Effect of heat stress on muscle energy metabolism during exercise

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Febbraio, M. A., R. J. Snow, C. G. Stathis, M. Hargreaves, and M. F. Carey. Effect of heat stress on muscle energy metabolism during exercise. *J. Appl. Physiol.* 77(6): 2827–2831, 1994.—To examine the effect of heat stress on muscle energy metabolism during submaximal exercise, 12 endurance-trained men cycled on two occasions for ~40 min at 70% maximal O_2 uptake in an environmental chamber at either 20°C and 20% relative humidity (T_{20}) or 40°C and 20% relative humidity (T_{40}). Trials were conducted ≥1 wk apart in random order. No difference in mean O_2 uptake was observed when exercise in T_{40} was compared with that in T_{20}. In contrast, exercise in T_{40} resulted in a higher mean heart rate ($P < 0.01$) and respiratory exchange ratio ($P < 0.05$) compared with that in T_{20}. Postexercise rectal and muscle temperatures were also higher ($P < 0.01$) in T_{40} than in T_{20}. Lower ($P < 0.01$) postexercise creatine phosphate and higher creatine ($P < 0.01$) and ammonia ($P < 0.05$) were observed in muscle after exercise in T_{40} compared with T_{20}. In addition, an increased ($P < 0.01$) muscle glycogenolysis and higher ($P < 0.01$) postexercise muscle lactate accumulation were observed during exercise in T_{40} compared with T_{20}. In contrast, no differences were observed in postexercise concentrations of total adenine nucleotide pool (ATP + ADP + AMP), ATP/ADP ratio, or inosine 5′-monophosphate (IMP) when T_{40} was compared with T_{20}. These results indicate that the rate of ATP utilization may be increased during exercise in the heat but that this increased energy demand is predominantly met by an increase in anaerobic glycolysis and creatine phosphate hydrolysis, preventing a reduction in total adenine nucleotide pool. In addition, the higher ($P < 0.05$) postexercise concentration of muscle ammonia observed in T_{40}, in the absence of any differences in muscle IMP accumulation, suggests that ammonia is produced by sources other than net adenine nucleotide degradation.

adenine nucleotides; high-energy phosphates; ammonia; glycogenolysis; muscle temperature

AN ENHANCED INTRAMUSCULAR ATP and creatine phosphate (CrP) degradation in humans along with an increased tissue temperature has been observed during repeated isometric contractions to fatigue after passive heating of the leg (5). The experimental model used in the study by Edwards et al. (5) cannot, however, be representative of the physiological and metabolic responses to whole body heating during dynamic exercise. During the latter type of exercise, blood continues to perfuse the contracting muscle. In these circumstances, alterations in hormone (9), oxygen (19), and substrate (24) levels may have an effect on muscle energy metabolism. Furthermore, the total energy liberation in an isometric contraction is less compared with that in an isotonic contraction (8). A reduction in muscle contents of ATP and CrP and increases in AMP and ADP accumulation have been observed during exercise-induced hyperthermia in dogs (13). Whether such metabolic changes occur in humans during dynamic exercise in the heat has not been studied. We have previously observed an increased muscle ammonia (NH_3) accumulation during submaximal dynamic exercise in the heat in humans (23), which may indicate an increased degradation of intramuscular ATP and increased inosine 5′-monophosphate (IMP) accumulation, although adenine nucleotides and their degradation products were not measured in our earlier study. Furthermore, we have also observed an increase in muscle glycogenolysis and lactate accumulation during exercise in the heat (7). The aim of the present study was to examine the effect of whole body heat stress on anaerobic energy metabolism during submaximal dynamic exercise in humans. We hypothesized that exercise in the heat would result in an increased ATP utilization in contracting muscle, leading to reduced CrP and total adenine nucleotide concentrations ([TAN]), increased NH_3 and IMP accumulation, and enhanced glycogenolysis and glycolysis.

