Substrate utilization in leg muscle of men after heat acclimation

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KIRWAN, JOHN P., DAVID L. COSTILL, HARM KUIPERS, MICHAEL J. BURRELL, WILLIAM J. FINK, JOHN E. KOVALESKI, AND ROGER A. FIELDING. *Substrate utilization in leg muscle of men after heat acclimation.* J. Appl. Physiol. 63(1): 31–35, 1987.—Eight men were heat acclimated (39.6°C and 29.2% rh) for 8 days to examine changes in substrate utilization. A heat exercise test (HET), (cycling for 60 min; 50% maximal O_2 consumption) was performed before (UN-HET) and after (ACC-HET) the acclimation period. Muscle glycogen utilization (67.0 vs. 37.6 mmol/kg wet wt), respiratory exchange ratio (0.85 ± 0.002 vs. 0.83 ± 0.001), and calculated rate of carbohydrate oxidation (75.15 ± 1.38 vs. 64.80 ± 1.52 g/h) were significantly reduced ($P < 0.05$) during the ACC-HET. Significantly lower ($P < 0.05$) femoral venous glucose (15, 30, and 45 min) and lactate (15 min) levels were observed during the ACC-HET. No differences were observed in plasma free fatty acid (FFA) and glycerol concentrations or glucose, lactate and glycerol arteriovenous uptake/release between tests. A small but significant increase ($P < 0.05$) above resting levels in FFA uptake was observed during the ACC-HET. Leg blood flow was slightly greater ($P > 0.05$) during the ACC-HET (4.64 ± 0.13 vs. 4.80 ± 0.13 l/min). These findings indicate a reduced use of muscle glycogen following heat acclimation. However, the decrease is not completely explained by a shift toward greater lipid oxidation or increased blood flow.

muscle glycogen; blood glucose; lactate; plasma free fatty acid; glycerol; blood flow

PREVIOUS STUDIES have demonstrated the role of central circulation (1, 22, 24, 30, 31), body fluid spaces (1, 18, 30), and temperature regulation (18) in the process of heat acclimation. However, few studies have examined the metabolic adaptations in muscle that may accompany acclimation (15, 32). Recently, King et al. (15) observed a significant reduction in muscle glycogen utilization after 8 days of heat acclimation. The sparing of glycogen was not associated with differences in blood glucose or lactate concentrations or by differences in the respiratory exchange ratio (RER).

Heat acclimation results in a redistribution of cardiac output which may facilitate a greater blood supply to skeletal muscle (22). An increased glucose or free fatty acid (FFA) uptake, and/or greater blood flow to the active muscle, could facilitate a reduction in muscle glycogen utilization. The purpose of this study was to determine whether differences in substrate uptake/release or leg blood flow by the working muscle could explain the glycogen sparing associated with heat acclimation.

METHODS

Subjects. Eight active male college students volunteered to participate in this study after being informed of the risks and stresses associated with the procedures and signing a letter of informed consent. Subject characteristics are presented in Table 1.

Protocol. Each subject performed a maximal O_2 consumption ($\dot{V}O_{2\,max}$) test before an initial preacclimation heat exercise test (UN-HET) and within 2 days after completing the postacclimation test (ACC-HET). The heat exercise test (HET) consisted of cycling on an electrically braked cycle ergometer at ~50% $\dot{V}O_{2\,max}$ for 60 min. Heat acclimation was achieved by cycling at ~50% $\dot{V}O_{2\,max}$, 90 min/day for 8 days. Water was provided ad libitum during the acclimation period. Both acclimation and the HET were performed in an environmental chamber maintained at an average of 39.6°C and 29.2% rh. The study was completed in the winter months (December through January) to avoid natural heat acclimatization.

Before each HET the subjects arrived at the laboratory following a 4-h fast. A needle biopsy sample (2) was obtained from the vastus lateralis before and after the HET. The sample was immediately frozen in liquid nitrogen and stored at −80°C until analyzed for glycogen concentration (19) and citrate synthase activity (26). Nude body weight was recorded before and after each HET. Subscapular and thigh skinfolds were measured for estimation of percent body fat according to the equation of Sloan (25). A thermistor probe (Yellow Springs Instrument) was positioned to a depth of 10 cm to monitor rectal temperature.

