

Magni Mohr · Peter Rasmussen · Barry Drust  
Bodil Nielsen · Lars Nybo

## Environmental heat stress, hyperammonemia and nucleotide metabolism during intermittent exercise

Accepted: 24 January 2006 / Published online: 17 February 2006  
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**Abstract** This study investigated the influence of environmental heat stress on ammonia (NH<sub>3</sub>) accumulation in relation to nucleotide metabolism and fatigue during intermittent exercise. Eight males performed 40 min of intermittent exercise (15 s at 306 ± 22 W alternating with 15 s of unloaded cycling) followed by five 15 s all-out sprints. Control trials were conducted in a 20°C environment while heat stress trials were performed at an ambient temperature of 40°C. Muscle biopsies and venous blood samples were obtained at rest, after 40 min of exercise and following the maximal sprints. Following exercise with heat stress, the core and muscle temperatures peaked at 39.5 ± 0.2 and 40.2 ± 0.2°C to be ~ 1°C higher ( $P < 0.05$ ) than the corresponding control values. Mean power output during the five maximal sprints was reduced from 618 ± 12 W in control to 558 ± 14 W during the heat stress trial ( $P < 0.05$ ). During the hot trial, plasma NH<sub>3</sub> increased from 31 ± 2 μM at rest to 93 ± 6 at 40 min and 151 ± 15 μM after the maximal sprints to be 34% higher than control ( $P < 0.05$ ). In contrast, plasma K<sup>+</sup> and muscle H<sup>+</sup> accumulation were lower ( $P < 0.05$ ) following the maximal sprints with heat stress compared to control, while muscle glycogen, CP, ATP and IMP levels were similar across trials. In conclusion, altered levels of “classical peripheral fatiguing agents” does apparently not explain the reduced capacity for performing repeated sprints following intermittent exercise in the heat, whereas the augmented systemic NH<sub>3</sub> response may be a factor influencing fatigue during exercise with superimposed heat stress.

**Keywords** Sprint performance · Fatigue · Muscle metabolism · Exercise · Hyperthermia

### Introduction

Prolonged exercise performances are impaired by whole-body hyperthermia (González-Alonso et al. 1999; Nybo et al. 2001), whereas the capacity for short-term (sprint) exercise appears to be improved when the muscle temperature is elevated by either passive or active warm-up (Assmussen and Bøje 1945). Thus, there seems to be a paradox, where elevations in body temperatures on one hand are beneficial for peripheral factors of importance for exercise performance, but on the other hand could provoke so-called central fatigue as supported by the observation that maximal voluntary activation of the motorneurons is reduced by both exercise-induced and passive hyperthermia (Nybo and Nielsen 2001; Morrison et al. 2004).

The aetiology of fatigue appears to be complex and it is likely that several factors interact. This may be the case especially during exercise with superimposed heat stress, since this is associated with both central and peripheral metabolic and circulatory perturbations that could contribute to reduce exercise performance (Parkin et al. 1999; Nybo and Secher 2004). A link between the peripheral changes and the CNS fatigue that arises with hyperthermia could involve ammonia (NH<sub>3</sub>) produced by the exercising muscles as the result of enhanced purine nucleotide degradation may be in combination with amino acid catabolism (Graham 1994). Hence, exercise may elevate the circulating level of NH<sub>3</sub> several fold and since NH<sub>3</sub> can cross the blood–brain barrier it may enter the brain where excessive accumulation could have detrimental effects on cerebral function due to disturbances in cerebral neurotransmitter homeostasis (Banister and Cameron 1990). In support of this notion, a cerebral uptake of NH<sub>3</sub> and accumulation in the cerebrospinal fluid has been observed during prolonged exercise (Nybo et al. 2005), and the production

