may have been underestimated slightly in this study. In this study, no correction was made for the background shift (change in $^{13}CO_2$) from endogenous substrate stores. The higher rate of muscle glycogen oxidation in the heat trial resulted in an increased release of ^{13}C from endogenous stores and hence may have resulted in a small overestimation of the rate of exogenous glucose oxidation in the heat trial. Therefore, any background correction would have reduced the rate of exogenous glucose oxidation in the heat more than in the cool trial, which would have resulted in a greater difference in rate of exogenous glucose oxidation between the heat and cool trials. However, this effect is likely to be small (<0.05 g/min), because subjects were instructed to deplete their muscle glycogen stores 5–7 days before each exercise trial and to avoid food products with a high natural ^{13}C abundance during the experimental period. This dietary-exercise regimen has previously been shown to minimize a background shift from endogenous substrate stores (42, 48, 49)

In the present study, total CHO oxidation during the last 30 min of exercise tended to be higher in the the heat compared with the cool trial (Table 2), but this did not reach statistical significance ($P = 0.087$) However, previous studies have reported a greater reliance on CHO metabolism during exercise in the heat compared with exercise in the cool trial (14, 15, 18). This effect was attributed to increased muscle glycogen utilization, which was associated with higher blood lactate concentrations (14, 15). Also, in the present study, exercise in the heat trial was accompanied by increased muscle glycogen utilization and higher lactate concentrations compared with exercise in the cool trial. A number of mechanisms have been proposed to account for the shift toward increased CHO metabolism during exercise and heat stress (10). It has been suggested that the increase in muscle glycogen utilization is due to an elevation in muscle temperature that occurs during exercise and heat stress (46). The mechanism(s) for an increase in muscle glycogen utilization with elevations in muscle temperature is(are) not known at this time but may be related to the activity of key enzymes involved in CHO metabolism, mitochondrial function, cross-bridge cycling, and motor unit recruitment (46). Furthermore, there is also evidence to suggest a potential role for epinephrine as a mechanism for increased muscle glycogenolysis during exercise in the heat (12, 13, 18). It is well known that the secretion of epinephrine is increased during exercise in

the heat compared with exercise in cooler environments (13, 18, 33). Febbraio et al. (12) demonstrated that a twofold increase in circulating epinephrine increased muscle glycogen utilization, glycolysis, and CHO oxidation when subjects were exercising at 70% $\dot{V}O_{2\,max}$. The magnitude of the increase in epinephrine in that study was similar to those observed in previous studies that compared hot and thermoneutral environments (13, 18). It has been hypothesized now that the increase in core temperature during exercise in the heat may result in an increased epinephrine secretion and this in addition to the effect on increased muscle temperature per se may increase muscle glycogen utilization (11). Although we did not measure epinephrine and muscle temperature, the observation that HR, T_{re}, T_{sk}, and RPE were higher in the heat compared with the cool trial indicates that sympathoadrenal activity and thermal stress were increased. It is therefore not unlikely that higher epinephrine levels and muscle temperature in the heat trial have contributed to the augmented glycogen utilization in the present study. It should be noted that no difference was found in plasma FFA and insulin concentrations between the heat and cool trials (Fig. 5, *A* and *B*). Insulin has been shown to be a potent inhibitor of lipolysis and the rate of appearance of FFA (20). The increased plasma insulin levels after glucose ingestion may have reduced whole body lipolysis, as indicated by the drop in plasma FFA during the early part of exercise. The similar plasma FFA levels during exercise in the heat and cool trials are in agreement with the findings of previous studies in which no CHO was ingested (13, 15, 18, 52).

Several studies have reported higher blood glucose concentrations during exercise in the heat compared with thermoneutral or cool environments (13, 15, 18, 52). In the present study, plasma glucose concentrations were only slightly higher in the heat compared with cool trial (Table 1, Fig. 4*A*). The relative hyperglycemia observed during exercise in the heat is most likely due to an imbalance between glucose production by the liver and glucose uptake by the muscle and/or other tissues. Hargreaves et al. (18) demonstrated a greater increase in HGP without any alteration in glucose disappearance, when subjects were exercising at 40°C compared with 20°C. The increased HGP during exercise in the heat in that study may have been caused, in part, by increased plasma epinephrine levels (18, 21). In the present study, we did not measure rate of appearance and disappearance of glucose, HGP, and epinephrine; hence, we can only speculate what might have caused the increased glucose levels during exercise in the heat. Because plasma glucose levels were only slightly higher in the heat compared with the cool trial, in the presence of similar liver-derived glucose oxidation rates (Table 2), it seems unlikely that HGP was significantly higher in the heat compared with the cool trial. A small mismatch between glucose appearance and disappearance may have caused the observed difference in plasma glucose levels between the heat and cool trials.

The magnitude of the difference in glucose concentration between heat and cool trials in the present study may have been masked by the intake of a relatively large amount of CHO during exercise (~88 g/h). CHO intake during exercise has the potential to reduce HGP to very low levels, especially at high rates of intake (4, 30). In this study, hepatic glucose oxidation rates were relatively low and similar in both trials, whereas in other studies differences in HGP could probably account for the differences in blood glucose concentration (18).

Although the mechanism is largely unknown, the present data show that intake of regular CHO feedings during exercise in the heat helps to maintain plasma glucose levels in a narrow range of 5.0–5.5 mmol/l and diminishes the difference in plasma glucose between the heat and cool trials. Dual-tracer studies (1, 27) are required to assess the effect of ambient temperature on hepatic glucose output and glucose uptake when feeding CHO. Furthermore, measurements of catecholamines are needed to gain more insight into the regulating mechanisms.

In summary, the present data demonstrate that exogenous CHO oxidation is reduced during exercise in the heat compared with exercise in a cool environment. The data also suggest that CHO oxidation in heat is increased, most likely because of an increased muscle glycogen utilization. The higher gastrointestinal discomfort reported during exercise in the heat when feeding CHO suggests that intestinal absorption may be a potential factor contributing to the reduction in exogenous CHO oxidation. However, the present findings cannot exclude that reduced muscle glucose uptake is responsible for the lower exogenous CHO oxidation rates in the heat. These results suggest that the recommendations for CHO feeding during exercise in the heat should be adapted to include the ingestion of less CHO (50–60 g/h) compared with during exercise in cool and thermoneutral environments (60–70 g/h).

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