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EFFECT OF TEMPERATURE ON MUSCLE METABOLISM DURING SUBMAXIMAL EXERCISE IN HUMANS

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SUMMARY

To study the effect of temperature on muscle metabolism during submaximal exercise, six endurance-trained men had one thigh warmed and the other cooled for 40 min prior to exercise using water-perfused cuffs. One cuff was perfused with water at 50–55 °C (HL) with the other being perfused with water at 0 °C (CL). With the cuffs still in position, subjects performed cycling exercise for 20 min at a work load corresponding to 70 % $\dot{V}_{O_2,peak}$ (where $\dot{V}_{O_2,peak}$ is peak pulmonary oxygen uptake) in comfortable ambient conditions (20–22 °C). Muscle biopsies were obtained prior to and following exercise and forearm venous blood was collected prior to and throughout the exercise period. Muscle temperature (T_{mus}) was not different prior to treatment, but treatment resulted in a large difference in pre-exercise T_{mus} (difference = 6·9 ± 0·9 °C; $P < 0·01$). Although this difference was reduced following exercise, it was nonetheless significant (difference = 0·4 ± 0·1 °C; $P < 0·05$). Intramuscular [ATP] was not affected by either exercise or muscle temperature. [Phosphocreatine] decreased ($P < 0·01$) and [creatine] increased ($P < 0·01$) with exercise but were not different when comparing HL with CL. Muscle lactate concentration was not different prior to treatment nor following exercise when comparing HL with CL. Muscle glycogen concentration was not different when comparing the trials before treatment, but the post-exercise value was lower ($P < 0·05$) in HL compared with CL. Thus, net muscle glycogen use was greater during exercise with heating (208 ± 23 *vs.* 118 ± 22 mmol kg^{-1} for HL and CL, respectively; $P < 0·05$). These data demonstrate that muscle glycogen use is augmented by increases in intramuscular temperature despite no differences in high energy phosphagen metabolism being observed when comparing treatments. This suggests that the increase in carbohydrate utilization occurred as a direct effect of an elevated muscle temperature and was not secondary to allosteric activation of enzymes mediated by a reduced ATP content.

INTRODUCTION

It has been previously demonstrated that the combination of exercise and heat stress results in an increase in muscle glycogenolysis (Fink *et al.* 1975; Febbraio *et al.* 1994*a*,*b*; Hargreaves *et al.* 1996*b*) and lactate accumulation (Young *et al.* 1985; Febbraio *et al.* 1994*a*,*b*; Hargreaves *et al.* 1996*b*), along with an elevated intramuscular temperature, compared with similar exercise in a cooler environment. However, exercise and heat stress also result in an augmented circulating adrenaline (Nielsen *et al.* 1990; Febbraio *et al.* 1994*a*; Hargreaves *et al.* 1996*a*) and may (Bell *et al.* 1983) or may not (Savard *et al.* 1988; Nielsen *et al.* 1990) alter active

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muscle blood flow. Since these factors may affect glycogenolytic processes, the influence of elevated muscle temperature *per se* on metabolism during exercise is unclear.

Only two previous studies have attempted to examine the effect of alterations in muscle temperature on metabolic processes in humans. Edwards *et al.* (1972) demonstrated that an elevation in intramuscular temperature increased glycogen breakdown and lactate accumulation during isometric contractions to fatigue. During this study, however, core temperature also increased when the limb was heated, whereas it was reduced in the control trial, a factor which may also have influenced metabolism. In addition, because the energy turnover during isometric contractions is lower compared with dynamic exercise (Fenn, 1923), the results of Edwards *et al.* (1972) are not a true indication of the effect of temperature *per se* on metabolic processes during prolonged exercise.

