FATIGUE during prolonged, submaximal exercise often coincides with glycogen depletion (9, 10, 37), and endurance can be increased by providing exogenous carbohydrate during exercise (7, 10). During prolonged submaximal exercise, glycogen stores within the muscle are lowered, eventually giving rise to reduced glycolytic flux, leading to a fall in pyruvate formation (37) and a reduction in tricarboxylic acid cycle (TCA) intermediates (TCAI) (18). It has been hypothesized that this reduction in flux through the TCA cycle decreases mitochondrial NADH production and energy turnover via oxidative phosphorylation, leading to ATP generation from alternative pathways (40). One such pathway, the adenylate kinase reaction, also results in the formation of AMP, which is rapidly deaminated to IMP. Accordingly, many studies have noted the accumulation of IMP at fatigue during prolonged exercise in the presence of low intramuscular glycogen stores (4, 34, 37, 39, 40) but not earlier during exercise when glycogen stores are adequate (34, 37). Although exercise in the heat often results in an increase in intramuscular glycogen utilization (13, 14, 16), fatigue, in these circumstances, appears to be related to factors other than carbohydrate availability. It has been demonstrated that intramuscular glycogen concentration is >300 mmol/kg dry wt at fatigue during submaximal exercise in the heat (33), whereas carbohydrate supplementation provides no ergogenic benefit in these circumstances (12). It is likely, therefore, that at fatigue during exercise in the heat there would be no significant accumulation of IMP, although this has never been investigated.

When the rise in body temperature is attenuated during prolonged exercise by either reducing the ambient temperature (15), providing external cooling (26), or preventing dehydration (20), contracting muscle glycogen utilization is reduced. It is possible that the sparing of glycogen may be due to enhanced lipid oxidation because both plasma free fatty acid mobilization and fatty acid oxidation increase with cold exposure (24). In addition, when the rise in body temperature is attenuated, exercise performance is increased (12, 17, 23, 28). Although no previous studies have examined muscle metabolism at fatigue during prolonged exercise in cooler ambient temperatures, it is likely that intramuscular glycogen stores would be depleted and IMP elevated because providing exogenous carbohydrate in these conditions results in increased endurance (15).

The present study examined metabolism during prolonged submaximal exercise to exhaustion at a range of ambient temperatures: cool (3°C), thermoneutral (20°C), and hot (40°C). We hypothesized that at fatigue during prolonged submaximal exercise in a hot environment, muscle glycogen levels would be adequate, resulting in no significant formation of IMP. In contrast, we expected glycogen to be depleted after prolonged submaximal exercise in both cool and thermoneutral environments, resulting in marked increases in IMP at fatigue.
but that exercise duration would be prolonged in the cooler environment due to a reduction in glycolytic rate.

**MATERIALS AND METHODS**

Subjects. Eight endurance-trained men [age 22.6 ± 4.5 (SD) yr; height 176.4 ± 4.8 cm; weight 75.9 ± 8.5 kg; peak pulmonary oxygen uptake (V\(\text{O}_2\)peak) 4.2 ± 0.6 l/min] volunteered as subjects for this study. The subjects were informed of the purpose and the risks associated with the procedures and were free to withdraw from the study at any time. Written informed consent was obtained from all subjects before they commenced the experiment. The study was approved by the Victoria University of Technology Human Research Ethics Committee.

Experimental procedures. V\(\text{O}_2\)peak was determined on a friction-braked bicycle ergometer (Ergomatic 814E, Monark, Varberg, Sweden) by using an incremental cycling exercise test to volitional fatigue at 20–22°C as previously described (13).

At least 7 days after the V\(\text{O}_2\)peak test, subjects arrived at the laboratory to participate in one of three trials. Each trial required subjects to cycle at 70% V\(\text{O}_2\)peak in a temperature- and humidity-controlled chamber maintained at temperatures of 3°C (CT), 20°C (NT), or 40°C (HT) with a relative humidity of <50% in each condition. The trials were conducted in a counterbalanced fashion to remove any chance of an order effect. The subjects arrived after an overnight fast, having refrained from strenuous exercise, alcohol, caffeine, and tobacco for a period of 24 h. To minimize differences in resting muscle glycogen concentration, subjects completed a 48-h diet and activity log before the first trial and were then instructed to follow the same diet and activities before the second and third trials. To further minimize differences, subjects were provided with a standardized carbohydrate meal, which they consumed the night before each exercise trial.