METHODS

These experiments were conducted during the cooler months to reduce any natural heat acclimatization. Mean daily maximum temperatures during these months ranged between 14.8 and 19.5°C.

Subjects. Twelve endurance-trained males [21.6 ± 0.5 yr; 176.1 ± 1.9 cm; 70.4 ± 2.0 kg; maximal O_2 uptake ($\dot{V}O_{2\,max}$) = 65.2 ± 1.5 ml · kg^{-1} · min^{-1}] volunteered as subjects for this study after being informed of the risks associated with the procedures and signing a letter of informed consent. The study was approved by the Victoria University of Technology Human Experimentation Ethics Committee.

$\dot{V}O_{2\,max}$. $\dot{V}O_{2\,max}$ was determined during incremental cycling exercise to volitional fatigue on either an electrically braked (Mijnhart KEM 2) or friction-braked (Monark Ergomedic 814E) cycle ergometer. Expired air was directed by a Hans Rudolph valve through a ventilometer (Pneumoscan S30) into a mixing chamber and analyzed for O_2 and CO_2 by gas analyzers (Applied Electrochemistry S-3A O_2 and CD-3A CO_2, respectively) that were calibrated before each test with commercially prepared gas mixtures. The criterion used to determine the attainment of $\dot{V}O_{2\,max}$ was the achievement of a plateau in O_2 uptake ($\dot{V}O_2$; <2 ml · kg^{-1} · min^{-1} increase) with an increase in work rate. All subjects fulfilled this criterion.

Exercise trials. A workload was selected that would elicit ~70% $\dot{V}O_{2\,max}$ from the predetermined $\dot{V}O_{2\,max}$ test. Each subject performed two trials at this work rate on the cycle ergometer used during the $\dot{V}O_{2\,max}$ test. One trial was conducted at 20°C with a relative humidity of 20% (T_{20}) and the other at 40°C with a relative humidity of 20% (T_{40}). These trials were conducted ≥1 wk apart in random order. Subjects were instructed to cycle at the predetermined work rate for 40 min. Eight of the 12 subjects cycled for 40 min in both trials, whereas the remaining four were unable to complete this task during the first trial. These subjects completed 32, 35, 38, and 39 min of exercise, respectively. Because these subjects performed T_{40} first, T_{20} was terminated at the same time. The postexercise muscle data for all 12 subjects were treated in the same way. A further subject was unable to complete T_{40} after having completed 40 min of exercise in T_{20}. As a consequence, these results were not included in this study, since we were unable to compare the data with validity. Before each submaximal exercise

TABLE 1. *Mean $\dot{V}O_2$, mean heart rate, mean RER, and rectal and muscle temperatures during exercise T_{20} and T_{40}*

	T_{20}	T_{40}
$\dot{V}O_2$, l/min	2.94±0.50	2.94±0.60
Heart rate, beats/min	150±2	168±2†
RER	0.88±0.01	0.91±0.01*
T_r, °C		
Preex	37.0±0.1	37.2±0.1
Postex	38.6±0.1	39.6±0.1†
T_m, °C		
Preex	35.6±0.2	36.0±0.4
Postex	39.0±0.1	40.7±0.1†

Values are means ± SE; n = 12 men. T_{20}, 20°C and 20% relative humidity; T_{40}, 40°C and 20% relative humidity; $\dot{V}O_2$, O_2 uptake; RER, respiratory exchange ratio; T_r, rectal temperature; T_m, muscle temperature; preex, preexcerise; postex, postexercise. Significant difference between T_{40} and T_{20}: * $P < 0.05$; † $P < 0.01$.

trial, subjects arrived at the laboratory after an overnight fast, having refrained from exercise, alcohol, tobacco, and caffeine for 24 h. In an attempt to minimize differences in resting muscle glycogen concentration, subjects were given food packages for the 24 h before each test. The energy content of these packages was ~3,100 kcal, consisting of 80% carbohydrate, and the subjects consumed all of the food before each trial. Subjects were weighed nude before each exercise trial and wore cycling shorts and running shoes during exercise.