We have recently observed that an elevated muscle temperature results in an augmented decrease in the total adenine nucleotide (TAN = ATP + adenosine 5′-diphosphate (ADP) + adenosine 5′-monophosphate (AMP)) pool along with increases in muscle glycogen utilization and lactate accumulation during intense, dynamic exercise (Febbraio *et al.* 1996). The decrease in TAN in this previous study may have been due to a temperature effect on the activity of several enzymes, namely adenosine triphosphatases, adenosine 5′-monophosphate deaminase, 5′-nucleotidase and nucleoside phosphorylase. Whilst it is likely that the increase in glycogenolysis and anaerobic glycolysis was the result of a direct temperature effect on glycogenolytic and glycolytic processes, we cannot rule out the possibility that the decrease in TAN mediated these alterations in carbohydrate metabolism. It has been demonstrated that TAN degradation, in particular increases in free adenosine 5′-diphosphate (ADP), results in allosteric activation of key glycogenolytic and glycolytic enzymes, namely phosphofructokinase (PFK) (Uyeda, 1979) and phosphorylase (Ren & Hultman, 1990), thereby increasing flux through glycolysis. The purpose of the present study was, therefore, to directly test the hypothesis that an augmented muscle temperature would result in greater net glycogen utilization during steady-state, submaximal exercise in trained men, a circumstance which has been demonstrated to result in no net change to the TAN pool irrespective of thermal stress (Febbraio *et al.* 1994*b*).

METHODS

Subjects

Six endurance-trained men (24·3 ± 5·4 years; 80·1 ± 7·6 kg; $\dot{V}_{O_2,peak} = 4{\cdot}79 \pm 0{\cdot}34\ l\ min^{-1}$ (mean ± S.D.)) volunteered as subjects for this study. Each subject was informed of the risks associated with the procedures and signed a letter of informed consent prior to participation. The study was approved by the Human Research Ethics Committee of The University of Melbourne.

Experimental procedures

Peak pulmonary oxygen uptake ($\dot{V}_{O_2,peak}$) was determined during an incremental cycling test to volitional exhaustion on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands). A power output estimated to require 70 % $\dot{V}_{O_2,peak}$ was determined from a linear regression equation which plotted the first four steady-state submaximal $\dot{V}_{O_2}$ values against corresponding workloads. At least one week following the $\dot{V}_{O_2,peak}$ test, subjects arrived in the laboratory to participate in the experimental trial in the morning after an overnight fast. Subjects were instructed to abstain from alcohol, caffeine, tobacco and strenuous exercise and to consume their normal diet for 24 h prior to the experiment. The laboratory temperature was maintained at 20–22 °C. Upon arrival, subjects voided, were weighed and then positioned a rectal thermometer (Monatherm Mallinckrodt Medical, St Louis, MO, USA) 10–15 cm beyond the anal sphincter. The subjects then moved to an examination table and rested supine. A 20 gauge

indwelling Teflon catheter (Terumo, 20G, 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. This small amount of heparin does not influence blood metabolites (Ahlborg & Hagenfeldt, 1977). A resting muscle biopsy was then obtained from the vastus lateralis of each limb using the percutaneous needle biopsy technique. Briefly, local anaesthetic (1 % xylocaine) was injected approximately 10 cm and 13 cm proximal to the lateral epicondyle of the femur of both legs. Incisions were then made over the anaesthetized areas. Biopsies were taken from the distal incision first, with the right leg being biopsied prior to the left both pre- and post-exercise. Immediately following the resting biopsy, muscle temperature (T_{mus}) was obtained using a needle thermistor (YSI 525, Yellow Springs Instruments, Yellow Springs, OH, USA) inserted to a depth of 4 cm through the biopsy incision. The subjects were then seated on the cycle ergometer and water-perfused cuffs (Aircast Autochill System, Aircast, NJ, USA) were wrapped around each limb over the vastus lateralis. One cuff was perfused with water at 50–55 °C (HL), while the other was perfused with water at 0 °C (CL). The cuffs remained on the limbs for 40 min prior to exercise and throughout the 20 min exercise period. Three subjects had their left leg cooled, while the order was reversed for the remaining three subjects. The right leg was always biopsied first to ensure that the heated leg was sampled first in three subjects and the cooled leg was sampled first in the remaining three. The subjects wore swimming trunks during the experiment so that there would be no contact between clothing and the cuffs.