On arrival at the laboratory, the subjects voided, were weighed nude, and positioned a rectal thermometer (Thermometer, Mallinckrodt Medical, St. Louis, MO) 10–15 cm beyond the anal sphincter. The subjects then moved into the environmental chamber and lay supine. A 20-gauge indwelling Teflon catheter (Terumo, Tokyo, Japan) was inserted into an antecubital vein of one arm, and a resting blood sample was obtained. The catheter was kept patent by flushing with 0.5 ml NaCl containing 5 U of heparin after each sample collection. After anesthesia, two incisions were made ~10 and 13 cm proximal to the lateral epicondyle of the femur, and a muscle sample was removed from the vastus lateralis (distal incision) by using the percutaneous needle biopsy technique (1) modified to include suction. The sample was quickly frozen (incision) by using the percutaneous needle biopsy technique and then weighed. After anesthesia, two incisions were made ~10 and 13 cm proximal to the lateral epicondyle of the femur, and a muscle sample was removed from the vastus lateralis (distal incision) by using the percutaneous needle biopsy technique (1) modified to include suction. The sample was quickly frozen. Muscle temperature (T\(\text{mus} \)) was measured immediately after the biopsy by using a needle thermometer (YSI 525, Yellow Springs Instruments, Yellow Springs OH) inserted to a depth of 4 cm through the biopsy incision. Subjects then moved to the cycle ergometer, a heart rate monitor (Sports Tester, Polar) was positioned, and exercise commenced. The friction-braked cycle ergometer was interfaced with a computer by using a data-acquisition operating system software. Subjects were instructed to cycle at 80 rpm, which allowed for the maintenance of a work rate that was equivalent to 70% V\(\text{O}_2\)peak. Fatigue was defined as the point when subjects were unable to maintain 70 rpm for 20 s consecutively. At the point of fatigue, a muscle biopsy was sampled and immediately frozen in liquid N\(\text{2} \). T\(\text{mus} \) was subsequently measured. Blood samples were obtained at 20 min of exercise and at fatigue. Heart rate and rectal temperature (T\(\text{re} \)) were recorded at rest; at 5, 10, and 20 min of exercise; and then every 20 min until fatigue. Pulmonary gases were collected at the same time points by using Douglas bags as previously described (13). Subjects wore cycling shorts and shoes during all trials and were not supplied with fluid or circulating air throughout the period of the exercise.

Analytic techniques. Oxygen uptake (V\(\text{O}_2 \)) and respiratory exchange ratio (RER) were calculated from expired gases by using standardized equations (8). For sampling, an aliquot (1.5 ml) of whole blood was placed in a tube containing 30 μl of EGTA and reduced glutathione, mixed, and spun at 1,500 rpm at 4°C for 15 min, and the supernatant was stored at −80°C until analysis. Samples were analyzed for plasma catecholamines by using the single-isotope \(^{3}H \) radioenzymatic assay as described in the Amersham Catecholamines Research Assay System (code TRK 995). Each muscle sample was divided into two portions and weighed at −20°C. One portion was extracted, neutralized, and analyzed for NH\(\text{3} \) by the flow-injection analysis technique as described by Katz et al. (25). The remaining muscle was subsequently freeze-dried, dissected free of any blood and connective tissue, powdered, and divided into two portions. Glycogen concentrations were determined from one portion after acid hydrolysis and neutralization according to the procedure of Passonneau and Lauderdale (35). The second portion was extracted according to the procedure of Harris et al. (21) and analyzed enzymatically for lactate (La), creatine (Cr), and creatine phosphate (PCr) by using fluorometric detection, according to the methods of Lowry and Passonneau (29). Reverse-phase high-performance liquid chromatography was used to quantify ATP, ADP, AMP, and IMP according to the method of Wynnans and Van Belle (43). Muscle NH\(\text{3} \) was corrected for water content on the basis of the wet-to-dry weight ratio determined from the freeze-dried sample. Muscle metabolites, except for La, glycogen, and NH\(\text{3} \) (because of their extracellular presence) were adjusted to peak total Cr for each subject to correct for variability in blood, connective tissue, and other nonmuscle constituents between biopsies.