Heart rate, $\dot{V}O_2$, and body temperature measurement. Heart rate was recorded during each trial at 5, 15, 25, and 35 min of exercise by a monitor (Sports Tester PE3000). $\dot{V}O_2$ and respiratory exchange ratio were also measured at these times with Douglas bags. The expired gases were measured on the same analyzers used for the $\dot{V}O_{2\,max}$ test. Volumes were determined with a gas meter (Parkinson-Cowan). Rectal temperature (T_r) was monitored throughout each trial by a rectal thermistor probe (YSI 401) inserted 10 cm beyond the anal sphincter. Muscle temperature (T_m) was measured before and within 30 s of the cessation of exercise by a 25-gauge needle thermistor (YSI 524) inserted 4 cm into the vastus lateralis.

Muscle sampling and analyses. Resting muscle samples were obtained after the subjects lay supine in the appropriate environmental condition for 20 min. Muscle biopsies were obtained from the vastus lateralis before and immediately (<5 s) after exercise by the percutaneous needle biopsy technique modified to include suction. Muscle samples were frozen in liquid nitrogen within 10 s of being obtained. Each sample was divided into two portions that were weighed at −30°C. One portion weighing 8–15 mg was extracted at −20°C using 0.6 M perchloric acid-10% methanol, neutralized with KOH, and analyzed for NH_3 by flow injection analysis according to the method described by Katz et al. (12). Muscle NH_3 was corrected for water content based on the wet-to-dry weight ratio determined on the second portion of the sample. The second muscle portion was freeze dried, weighed, dissected free of any connective tissue, powdered, and divided into two aliquots. One aliquot was extracted according to the method of Harris et al. (11) and was analyzed for CrP, creatine (Cr), and lactate as described by Lowry and Passonneau (14). In addition, reverse-phase high-performance liquid chromatography was used to quantify ATP, ADP, AMP, and IMP according to the method of Wynants and van Belle (26). The second powdered aliquot was hydrolyzed, neutralized, and analyzed for glycogen according to the procedure of Lowry and Passoneau. Muscle metabolites, except for glycogen (measured as glucose), lactate, and NH_3 (due to their extracellular presence), were adjusted to the peak total Cr for each subject. This was done to correct for variability in blood, connective tissue, or other nonmuscle constituents between biopsies.

Statistical analyses. The data from the two trials were compared by using a two-factor (time and temperature) analysis of variance with repeated measures. Simple main effects analyses and Newman-Keuls post hoc tests were used to locate differences when analysis of variance revealed a significant interaction. A Student's t-test for paired samples was used to compare muscle glycogenolysis between T_{20} and T_{40}. A biomedical data processing (BMDP) computer software program was used to compute these statistics. The level of probability to reject the null hypothesis was set at $P < 0.05$. All data are reported as means ± SE.

RESULTS

Mean exercise $\dot{V}O_2$ was not different when T_{40} was compared with T_{20}. In contrast, mean heart rate ($P < 0.01$) and respiratory exchange ratio ($P < 0.05$) were higher during T_{40} than during T_{20} (Table 1). Neither T_r nor T_m was different at rest when T_{40} was compared with T_{20}. In contrast, postexercise measurements for both of these parameters were higher ($P < 0.01$) in T_{40} compared with T_{20} (Table 1).

No differences were observed in resting concentrations of muscle CrP and Cr between T_{40} and T_{20}. Exercise resulted in lower ($P < 0.05$) CrP and higher ($P < 0.01$) Cr concentrations in both trials. Postexercise concentrations of CrP were lower ($P < 0.01$) and those of Cr were higher ($P < 0.01$) when T_{40} was compared with T_{20} (Table 2). Muscle lactate concentrations were not different at rest when T_{40} was compared with T_{20}. Postexercise muscle lactate concentrations were, however, higher ($P < 0.01$) in T_{40} than in T_{20} (Table 2). Although preexercise muscle glycogen concentrations were not different when the two trials were compared, postexercise muscle glycogen concentrations were lower ($P < 0.01$) in T_{40} than in T_{20} (Table 3). In addition, muscle glycogenolysis was higher ($P < 0.01$) when T_{40} was compared with T_{20} (Table 3). Intramuscular ATP concentration was unaffected by