Immediately prior to exercise, a further blood sample was obtained and T_{mus} from each vastus lateralis was again recorded. In addition, rectal temperature (T_{rec}) was recorded at this point. A muscle biopsy was not obtained because resting muscle metabolite concentrations are unaffected by passive heating (Febbraio *et al.* 1996). Exercise was conducted on the cycle ergometer used during the $\dot{V}_{O_2,peak}$ test for 20 min. Throughout exercise, T_{rec} and heart rate (PE3000, Polar, Finland) were recorded at 5 min intervals and expiratory gas samples were obtained at 5 and 15 min. Further blood samples were obtained at 10 and 20 min. Immediately post-exercise, muscle biopsies and T_{mus} were again obtained from the vastus lateralis of each thigh. The times from the cessation of exercise to freezing in the post-exercise muscle samples were 36 ± 1 s for HL and 33 ± 5 s for CL ($P > 0{\cdot}05$). The time between cessation of exercise and the recording of T_{mus} in both limbs was < 90 s. It has been demonstrated that muscle temperature is unchanged for 2 min when the post-exercise muscle temperature is comparable to the values obtained in the present study (Allsop *et al.* 1990).

Analytical techniques

Expired gas samples were directed via a Hans Rudolf valve and plastic tubing into Douglas bags which were analysed using oxygen (Applied Electrochemistry S-3A, Ametek, Pittsburg, PA, USA) and carbon dioxide (Applied Electrochemistry CD-3A, Ametek) analysers. The volumes of expired gases were determined using a gas meter (Parkinson-Cowan, Manchester, UK) calibrated against a Tissot spirometer. Blood samples were analysed for plasma glucose, lactate and catecholamines. After each blood sample collection, 3 ml of whole blood was placed in a tube containing fluoride heparin and mixed. Aliquots of blood were transferred into tubes and spun in a centrifuge for 2 min at 1200 *g*. A 250 μl volume of plasma was transferred into a tube containing 500 μl of 3 M perchloric acid, mixed and spun for 5 min at 1200 *g*. The supernatant was stored at −80 °C until analysis for plasma lactate. The analysis was performed on a spectrophotometer using an enzymatic technique (Lowry & Passonneau, 1972). The remainder of the anticoagulated plasma was stored at −80 °C until analysis for plasma glucose using an automated glucose oxidase method (YSI 23AM Glucose Analyser, Yellow Springs, OH, USA). A further 1·5 ml of blood was placed in a tube containing 30 μl of ethylene glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) and reduced glutathione, mixed and spun at 900 *g* at 4 °C for 15 min and the supernatant stored at −80 °C until analysis. Samples were analysed for plasma catecholamines using the single isotope [^{3}H] radioenzymatic assay as described in the Amersham Catecholamines Research Assay System (code TRK 995). Muscle samples were freeze dried and divided into two portions. One portion (approximately 2 mg) was extracted, neutralized (Harris *et al.* 1974) and analysed for lactate, ATP, phosphocreatine (PCr) and creatine (Cr) using enzymatic analysis with fluorometric detection (Lowry & Passonneau, 1972). The second portion was hydrolysed in 250 μl of 2 M HCl, incubated for 2 h at 100 °C, subsequently neutralized with 750 μl of 0·667 M NaOH and analysed for muscle glycogen (glucose) according to the procedure described in Passonneau & Lauderdale (1974). The concentrations of ATP, PCr and Cr were adjusted to the peak total creatine (PCr + Cr) concentration for each subject to minimize

the error in measuring non-muscle compartments of the tissue not visible in the sample. Lactate and glycogen (measured as glucose) were not corrected due to their extracellular presence.