Statistics. A biomedical statistical software package was used for all statistical calculations. A two-way (time and treatment) ANOVA with repeated measures was used to compare the data collected in the three trials. When the two-way ANOVA revealed a significant interaction, simple main-effects analysis was used to locate the differences. When the analyses indicated a significant difference, a Newman-Keuls test was used to locate the difference. The level of probability to reject the null hypothesis was set at \(P < 0.05 \). All comparative data are expressed as means ± SE.

**RESULTS**

Exercise time was longer (\(P < 0.05 \)) in CT compared with NT which, in turn, was longer (\(P < 0.05 \)) compared with HT (Fig. 1). Neither V\(\text{O}_2 \) nor RER was different when the three trials were compared at any measurement point (data not shown). Mean heart rate during exercise was higher (\(P < 0.05 \)) in HT compared with NT and CT. Heart rate was not different in NT compared with CT (181 ± 2 vs. 173 ± 2 vs. 168 ± 2 beats/min for HT, NT, and CT, respectively).

T\(\text{mus} \) was not different when the three trials were compared at rest, but it was higher (\(P < 0.01 \)) at fatigue compared with rest in all trials. T\(\text{mus} \) was higher (\(P < 0.05 \)) at fatigue in HT compared with NT and CT (40.7 ± 3 vs. 39.4 ± 2 vs. 39.4 ± 2°C for HT, NT, and CT, respectively). The values at fatigue were not different when the latter two trials were compared (Fig. 2). T\(\text{re} \)
was not different when the three trials were compared at rest. No differences were observed when NT was compared with CT at any point during exercise. In contrast, $T_{re}$ was higher ($P < 0.05$) at 10 min and thereafter when HT was compared with the other trials (Fig. 2).

Plasma epinephrine concentration was not different at rest when the three trials were compared. The concentration of this hormone was, however, higher ($P < 0.05$) after 20 min of exercise in HT compared with NT and CT. Furthermore, the plasma epinephrine concentration was higher ($P < 0.05$) in NT compared with CT at this time. No differences were observed in plasma epinephrine concentration at fatigue when the three trials was compared (Fig. 3). Plasma norepinephrine concentration was not different when the three trials were compared at any point (data not shown).

Concentrations of the total adenine nucleotide pool (ATP + ADP + AMP) were not different when the three trials were compared. (Table 1). Concentrations of Cr were higher ($P < 0.05$) and of PCr lower ($P < 0.05$) when resting values were compared with those at fatigue, but the values were not different when the three trials were compared (Table 1). Muscle La concentrations were not different when the three trials were compared at rest. Concentrations of this metabolite were higher ($P < 0.05$) at fatigue in all trials compared with rest. Although there was a graded response in muscle La concentration when the three trials at fatigue were compared, a significant difference ($P < 0.05$) at fatigue, was only observed when HT was compared with CT (Table 1). Muscle NH$_3$ concentrations were not different when the three trials were compared at rest. Concentrations of this metabolite were higher ($P < 0.05$) at fatigue in all trials compared with rest. Postexercise muscle NH$_3$ concentration was greater ($P < 0.05$) in CT when compared with both NT and HT. Postexercise muscle NH$_3$ concentrations were not different when NT was compared with HT (Table 1). Intramuscular glycogen content was not different when the three trials were compared at rest. Postexercise muscle glycogen content was lower ($P < 0.05$) when compared with rest for all trials, although it was greater ($P < 0.05$) in HT when compared with both NT and CT. Concentrations of this metabolite were not different between these two trials postexercise (Fig. 4), but the longer exercise duration rendered the glycogenolytic rate to be greater ($P < 0.05$) in NT compared with CT ($6.1 \pm 0.9$ vs. $4.3 \pm 0.5$ mmol glucosyl units·kg$^{-1}$·min$^{-1}$). Although IMP concentrations were not statistically different before or after exercise when the three trials were compared, there was a main effect ($P < 0.05$) for exercise for this metabolite (Fig. 4).