TABLE 2. *Intramuscular concentrations before and immediately after exercise in T_{20} and T_{40}*

	T_{20}		T_{40}	
	Preex	Postex	Preex	Postex
ATP	25.3±0.7	26.0±0.8	24.5±0.7	25.7±1.1
ADP§	2.5±0.1	2.7±0.2	2.4±0.1	2.9±0.2
AMP	0.09±0.02	0.10±0.02	0.06±0.01	0.13±0.02*
IMP§	0.06±0.01	0.14±0.05	0.14±0.03	0.29±0.06
TAN	27.5±3.3	28.5±4.0	26.5±3.3	27.5±5.7
NH_3	0.32±0.06	0.98±0.09*	0.34±0.05	1.25±0.13*†
ATP/ADP ratio	10.7±1.2	10.0±1.7	10.3±1.1	9.0±2.0
EC	0.96±0.01	0.96±0.01	0.96±0.01	0.96±0.01
Lactate	5.5±0.6	12.0±2.0*	7.2±0.9	20.7±2.2*‡
CrP	84.6±3.8	67.2±3.6*	84.4±3.7	50.9±4.0*‡
Cr	41.5±1.8	60.5±4.2*	43.2±2.2	77.7±4.8*‡

Values are means ± SE, expressed in mmol/kg dry wt; n = 12 men. IMP, inosine 5'-monophosphate; TAN, total adenine nucleotides (= ATP + ADP + AMP); EC, energy charge potential (= ATP + 0.5 ADP/TAN); CrP, creatine phosphate; Cr, creatine. * Significant difference between postex and preex, $P < 0.05$. Significant difference between postex T_{20} and postex T_{40}: † $P < 0.05$; ‡ $P < 0.01$. § Main effect for exercise, $P < 0.05$.

TABLE 3. *Muscle glycogen concentrations before and immediately after exercise in T_{20} and in T_{40}*

	Preex	Postex	Δ
T_{20}	567±46	401±45	166±20
T_{40}	545±34	326±33†	218±18*

Values are means ± SE, expressed in mmol glucosyl units/kg dry wt; n = 12 men. * Significant difference between T_{40} and T_{20}, $P < 0.01$. † Significant difference between T_{40} postex and T_{20} postex, $P < 0.01$.

either exercise or temperature (Table 2). Concentrations of muscle ADP and AMP were not different at rest. Although a main effect ($P < 0.05$) for exercise was observed in the concentration of ADP, no differences were observed in postexercise concentrations between the two trials (Table 2). No difference was observed between pre- and postexercise AMP concentration in T_{20}. In contrast, postexercise AMP levels were higher ($P < 0.05$) compared with resting concentrations in T_{40}. Postexercise AMP concentrations were not different between the two trials (Table 2). Intramuscular IMP concentrations were not different at rest or after exercise when T_{40} was compared with T_{20}, although a main effect for exercise ($P < 0.05$) was observed for IMP concentration (Table 2). The ATP/ADP molar ratio tended ($P = 0.09$) to be lower when postexercise concentrations were compared with preexercise concentrations in both trials, and no differences were observed in this measurement when T_{40} was compared with T_{20}. Neither the energy charge potential, calculated according to Atkinson (1) as ATP + 0.5ADP/ATP + ADP + AMP, nor [TAN] (=ATP + ADP + AMP) was affected by either exercise or temperature (Table 2). Muscle NH_3 levels were not different at rest between the two trials. Postexercise muscle NH_3 levels were higher ($P < 0.05$) in T_{40} than in T_{20}. Postexercise concentrations for this metabolite were higher ($P < 0.01$) in both trials compared with resting values (Table 2).