M. Mohr · P. Rasmussen · B. Nielsen · L. Nybo (✉)  
Institute of Exercise and Sport Sciences, Department of Human Physiology, August Krogh Institute, University of Copenhagen, Universitetsparken 13, 2100, Copenhagen Ø, Denmark  
E-mail: lnnielsen@aki.ku.dk  
Tel.: +45-35-321620  
Fax: +45-35-321600

B. Drust  
Research Institute for Sport and Exercise Sciences,  
John Moore University, Liverpool, England

of  $\text{NH}_3$  appears to be augmented by hyperthermia (Snow et al. 1993). However, continuous exercise at a moderate intensity will only induce minor elevations of plasma  $\text{NH}_3$  (Parkin et al. 1999), whereas intermittent exercise with repeated bouts of intense efforts separated by brief periods of rest may elevate the systemic  $\text{NH}_3$  concentrations to a much larger extent (Krustrup et al. 2003).

As the influence of heat stress on circulating  $\text{NH}_3$  during intermittent exercise has not been investigated, it remains unknown if this could constitute a link between central and peripheral factors of importance for fatigue and to what extent it relates to enhanced purine nucleotide degradation. Furthermore, peripheral fatigue and inhibitory feed-back from the exercising muscles to the central nervous system could involve low muscle glycogen, CP and ATP levels, as well as accumulation of muscle  $\text{H}^+$  and extracellular  $\text{K}^+$  (Fitts 1994; Cairnes and Dulhunty 1995). Therefore, the present study utilised a protocol with submaximal and maximal intermittent exercise with or without environmental heat stress to evaluate the relationship between the development of fatigue and changes in circulating ammonia, muscle purine nucleotide degradation, plasma potassium and muscle proton accumulation.

## Materials and methods

### Subjects

Eight males (age;  $23.4 \pm 1.0$  years, height;  $182.4 \pm 1.5$  cm, weight;  $73.9 \pm 2.2$  kg, maximal oxygen uptake;  $55.8 \pm 2.0$  ml  $\text{O}_2$   $\text{kg}^{-1}$   $\text{min}^{-1}$ ; mean  $\pm$  standard error) volunteered as subjects. They were informed of any risks and discomforts associated with the experiments before giving their written consent to participate. The experiments were carried out in accordance with the Declaration of Helsinki and approved by the Ethics committee of Copenhagen and Frederiksberg (KF 01-135/00).

### Pre-experimental procedures

Each subject visited the laboratory on two occasions prior to the experimental trials. These visits included the assessment of maximal oxygen uptake (visit 1) and familiarisation to the intermittent and the repeated sprint protocols (visits 1 and 2) to be used in the experimental trials. Maximal oxygen consumption was determined for each subject by means of an incremental cycling test to volitional exhaustion on an electronically braked cycle ergometer (Monark Ergomedic 839 E, Varberg, Sweden). The test was initiated at 200 W and increased by 25 W every min until the subjects could no longer complete the given load. Pulmonary oxygen uptake was continuously measured throughout the test using an automated on-line gas analysis system (model CPX/D, Medgraphics, St Paul, MN, USA). After the completion

of this test, and following 30 min of recovery, subjects were required to perform both the intermittent (20 min of intermittent exercise) and the repeated sprint protocols. In addition, all subjects completed a second familiarisation session for the intermittent and repeated sprint exercise. These familiarisation sessions served to accustom the subjects with the activity pattern, the intense nature of the exercise and the procedures involved in data collection used in the experimental trials. All pre-trials were performed at an environmental temperatures of  $21.7 \pm 2.5^\circ\text{C}$ .