**DISCUSSION**

The results from this study demonstrate that glycogen content within contracting muscle at fatigue during exercise in the heat is not reduced to the low levels observed during exercise in comfortable ambient temperatures, and, therefore, fatigue during exercise in the heat is related to processes other than carbohydrate...
availability. In addition, the main effect for exercise in IMP within contracting muscle indicates that the accumulation of this metabolite during prolonged exercise in the heat is not related to substrate availability. Because no differences were observed in muscle glycogen content or energy metabolism when NT was compared with CT, the 30% improved performance in CT probably reflects a lower rate of glycogen utilization for the exercise duration.

Several studies have previously demonstrated that attenuating body core temperature by employing a preclooding maneuver (3, 23, 28), lowering ambient temperature (12, 17), or cooling with ice packs (26, 27) improves exercise performance. No studies, however, have examined intramuscular metabolism in these circumstances. The data from the present study suggest that the reason for the improved performance with cooling is likely to be related to carbohydrate availability because we observed a lower glycogenolytic rate. The mechanism for such a lower glycogenolytic rate is probably due to the attenuated epinephrine concentration observed early during exercise (Fig. 3), because epinephrine concentration influences glycogen use during submaximal exercise in trained men (11). The present data are consistent with our earlier findings that demonstrated that an attenuated rise in body core temperature was associated with a blunted epinephrine response and concomitant reduction in glycogen use during exercise in humans (15).

It has been previously demonstrated that prolonged, submaximal exercise to fatigue in comfortable ambient temperatures results in muscle glycogen depletion (2, 4, 10, 22). The reduction in glycogen availability causes a decrease in the TCAI (18, 37), a reduction in flux through the TCA cycle, and a decrease in NADH formation, leading to reduced ADP phosphorylation (37). As a result, the transient increase in free ADP stimulates the adenylate kinase reaction, resulting in the formation of ATP and AMP. Subsequently, the AMP thus formed activates AMP deaminase, producing IMP and NH₃ (4, 25, 34, 39). Results from the present study support these previous observations. Muscle glycogen content was reduced to low levels in both NT and CT (Fig. 3) Accordingly, both IMP and NH₃ concentrations were elevated in these trials at fatigue.

Despite the fact that exercise in the heat accelerates the rate of glycogen utilization (13, 14, 16, 26), the muscle glycogen concentration at fatigue in HT, ~300 mmol glucosyl units/kg, was higher compared with CT and NT (Fig. 3). These data support previous findings by Nielsen et al. (33), who have demonstrated that exercise duration in a hot environment is reduced, despite the presence of adequate muscle glycogen stores, and is, therefore, not related to the depletion of this substrate. It is clear, therefore, that factors other than substrate availability are related to fatigue at high ambient temperatures. It has previously been suggested that fatigue in these circumstances is related to a diminished central drive to exercise (6). Indeed, Nielsen et al. have demonstrated that when subjects underwent 9 days of heat acclimation they increased their exercise capacity in the heat twofold but fatigued with the same core temperature on each occasion. In addition, when subjects exercised to exhaustion in a hot environment while ingesting various beverages, they also fatigued at the same core temperature (12). The data from the present study, however, suggest that fatigue may be related, at least in part, to metabolic processes. Despite the relatively high concentration of

Table 1. Muscle metabolite concentrations before and after cycling exercise at 70% VO₂peak in 40°C (HT), 20°C (NT), and 3°C (CT)