DISCUSSION

The results of this study demonstrate that muscle adenine nucleotide metabolism during submaximal dynamic exercise in trained endurance athletes is unaffected when exercise at T_{40} is compared with that at T_{20} (Table 2). In contrast, muscle CrP degradation (Table 2), muscle lactate (Table 2), and muscle glycogenolysis (Table 3) are increased during exercise in the heat. In addition, the larger accumulation of muscle NH_3 compared with the accumulation of IMP in both trials and the higher postexercise muscle NH_3 concentration (Table 2) observed in T_{40}, in the absence of any statistical difference in postexercise IMP accumulation between the two trials, may indicate that NH_3 is produced from sources other than net adenine nucleotide degradation during exercise in a hot environment.

Increases in muscle adenine nucleotide and CrP degradation, glycogenolysis, and lactate accumulation have been observed with an elevated T_m during isometric exercise to fatigue in humans (5) and during submaximal exercise in dogs (13). The results from the present study show an increase in postexercise T_m (Table 1), a lower postexercise CrP concentration (Table 2), and increased glycogenolysis (Table 3) and lactate accumulation (Table 2) when T_{40} and T_{20} are compared but no difference in TAN catabolism between the two trials (Table 2). The present data suggest that muscle ATP utilization may have increased during exercise in the heat but that this increase was met, at least in part, by resynthesis of ATP from the creatine phosphokinase reaction and an increase in anaerobic glycolysis, such that [TAN] was unaltered. This contrasts with the studies above (5, 13), which observed increased adenine nucleotide degradation. Possible explanations for this difference include a lower relative workload, and therefore ATP turnover rate, in the present study and the use of endurance-trained subjects whose muscles may be able to meet the energy demand by sources other than [TAN]. Furthermore, Sahlin et al. (20) demonstrated that, in contracting human skeletal muscle, large increases in IMP accumulation occur when the CrP content is reduced to ~40 mmol/kg dry wt. Postexercise muscle CrP content was not reduced to this concentration in either trial, and the exercise-induced increase in IMP during both trials was relatively small (Table 2). Because the activity of AMP deaminase within muscle is low in comparison to that of creatine phosphokinase (4), it is likely that any rise in free ADP is utilized, along with increased H^+ concentration, in the creatine phosphokinase reaction until CrP concentrations reach a critically low level. Although IMP accumulation appeared to be higher in T_{40}, the results were not significant ($P = 0.28$ for the interaction between time and temperature), and four of eight subjects had higher postexercise IMP concentrations in T_{20}. In addition, [TAN], ATP/ADP, and the energy charge potential all indicated that the energy state of the muscle was not compromised in either trial (Table 2). Although muscle CrP degradation is similar when the results of Kozlowski et al. (13) are compared with those of the present study, the concentration of CrP at which adenine nucleotide catabolism is elevated during exercise and heat stress may be species dependent.

There are several possible mechanisms that may explain the effect of exercise in the heat on CrP degradation, glycogenolysis, and lactate accumulation. *1*) An increased T_m may have increased ATP utilization by directly enhancing the activities of several adenosinetriphosphatases (e.g., myosin, Na^+-K^+, Ca^{2+}) or by altering the rate and/or efficiency of cross-bridge cycling as suggested previously (5, 6). *2*) Although pulmonary $\dot{V}O_2$ during exercise was not different between the two trials, it is possible that mitochondrial ATP production was impaired by an elevated T_m. In examining the ratio between ADP production and mitochondrial $\dot{V}O_2$ (ADP/O ratio) in isolated skeletal muscle mitochondria, Brooks et al. (3) observed a constant ADP/O at temperatures ranging from 25 to 40°C. Above 40°C, however, ADP/O declined linearly with an increase in temperature, suggesting that for a given $\dot{V}O_2$ the increase in ADP rephosphorylation was lower than the rate of ATP degradation. It should be noted that postexercise T_m was >40°C in the T_{40} but below this temperature in the T_{20} (Table 1). *3*) It is possible that a direct effect of elevated T_m (Q_{10}) on the enzymes responsible for CrP and glycogen degradation was,