Leg blood flow was measured by the thermodilution technique (13). A catheter was inserted into the femoral vein and positioned below the bifurcation of the common iliac vein. The position of the catheter was checked by roentgenography in two subjects to verify proper positioning. An injectate port in the catheter facilitated the manual injection of cold sterile saline into the circulation. The change in blood temperature was detected by a thermistor located distal to the injectate port. A COM-1 cardiac output computer (American Edwards) was used to determine flow rate. Since femoral venous blood flow is pulsatile in nature, five determinations were made at

TABLE 1. *Characteristics of subjects*

Characteristic	
Age, yr	24.8±1.0
Ht, cm	179.6±1.3
Wt, kg	76.4±1.5
Body fat, %	13.0±1.6
$\dot{V}O_{2\,max}$, ml·kg^{-1}·min^{-1}	54.5±2.2

Values are means ± SE; $n = 8$. $\dot{V}O_{2\,max}$, maximal O_2 consumption.

each time point (rest, 15, 30, 45, and 60 min of exercise). The mean of the three closest values was taken as the blood flow. A second catheter was positioned in a forearm vein to facilitate sampling of blood from an inactive limb. Both catheters were kept patent with a 0.9% solution of sterile saline. Arterial blood samples were obtained from the radial artery at 30 and 60 min of the exercise bout. Since no differences were observed between the measures obtained using arterial blood samples and those obtained using the inactive forearm, the latter values were used for arteriovenous determination.

Blood samples were obtained from both the arm and leg at rest and at 15-min intervals throughout the 60-min test. Samples were analyzed for glucose, lactate, FFA, and glycerol. Exercising $\dot{V}O_2$ and RER values were determined from expired air collected for 90 s (Douglas bags) at 15-min intervals throughout the test. Gas volumes were measured on a Parkinson-Cowen gas meter. Oxygen and carbon dioxide concentrations were measured on an Applied Electrochemistry S-3A O_2 analyzer and Beckman LB-2 CO_2 analyzer, respectively. Heart rate and rectal temperatures were recorded at 10-min intervals.

Blood and muscle analyses. Muscle glycogen was determined fluorometrically (19) after acid hydrolysis (2.0 N HCl). Muscle citrate synthase activity was determined as described by Srere (26). Blood glucose and lactate were determined enzymatically in perchloric acid extracts (16). FFA concentrations were determined by the microfluorometric method described by Miles et al. (17). Blood glycerol concentrations were determined according to Wieland as described by Bergmeyer (2). Hemoglobin and hematocrit concentrations were determined from heparinized blood using the cyanmethemoglobin method and microcentrifugation, respectively. Changes in plasma volume were estimated from hemoglobin and hematocrit values (6). The rate of carbohydrate oxidation was calculated from respiratory exchange data. Substrate uptake/release was calculated from venous and arterial concentrations and leg blood flow.

Statistical analysis. All values are reported as means ± SE. Differences between means were determined by repeated measures analysis of variance. Specific mean differences were located using a Newman-Keuls post hoc test. Differences between paired means were determined by a Student's t test. Significance was set at the $P < 0.05$ level.

RESULTS

Heat acclimation. Changes in rectal temperature and heart rate were used as criteria to demonstrate heat acclimation. During the acclimation period exercising heart rates were significantly decreased ($P < 0.05$) from 150 ± 3 beats/min on *day 1* to 135 ± 4 beats/min on *day 8*. The change in rectal temperature was also significantly reduced ($P < 0.05$) from 1.13 ± 0.11°C on *day 1* to 0.75 ± 0.08°C on *day 8*. In addition, plasma volume estimated from hemoglobin and hematocrit was increased by 6.0 ± 2.2% after acclimation. To demonstrate that the physiological and metabolic changes were not due to a training effect, a $\dot{V}O_{2\,max}$ test was performed within 2 days of the UN-HET and ACC-HET. In addition, muscle samples from the vastus lateralis were analyzed for citrate synthase activity. The heat exercise bouts had no measurable effect on either of these parameters (Table 2).

Submaximal exercise in the heat. Mean values for leg blood flow, leg $\dot{V}O_2$, and pulmonary $\dot{V}O_2$ are presented in Table 3. No significant differences were observed in any of these variables after acclimation.

Resting muscle glycogen values were not different between the UN-HET or ACC-HET (133.22 ± 7.50 vs. 128.57 ± 6.61 mmol/kg wet wt, respectively). However, the amount of glycogen used during the exercise was significantly reduced ($P < 0.05$) from 67.0 to 37.6 mmol/kg wet wt as a consequence of the acclimation routine (Fig. 1). This difference represents a 47% reduction in glycogen utilization. The RER (0.85 vs. 0.83) and calculated rate of carbohydrate oxidation (75.15 vs. 64.80 g/h) were also significantly reduced ($P < 0.05$) after heat acclimation (Table 3).