Statistical analysis

A two-way (time × treatment) analysis of variance (ANOVA) with repeated measures was used to compare muscle metabolite and muscle temperature data. A one-way ANOVA was used to compare blood metabolite, rectal temperature, heart rate and oxygen uptake data throughout the trial. Simple main effects analysis and Newman–Kuels *post hoc* tests were used to locate differences when the ANOVA revealed a significant interaction. Student's *t* test was used to compare glycogen utilization between HL and CL using a spreadsheet with statistical measures. The level of significance to reject the null hypothesis was set at $P < 0{\cdot}05$.

RESULTS

The mean $\dot{V}_{O_2}$ during the experimental trial was $3{\cdot}32 \pm 0{\cdot}11$ l min^{-1} which was equivalent to 71 ± 2 % $\dot{V}_{O_2,peak}$. No difference was observed in T_{mus} when comparing HL with CL prior to treatment. Forty minutes of treatment prior to exercise resulted in a higher ($P < 0{\cdot}01$) T_{mus} in HL compared with CL. In addition, T_{mus} was higher ($P < 0{\cdot}05$) following exercise in HL compared with CL (Fig. 1). Pre-exercise treatment did not affect T_{rec} but T_{rec} increased ($P < 0{\cdot}05$) with exercise (Fig. 1). Heart rate increased ($P < 0{\cdot}05$) with exercise but reached a plateau after 10 min of exercise (65 ± 4, 154 ± 7, 164 ± 6, 166 ± 6, 170 ± 6 beats min^{-1} for rest and 5, 10, 15 and 20 min of exercise, respectively). Plasma glucose was unaffected by either pre-exercise treatment or exercise during the trial (Table 1). Although plasma lactate, adrenaline and noradrenaline concentrations were not affected by passive treatment, all increased ($P < 0{\cdot}05$) during exercise (Table 1). Intramuscular [ATP] (Table 2) was not affected by either exercise or treatment. [PCr] decreased ($P < 0{\cdot}01$) and [Cr] increased ($P < 0{\cdot}01$) with exercise but were not different when comparing HL with CL (Table 2). Muscle lactate concentration was not different either at rest or following exercise when comparing HL with CL with three of the six subjects demonstrating higher post-exercise lactate concentrations in HL. Muscle lactate was, however, elevated ($P < 0{\cdot}05$) by exercise in both HL and CL (Fig. 2). Muscle glycogen concentrations were not different when comparing HL with CL prior to exercise. Post-exercise concentrations of this metabolite were lower ($P < 0{\cdot}05$) in both HL and CL when compared with those at rest. In addition, post-exercise muscle glycogen content was lower ($P < 0{\cdot}05$) in HL compared with CL (Fig. 2). Hence, muscle glycogen utilization was greater ($P < 0{\cdot}05$) in HL compared with CL (208 ± 23 *vs.* 118 ± 22 mmol kg^{-1} for HL and CL, respectively). Of note, this increase in glycogenolysis was observed in all subjects.

DISCUSSION

The results from this study indicate that increased muscle temperature augments net muscle glycogen utilization during submaximal exercise. This suggests that the increase in carbohydrate utilization previously observed during exercise in a hot environment may be mediated, in part, by an elevated intramuscular temperature. In addition, since [ATP] was not reduced during exercise and was not affected by alterations in muscle temperature, the data also suggest that muscle temperature has a direct effect on glycogen utilization.

The marked difference in net muscle glycogen use when comparing the two trials was observed despite the relatively small, but nonetheless significant, difference in post-exercise T_{mus} (Fig. 1). It must be noted, however, that the difference in T_{mus} prior to exercise was substantial and this difference, although reduced, was maintained throughout the entire exercise period. We were

Table 1. *Plasma glucose, lactate, adrenaline and noradrenaline concentrations prior to treatment, prior to exercise and at 10 and 20 min during exercise at 70 % $\dot{V}_{O_2,peak}$*

			Exercise	
	Pre-treatment	Pre-exercise	10 min	20 min
Glucose (mmol l^{-1})	5·3 ± 0·2	5·2 ± 0·2	5·3 ± 0·4	5·7 ± 0·3
Lactate (mmol l^{-1})	1·4 ± 0·2	0·9 ± 0·1	5·0 ± 0·8 *	5·8 ± 1·2 *
Adrenaline (nmol l^{-1})	0·3 ± 0·1	0·4 ± 0·2	1·0 ± 0·1 *	1·8 ± 0·4 *
Noradrenaline (nmol l^{-1})	1·88 ± 0·21	2·96 ± 0·50	9·22 ± 1·59 *	10·37 ± 1·71 *

* Significant difference ($P < 0·05$) compared with pre-treatment. Values are means ± S.E.M. ($n = 6$).