### Experimental design

Subjects completed 40 min of intermittent cycling at an intensity corresponding to  $\sim 65\%$  of their maximal oxygen uptake utilising a repeated 15 s exercise ( $306 \pm 22$  W), 15 s unloaded cycling schedule on two occasions. This intensity was chosen as it is similar to the average intensity observed during intermittent team sports (E.g. soccer; Bangsbo 1994). Following the 40 min of submaximal intermittent exercise a muscle biopsy was obtained before the subjects were transferred to another cycle ergometer (Monark Ergomedic 824 E, Varberg, Sweden) where they performed  $5 \times 15$  s maximal sprints interspaced by 15 s recovery. On one occasion exercise was performed in a  $40.3 \pm 0.7^\circ\text{C}$  environment (environmental heat stress trial; EHS;  $17 \pm 1\%$  relative humidity), whereas the control trial (CON) was conducted at an ambient temperature of  $20.0 \pm 1.3^\circ\text{C}$  and  $24 \pm 6\%$  relative humidity. The two trials were separated by 5–7 days and completed in a randomised-counterbalanced order.

On the experimental days, the subjects arrived at the laboratory in a post absorptive state (at least 4 h), having refrained from vigorous exercise, alcohol, tobacco and caffeine during the previous 24 h. To ensure standardisation of nutritional and hydration status, the subjects recorded their food and fluid intakes for the 48-h period prior to the first trial, so the diet could be repeated for the remaining trial. The subjects did not consume any fluid during the experimental trials.

The subjects arrived at the laboratory approximately 45 min before the start of the exercise protocol and rested in a thermoneutral room while equipment was attached. An oesophageal probe was inserted through the nasal passage, a venous catheter was inserted into an antecubital vein, and a heart rate (HR) monitor (PE 4000, Polar, Finland) was attached. Under local anaesthesia (20 mg/ml lidocaine without adrenalin) two incisions ( $\sim 1$  cm) were made in the medial part of m. *vastus lateralis* of the right leg and a muscle biopsy taken from one of the incisions, while the other incision was used for the exercise biopsies.

The subjects entered the climatic chamber and commenced the intermittent exercise protocol. HR,  $T_c$ , rating of perceived exertion (RPE) (Borg 1975) were recorded every 5 min, whereas venous blood samples were collected at 0, 10, 20, 30 and 38 min.

Within  $\sim 6$  s after completion of the intermittent protocol with the subject still seated on the bike a post exercise muscle biopsy was obtained and muscle temperature ( $T_m$ ) was measured. Subjects were then transferred to another cycle ergometer and completed the maximal sprints before another muscle biopsy was obtained and  $T_m$  determined. The subjects were instructed to regard each sprint as an all-out sprint and to apply maximal effort from the onset.

### Body temperatures

Core temperature ( $T_c$ ) was measured continuously throughout the experiment with a thermocouple (model MOV-A, Ellab, Copenhagen, Denmark) inserted through the nasal passage to one-quarter of the subject's standing height.  $T_m$  was assessed by inserting a needle thermistor (model MKA-A, Ellab, Copenhagen, Denmark) to a depth of 3 cm into the vastus lateralis adjusting for the thickness of the skin fold.  $T_m$  measurements were made in the contra-lateral limb to that used for the biopsy. The measurements were performed at rest, as well as after the intermittent and sprint trial. Both thermocouples were connected to a recorder (CTF 9008 precision thermometer, Ellab) interfaced to an IBM computer. All thermistors had an accuracy of  $0.1^\circ\text{C}$  and were calibrated against a mercury thermometer.

### Repeated sprint trial

All subjects cycled against a resistance of 75 g kg ( $5.5 \pm 0.4$  kg; Ball et al. 1999) from a rolling start (with no load on the ergometer). The subjects were instructed and verbally encouraged to make a maximal effort during each sprint. A modified friction-loaded cycle ergometer (Monark Ergonomic 824 E, Varberg, Sweden), interfaced with a microcomputer, was used to attain high frequency logging of the flywheel angular velocity during each sprint. Power output was averaged over 1 s and mean power output for each sprint and the accumulated work during all five sprints was calculated.

### Measurements in blood and muscle

Blood samples were drawn at rest, every 10 min during the intermittent protocol and after the sprints. Muscle biopsies were obtained at rest and immediately after both exercise protocols (Bergström 1962).