Femoral venous glucose concentrations were lower during the ACC-HET. Significant differences ($P < 0.05$) were observed at 15 (5.30 ± 0.18 vs. 4.86 ± 0.16 mmol/

TABLE 2. *Aerobic capacity and muscle citrate synthase activity before and after heat acclimation*

	$\dot{V}O_{2\,max}$, l/min	Heart Rate, beats/min	Work Load, W	Citrate Synthase, μmol·g^{-1}·min^{-1}
UN	4.12±0.11	190±2	283±11	22.3±0.9
ACC	4.06±0.13	190±2	280±10	21.8±1.3

Values are means ± SE; $n = 8$. $\dot{V}O_{2\,max}$, maximal O_2 consumption; work load, maximal power output achieved during $\dot{V}O_{2\,max}$ test; UN and ACC, unacclimated and acclimated trials. Mean differences were not significantly different.

TABLE 3. *Respiratory, blood flow, and metabolic measurements before and after heat acclimation*

Variable	Condition	Value
Pulmonary $\dot{V}O_2$, l/min	UN	2.05±0.03
	ACC	2.19±0.02
RER	UN	0.85±0.001
	ACC	0.83±0.002*
CHO, g/h	UN	75.15±1.38
	ACC	64.80±1.52*
Lipid, g/h	UN	33.08±2.07
	ACC	38.82±1.15*
Leg blood flow, l/min	UN	4.64±0.13
	ACC	4.80±0.13
Leg $\dot{V}O_2$, ml/min	UN	664±35
	ACC	670±34

Values are means ± SE; $n = 8$. RER, respiratory exchange ratio; CHO, calculated carbohydrate oxidation; lipid, calculated lipid oxidation; leg $\dot{V}O_2$, leg O_2 consumption; UN and ACC, unacclimated and acclimated trials. * Significant difference between means ($P < 0.05$).

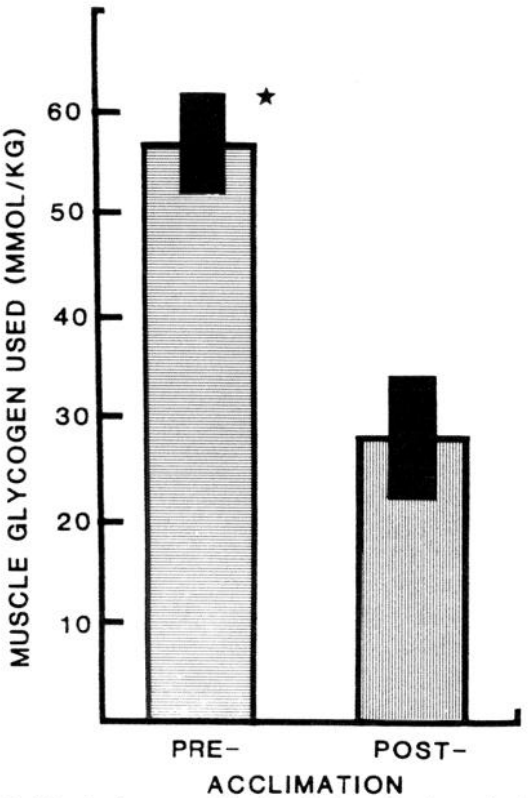

FIG. 1. Muscle glycogen utilization during a heat exercise test performed before (Pre-) and after (Post-) heat acclimation. *Significant difference between 2 tests ($P < 0.05$).

1), 30 (5.22 ± 0.22 vs. 4.79 ± 0.16 mmol/1), and 45 min (5.26 ± 0.16 vs. 4.36 ± 0.16 mmol/l) of exercise for the UN-HET and ACC-HET, respectively. Although the ACC-HET resulted in overall lower lactate concentrations, the only significant difference ($P < 0.05$) between tests occurred at 15 min (2.21 ± 0.42 vs. 1.47 ± 0.27 mmol/l) of exercise. No significant differences were observed in glucose or lactate uptake/release between tests.

Plasma FFA levels decreased during the first 15 min of exercise but returned toward resting concentrations by 60 min during the UN-HET (Fig. 2). During the ACC-HET, plasma FFA followed a similar pattern but with a tendency ($P > 0.05$) toward higher levels than in the UN-HET bout. Peak FFA uptake (Fig. 2) occurred at 30 min of exercise during the UN-HET (0.13 ± 0.06 mmol/min) and ACC-HET (0.16 ± 0.05 mmol/min) tests. Although no statistical differences were observed between the two tests, FFA uptake was significantly increased ($P < 0.05$) above resting values following the ACC-HET.