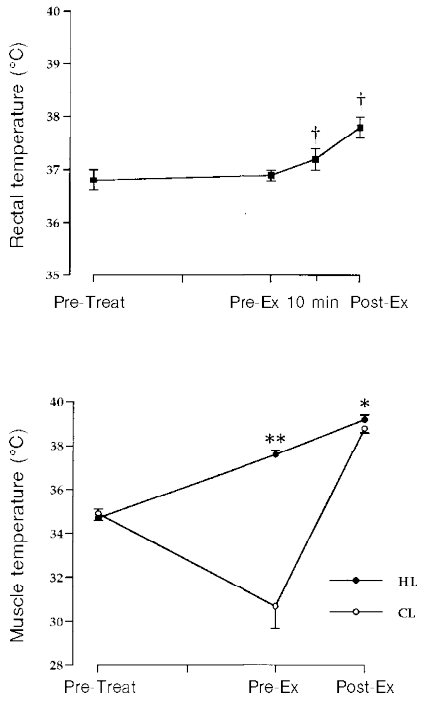

Fig. 1. Rectal temperature (top) and muscle temperature (bottom) prior to treatment (Pre-Treat), and before (Pre-Ex), during (10 min) and after (Post-Ex) 20 min of cycle exercise at 70 % $\dot{V}_{O_2,peak}$. HL indicates limb heating and CL indicates limb cooling for muscle temperature. † Significant difference ($P < 0·05$) compared with pre-treatment. Significant difference from CL: ** $P < 0·01$, * $P < 0·05$. Values are means ± S.E.M. ($n = 6$).

not able to determine the rate of increase in muscle temperature during the exercise period when comparing HL with CL. However, in a separate group of subjects ($n = 8$) similar exercise was performed for 10 min following the limb heating and cooling protocol described above. The difference in post-exercise T_{mus} at 10 min of exercise was 2·5 °C (39·0 ± 0·1 *vs.* 36·5 ± 1·0 °C; $P < 0·05$). In addition, in a recent experiment Koga *et al.* (1997) observed a marked difference in muscle temperature after 6 min of exercise when comparing a heated limb with a control. Given that the Q_{10} value (the temperature coefficient over a 10 °C temperature range)

Table 2. *Intramuscular concentrations of ATP, phosphocreatine (PCr) and creatine (Cr) before and following 20 min of exercise at 70 % $\dot{V}_{O_2,peak}$ with limb heating (HL) or limb cooling (CL)*

	HL		CL	
	Pre-treatment	Post-exercise	Pre-treatment	Post-exercise
ATP	26·0 ± 1·5	24·5 ± 2·0	25·3 ± 1·8	24·2 ± 1·5
PCr*	79·3 ± 4·8	54·8 ± 5·8	76·5 ± 6·0	53·8 ± 8·7
Cr*	39·1 ± 2·9	63·8 ± 10·5	42·1 ± 2·6	64·8 ± 7·1

* Main effect ($P < 0·01$) for exercise. All data are expressed in mmol (kg dry weight)$^{-1}$. Values are means ± S.E.M. ($n = 6$).