in part, responsible for the observed changes, although it is unlikely that this can account fully for the increases we have observed, as discussed previously (7). *4*) It is likely that the elevated circulating epinephrine observed during exercise in the heat will increase muscle glycogenolysis and lactate accumulation (7). As a result, it is also possible that muscle pH is lower, which would shift the equilibrium between CrP hydrolysis and rephosphorylation toward greater CrP degradation (11). Whether there is a direct effect of epinephrine on CrP degradation during exercise is unknown. *5*) It is unlikely that the increase in energy supply from CrP degradation and anaerobic glycolysis was due to decreased O_2 delivery secondary to a reduction in contracting muscle blood flow, as suggested by Kozlowski et al. (13). Active muscle blood flow is decreased in sheep (2) but not in humans (17, 21) during exercise in the heat, and hypovolemia results in an increase in O_2 extraction during hyperthermic exercise in dogs (22). Furthermore, although we have no measure of contracting muscle $\dot{V}O_2$, pulmonary $\dot{V}O_2$ during exercise was not different between T_{40} and T_{20}.

Although the energy charge of the muscle was unaltered during exercise at either temperature, the exercise-induced increase in AMP and the trend for a higher IMP content in T_{40} (Table 2) suggest that there may have been a change in the energy charge in some muscle fibers. Norman et al. (18) demonstrated that IMP content is elevated in glycogen-depleted but not in glycogen-filled fibers. In the present study and previously (7), we have observed increased muscle glycogenolysis during exercise in the heat. Although muscle glycogen content was >300 mmol glucosyl units/kg dry wt after 40 min of exercise in the heat, histochemical estimation of glycogen content revealed that ~19% of type I fibers contained either very little or no glycogen after exercise in the heat compared with only 5% after exercise in T_{20} conditions (7). Although speculative, it is possible that single-fiber analyses may have revealed a significant decrease in [TAN] and an increase in IMP in the glycogen-depleted type I fibers during exercise, but this increase was not large enough to result in altered adenine nucleotide metabolism in the mixed muscle fiber analysis.

The observation of a higher muscle NH_3 accumulation during exercise in T_{40} compared with T_{20} supports previous findings (23). Although muscle adenine nucleotides were not measured in the study by Snow et al. (23), the authors speculated that, in untrained men, the muscle NH_3 levels could reflect transient increases in intramuscular concentrations of free ADP and free AMP, which in turn resulted in elevated IMP concentrations. In the present study, the higher postexercise muscle NH_3 accumulation in T_{40} was observed in the absence of any differences in IMP levels between T_{40} and T_{20} (Table 2). In addition, the exercise-induced increase in muscle NH_3 levels was approximately five times higher than the exercise-induced increase in muscle IMP accumulation (Table 2). These results suggest that NH_3 came from sources other than net adenine nucleotide degradation, since there was no change in [TAN]. The mechanisms for NH_3 production during submaximal exercise are not well defined but may involve amino acid catabolism (10, 25). Muscle NH_3 concentration increases, despite an unaltered TAN catabolism, during submaximal exercise (16), and a higher plasma NH_3 has been observed in individuals who consumed amino acids before prolonged cycling compared with placebo feedings (15). The pathways responsible for the increased NH_3 formation from amino acid catabolism include the glutamate dehydrogenase reaction or purine nucleotide cycling (10), although it is unclear which pathway predominates during prolonged submaximal exercise.

In summary, the results of the present study suggest that exercise in the heat in trained men results in an increase in ATP utilization compared with that during exercise in a cooler environment. It appears that this increase is predominantly met by enhanced CrP degradation and anaerobic glycolysis, since TAN metabolism is unaltered. In addition, the higher postexercise concentration of muscle NH_3 observed in T_{40}, in the absence of any difference in muscle IMP accumulation, suggests that NH_3 could come from sources other than net adenine nucleotide degradation.

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