After the first 15 min, femoral venous glycerol concentrations increased during the UN-HET (Fig. 2). A similar pattern was observed during the ACC-HET. Although the values were slightly higher during the ACC-HET, the differences were not statistically significant. Peak glycerol release (Fig. 2) occurred at ~30 min during both the UN-HET and the ACC-HET (−0.08 ± 0.06 and −0.15 ± 0.09 mmol/min, respectively) tests.

DISCUSSION

This study was undertaken to investigate whether the exchange of substrates by the working leg muscles and/or an increase in leg blood flow could account for the previously reported (15) reduction in glycogen utilization after heat acclimation. The heat acclimation regimen was based on a slight modification of the original protocol described by Robinson et al. (21). Heat acclimation was confirmed by a decrease in heart rate and rectal temperature during the final days of acclimation and by an increase in plasma volume. The subjects did not demonstrate any improvements in $\dot{V}O_{2\,max}$, maximal work load, maximal heart rate, or citrate synthase activity as a result of the acclimation procedure. In addition, we have observed that a similar exercise protocol performed in a cold environment (10°C, 55% rh) for 3 days does not result in any change in glycogen values (4). This suggests that a learning effect is not a factor and provides further support that the reduced glycogen use is a function of heat acclimation.

The reduction in glycogen use previously demonstrated by King et al. (15) after 8 days of heat acclimation was confirmed by the data in this study. In contrast to the findings of King et al. (15), however, a small but statistically significant reduction in RER was observed following the ACC-HET. This is in agreement with observations by Young et al. (32), who used a similar heat acclimation protocol. The reduction in RER in the present study corresponds to a 10.4-g/h decrease in the amount of carbohydrate oxidized during the ACC-HET. This decrease in carbohydrate appears to be compensated by an increase in lipid oxidation. However, based on the amount of muscle glycogen spared (5.29 $g \cdot kg^{-1} \cdot h^{-1}$) and assuming an active muscle mass of ~4 kg, the RER values underestimate the amount of carbohydrate spared during the ACC-HET.

An increase in FFA oxidation has been shown to inhibit muscle glycogen utilization (5, 20). Changes in plasma FFA and glycerol concentrations did not indicate a greater lipid oxidation after acclimation. However, since FFA uptake was significantly greater than at rest during the ACC-HET, there appears to be a tendency toward greater lipid oxidation when acclimated. However, these small but significant changes are inadequate to explain the considerable reduction in glycogen utilization.

An increase in glucose uptake by the contracting muscle could also have contributed to the reduced dependence on muscle glycogen stores. Wahren et al. (28) demonstrated that blood glucose and FFA supply a considerable part of the metabolic demands during prolonged submaximal exercise. It has also been shown that the rate of muscle glycogen utilization is inversely related to hepatic glucose production (11) and to the amount of glycogen stored in the muscle (10). If increased hepatic glycogenolysis were responsible for sparing glycogen, then a considerable increase in glucose uptake should have been observed during the ACC-HET. However, no significant changes were observed during either test. Thus it does not appear that the muscle actively extracted glucose at a sufficiently rapid rate to account for the observed reduction in glycogen utilization.

During aerobic exercise blood lactate can also be used as a substrate and has been shown to be metabolized by