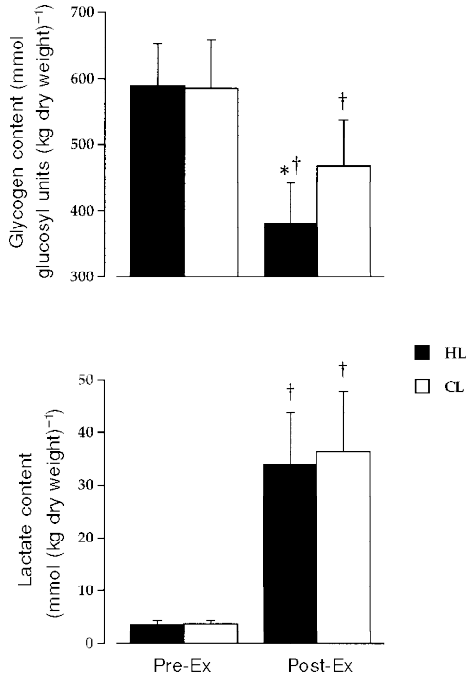

Fig. 2. Muscle glycogen content (top) and lactate content (bottom) before (Pre-Ex) and after (Post-Ex) 20 min of cycle exercise at 70 % $\dot{V}_{O_2,peak}$ with limb heating (HL) or limb cooling (CL). * Significant difference ($P < 0·05$) compared with CL. † Significant difference ($P < 0·05$) compared with rest. Values are means ± S.E.M. ($n = 6$).

commonly found for enzyme-mediated reactions is 2·0–3·0 (Florkin & Stolz, 1968) it is not surprising that a difference in glycogenolysis was observed given the magnitude of difference in T_{mus} at the onset of exercise (6·9 ± 0·9 °C). Indeed, it is likely that the largest change in glycogenolytic rate occurred early in the exercise period when the difference in T_{mus} was at its highest.

The mechanism/s for the increase in muscle glycogen utilization in HL compared with CL is/are unclear, but may be related to several factors. It has been suggested that elevations in muscle temperature may increase the rate, and/or decrease the efficiency, of cross-bridge

cycling (Edwards *et al*. 1972). Even though glycogenolysis was increased, this hypothesis seems unlikely given that ATP and PCr stores were unaffected. Alternatively, the difference in glycogen utilization when comparing HL with CL may have occurred due to alterations in the pathways which contribute to energy turnover during submaximal exercise, rather than an increase in ATP demand associated with higher muscle temperatures. The elevated T_{mus} in HL may have directly affected the activity of the key enzymes involved in carbohydrate utilization, such as glycogen phosphorylase, PFK and pyruvate dehydrogenase, via a Q_{10} effect as has been previously suggested (Young *et al*. 1985). This may have resulted in a substrate shift towards greater carbohydrate utilization and lesser lipid oxidation. Unfortunately, we were unable to measure intramuscular lipid utilization because of the difficulty in measuring triacylglycerol content in human skeletal muscle biopsy samples (Wendling *et al*. 1996). An alternative explanation for the increase in glycogen utilization observed with muscle heating may be related to mitochondrial function. Although whole body $\dot{V}_{O_2,peak}$ is not altered by body heating (Febbraio *et al*. 1994*a*), it is possible that in the present study leg $\dot{V}_{O_2}$ was affected by heating or cooling resulting in an altered energy supply from pathways other than aerobic metabolism. Brooks *et al*. (1971) have demonstrated that an increase in temperature of isolated skeletal muscle mitochondria resulted in a reduction in the ratio between ADP production and mitochondrial $\dot{V}_{O_2}$. We have recently observed intramuscular inosine 5′-monophosphate accumulation in the presence of an intramuscular glycogen content of >300 mmol (kg dry weight)$^{-1}$ during submaximal exercise with elevated T_{mus} (Parkin *et al*. 1999) which may suggest a temperature-induced perturbation of the tricarboxylic acid cycle. Hence, any impairment of mitochondrial function may result in an increase in flux through glycolysis.

There were no differences in high energy phosphagen concentrations when comparing the treatments (Table 2). It is apparent from these results, therefore, that the augmented glycogen utilization in HL relative to CL was not secondary to changes in the adenine nucleotide pool or free inorganic phosphate (P_i) concentrations. An increased plasma adrenaline concentration has been observed during exercise and heat stress (Nielsen *et al*. 1990; Febbraio *et al*. 1994*a*; Hargreaves *et al*. 1996*a*) which may partly account for the enhanced muscle glycogen utilization under such conditions (Febbraio *et al*. 1998). However, since in the present study both legs were exposed to the same plasma adrenaline level and core temperature, this could not account for the observed difference in muscle glycogen utilization between HL and CL.