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>27.9 ± 2.7</td>
<td>24.9 ± 1.9</td>
</tr>
<tr>
<td>ADP</td>
<td>2.8 ± 0.2</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>AMP</td>
<td>0.09 ± 0.06</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>La</td>
<td>5.2 ± 0.7</td>
<td>16.3 ± 3.5*</td>
</tr>
<tr>
<td>Cr</td>
<td>44.6 ± 3.4</td>
<td>74.7 ± 9.4*</td>
</tr>
<tr>
<td>PCr</td>
<td>91.4 ± 2.7</td>
<td>61.2 ± 6.7*</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.5 ± 0.1</td>
<td>3.1 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt for 8 men. VO₂peak, peak pulmonary O₂ uptake; HT, hot temperature; NT, thermoneutral temperature; CT, cold temperature; Rest, before cycling; Fatigue, after cycling; La, lactate; Cr, creatine; PCr, phosphocreatine. *Significantly different from rest, P < 0.05. †Significantly different from CT, P < 0.05.
muscle glycogen in HT at fatigue, the main effect for exercise indicates that IMP accumulation was significantly elevated above rest at this point. During the present study, we did not conduct the trials in an ordered fashion and thus could not compare muscle metabolism at the same time points in each trial. Therefore, we cannot rule out the possibility that IMP was also elevated in the presence of adequate glycogen stores in NT and CT. However, previous research has demonstrated that, during exercise in comfortable ambient temperatures, IMP is elevated at fatigue in the presence of low glycogen stores (34, 37, 40) but not earlier when glycogen stores are adequate (34, 37). It is unlikely, therefore, that were we to sample muscle earlier in NT and CT the same relationship between IMP and glycogen that we observed in HT would be prevalent. Hence, the theory that the increase in IMP at fatigue during prolonged exercise is related to substrate availability appears to be untrue during exercise in hot environments.

There are several possible explanations for the accumulation of IMP in the presence of adequate levels of muscle glycogen in HT at fatigue. It has been suggested (36) that as exercise in the heat progresses the increase in cardiac output is inadequate to meet the demands of increased blood flow to the skin for thermoregulation while maintaining active skeletal muscle blood flow. Potentially, therefore, this could result in a reduction in active skeletal muscle blood flow. In the absence of any rate change in oxygen extraction, it could explain the increase in IMP accumulation. It is unlikely, however, for two reasons, that alterations in blood flow to the active skeletal muscle would explain the present observations. First, active muscle blood flow during exercise in humans has been demonstrated to be unaffected by heat stress (33, 38). In addition, recent data suggest that, even when active muscle blood flow is reduced in the heat with the combination of exercise and dehydration, oxygen extraction is increased such that oxygen availability is not limiting (19).

Our data may suggest a temperature-induced perturbation in metabolism during fatiguing exercise in the heat. Brooks et al. (5) studied the phosphorylative efficiency of isolated rat skeletal muscle mitochondria by examining the ADP/O ratio over a range of temperatures. They observed a constant ADP/O ratio at temperatures ranging from 25 to 40°C; however, above 40°C the ADP/O ratio decreased linearly with increasing temperature. This suggests that for a given VO₂ the increase in ADP rephosphorylation was lower than the rate of ATP degradation. Similarly, Willis and Jackman (42), using rat and rabbit skeletal muscle mitochondria, found a 20% reduction in the ADP/O ratio at 43°C when compared with that at 37°C and suggested that the rise in muscle temperature with heavy exercise compromises the permselectivity of the inner mitochondrial membrane, increasing nonspecific proton leakage back across this membrane and decreasing the ADP/O ratio. Interestingly, in the present study, Tₘₐₜ was >40°C after exercise in the HT but was below this temperature in the other trials. Recent findings by Mills et al. (31), who observed an increase in the plasma concentration of lipid hyperoxides, an indicator of oxidative stress, in hyperthermic horses exercising to fatigue, may support the hypothesis that fatigue during exercise and heat stress may cause metabolic dysfunction. Of note, we have previously observed no increase in IMP accumulation after 40 min of submaximal exercise at 40°C, even though Tₘₐₜ was >40°C (14).

Despite there being no statistical difference in IMP when the three trials were compared at fatigue, intramuscular NH₃ accumulation was higher in CT relative to NT. This result can best be explained by the exercise duration and pathways for NH₃ production. The mechanisms for contracting skeletal muscle NH₃ production during submaximal exercise are related to the activation of AMP deaminase and amino acid catabolism (30, 41).
As exercise progresses, so too does NH₃ production from amino acid catabolism (30). Because exercise duration in CT was considerably longer relative to the other trials, it is likely that increased amino acid catabolism was responsible for the higher muscle NH₃ accumulation in this trial.

In summary, our observation of significant IMP accumulation at fatigue after submaximal exercise at 40°C, in the presence of adequate glycogen stores, suggests that fatigue under conditions of heat stress could reflect a temperature-induced metabolic perturbation. This may, therefore, influence in part, the reduction in performance during exercise in the heat.

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