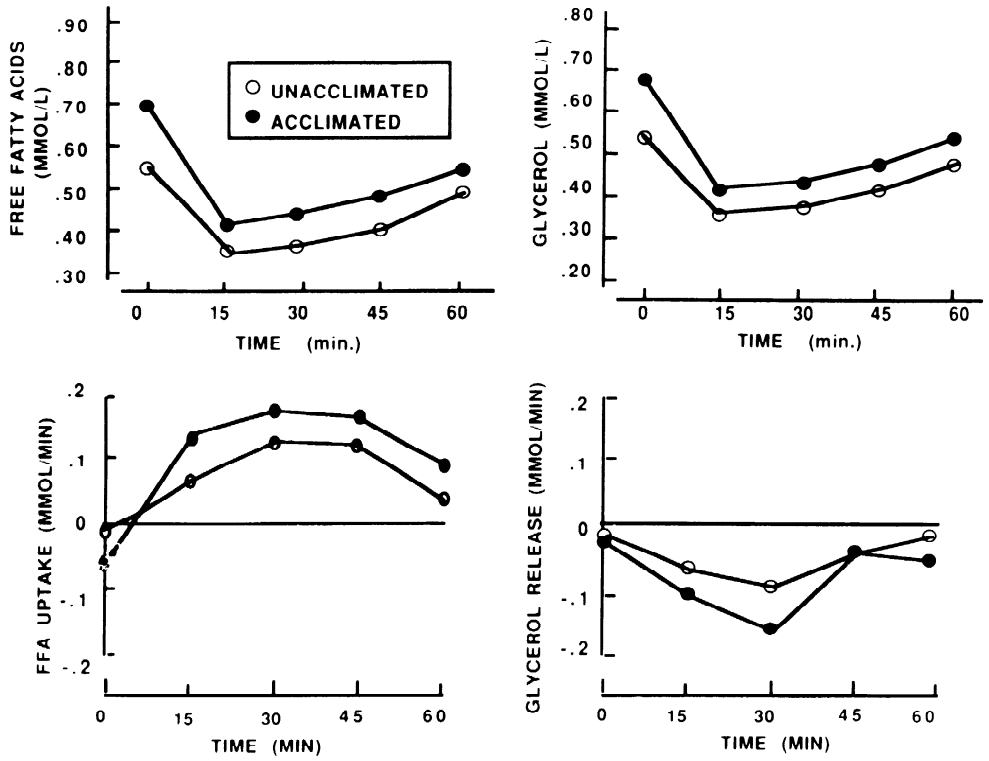

FIG. 2. Mean values for femoral venous free fatty acid (FFA) and glycerol concentrations (*top*) together with FFA uptake and glycerol release (*bottom*) before and after heat acclimation. Uptake and release were calculated from the product of arteriovenous differences and leg blood flow.

exercising skeletal muscle (14). Increased lactate uptake during the ACC-HET could account for a reduction in glycogen utilization. However, Essen et al. (7) have shown that the rate of lactate uptake increases when blood lactate concentration increases and muscle glycogen concentration decreases. In the present study, the highest femoral venous lactate values (2.47 mmol) were observed during the UN-HET. Furthermore, a consistent lactate release was observed throughout the ACC-HET. Thus lactate uptake does not appear to be responsible for the observed reduction in glycogen utilization.

The role of epinephrine in the enhancement of glycogenolysis in resting skeletal muscle is well established (14, 27). In a recent study, Jansson and co-workers (12) demonstrated that epinephrine also enhances glycogenolysis in skeletal muscle during submaximal exercise. Although training tends to reduce the catecholamine response to a given absolute work load (9, 27), and a considerable amount of the diminution in epinephrine has been shown to occur within the 1st wk of training (29), the subjects in this study did not appear to demonstrate a training effect. It is possible that heat acclimation may have led to a reduction in epinephrine levels during the ACC-HET, which may have contributed to a decreased rate of glycogenolysis.

There are no studies in which leg blood flow was determined directly in association with heat acclimation. Previously, Rowell et al. (23) suggested that blood flow to the exercising leg decreased during an acute exercise bout in the heat. A decrease in leg blood flow would lead to a reduced availability of oxygen to the working muscle. This may explain why previous investigations revealed that an acute exercise bout in a heated environment results in greater glycogen utilization than a similar exercise performed in a cold environment (8). After heat acclimation, less of the cardiac output is required for heat dissipation and consequently a greater fraction may be available for perfusion of muscle (22). Leg blood flow in this study was only slightly increased following acclimation and the difference between the UN-HET and ACC-HET was not statistically significant. Thus, within the design of this study, an increase in leg blood flow leading to greater oxygen and substrate availability does not appear to explain the reduction in carbohydrate oxidation.

In conclusion, heat acclimation did not significantly change leg blood flow during submaximal cycle ergometer exercise in the heat. A significant reduction in glycogen utilization, RER, and carbohydrate oxidation was observed after the acclimation period. Since leg blood flow was not significantly changed following acclimation, and since the increase in lipid oxidation is slight, the mechanism responsible for the shift away from carbohydrate oxidation is not readily apparent.

The authors acknowledge the technical assistance provided by M. Flynn, J. Hawley, S. Kaiseraur, D. Neuffer, and D. Porter.

This study was supported in part by US Army Research Grant 17-83-C-3009.

Received 22 July 1986; accepted in final form 28 January 1987.

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