The observation of a similar net muscle lactate concentration when comparing HL with CL was an unexpected finding, given that the possible mechanisms for enhanced glycogenolysis are also likely to promote lactate production. In addition, previous studies which have compared the effect of increased muscle temperature on carbohydrate metabolism during isometric contractions (Edwards *et al*. 1972) or supramaximal, dynamic exercise (Febbraio *et al*. 1996) have demonstrated increased muscle lactate accumulation with heating, while an increased muscle lactate accumulation during exercise and whole-body heat stress has been consistently observed (Young *et al*. 1985; Febbraio *et al*. 1994*a*, *b*; Hargreaves *et al*. 1996*a*, *b*). It is likely that the similar post-exercise muscle lactate concentration when comparing HL with CL was related to its removal from the muscle. It is possible that the production of lactate in HL exceeded that in CL but that lactate efflux was less in CL compared with HL, resulting in a similar post-exercise muscle lactate concentration. Any reduction in muscle lactate efflux in CL may be related to a reduction in muscle blood flow due to cooling and/or a temperature-induced impairment of lactate transport. A significant reduction in quadriceps muscle blood flow has been observed with local cold application both before and after exercise (Thorsson *et al*. 1985). Hence, the lower T_{mus} at rest and during exercise in CL may have impaired blood flow

and consequent lactate efflux. It has been suggested that a reduction in O_2 delivery secondary to an attenuated leg blood flow during localized cooling would promote anaerobic energy production by limiting O_2 availability (Beelen & Sargeant, 1991). Were this to be the case in the present study, it would have resulted in an increase in carbohydrate utilization in CL compared with HL. An increase in tissue O_2 extraction with decreased O_2 supply has been observed during exercise, however, in both heat-stressed humans (Gonzalez-Alonso *et al.* 1997) and hypovolaemic dogs (Schumaker *et al.* 1987). Hence, any reduction in muscle blood flow in CL was unlikely to affect muscle O_2 availability while still impairing muscle lactate efflux.

It is also possible that lactate transport was impaired, independent of blood flow kinetics. Lactate removal from skeletal muscle may have been impaired due to a temperature-induced reduction in sarcolemmal lactate transport (Hopfer, 1978) causing lactate accumulation in CL to appear to be equal to HL. Of note, when dynamic exercise with muscle cooling was compared with similar exercise without treatment, peak post-exercise blood lactate concentration occurred later (Blomstrand *et al.* 1986), leading these authors to speculate that the cooler muscle temperature impaired lactate efflux. In the current study, venous lactate concentrations were measured to provide an indication of the circulating lactate concentration. The plasma lactate data did not, however, allow us to determine either circulating arterial concentration or lactate release from the contracting limbs. In order to determine lactate efflux a measure of arterio-venous lactate concentrations in each limb would have been necessary. Hence, the suggestion that lactate efflux was impaired in CL is speculative.

Although less likely, it is also possible that the similar muscle lactate accumulation when comparing HL with CL was due to a change in motor unit recruitment favouring the greater use of fast twitch fibres in CL. A higher activation of fast twitch fibres has been previously observed in carp swimming at 10 °C compared with 20 °C (Rome *et al.* 1984). Hence, such a phenomenon may have resulted in a reduction in carbohydrate oxidation in CL relative to HL. However, we have previously observed no effect of hyperthermia on motor unit recruitment pattern in humans (Febbraio *et al.* 1994*a*) and, therefore, this explanation is speculative.

In summary, an increase in muscle temperature *per se* increases muscle glycogen utilization during submaximal exercise. These data suggest that the increased muscle temperature that accompanies exercise and heat stress may be partly responsible for the increase in net muscle glycogen utilization previously under such conditions.

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