### Blood analysis

Part of the blood samples ( $\sim 2.5$  ml) was immediately centrifuged for 30 s. The plasma was collected and stored at  $-20^\circ\text{C}$  until analysed for ammonia ( $\text{NH}_3$ ) using spectrophotometrical determinations (Kun and

Kearney 1974) and potassium using a flame photometer (Radiometer, Copenhagen, Denmark) with lithium as an internal standard.

### Muscle analysis

The muscle samples were frozen in liquid nitrogen within 5 s of sampling and stored at  $-80^\circ\text{C}$  for biochemical analysis. The frozen samples were weighed before and after freeze-drying to determine the water content. The freeze-dried sample was dissected free of blood, fat and connective tissue. Part of the muscle tissue (3–5 mg dw) was extracted in 1 M perchloric acid (PCA) and neutralised to pH 8.0 with 2 M KOH and analysed for ATP and IMP as described by Tullson et al. (1990). Another part of the sample ( $\sim 2$  mg dw) was extracted in a solution of 0.6 M PCA and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M  $\text{KHCO}_3$  and analysed for CP by fluorometric assays (Lowry and Passonneau 1972). Moreover, 1–2 mg dw of muscle tissue was extracted in 1 M HCl and hydrolysed at  $100^\circ\text{C}$  for 3 h, where after the glycogen content was measured by the hexokinase method (Lowry and Passonneau 1972). Muscle pH was measured by a small glass electrode (Radiometer GK2801) after homogenizing a freeze-dried muscle sample of about 2 mg dry wt. in a non-buffering solution containing 145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid (as described by Krstrup et al. 2003).

### Statistics

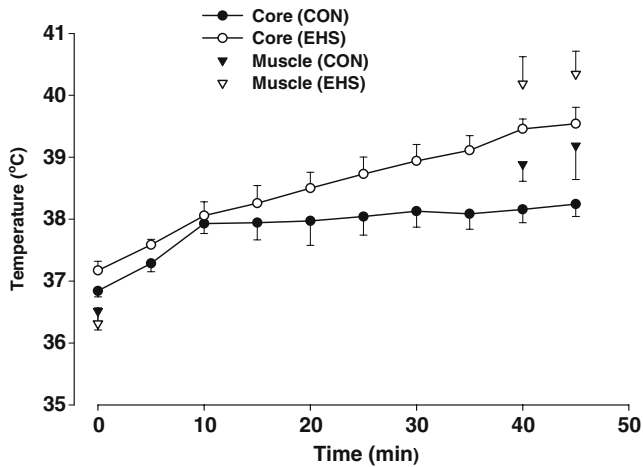
Data are presented as mean  $\pm$  standard error. Differences in core temperature, plasma and muscle metabolites between CON and EHS were tested using a two way (time-by-trial) ANOVA with repeated measurements. In case of significant main effects a Student Newman-Keuls post hoc test was used to identify the points of difference. Differences in muscle temperature and sprint performance between the two trials were tested with a paired *t*-test. Significance was set at the 0.05 level.

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## Results

### Body temperatures, heart rates and RPE

Both core and muscle temperatures were  $\sim 1^\circ\text{C}$  higher ( $P < 0.05$ ) following the 40 min of submaximal intermittent exercise in EHS ( $T_c = 39.3 \pm 0.0^\circ\text{C}$  and  $T_m = 40.1 \pm 0.2^\circ\text{C}$ ) as compared to CON ( $T_c = 38.1 \pm 0.1^\circ\text{C}$  and  $T_m = 38.9 \pm 0.1^\circ\text{C}$ ) and this difference was maintained during the maximal sprints (Fig. 1). Concomitantly with the elevation of  $T_c$ , RPE increased ( $P < 0.05$ ) from  $12 \pm 1$  at 5 min to  $18 \pm 1$  after 40 min of exercise in EHS to be higher ( $P < 0.05$ ) than the CON value of  $14 \pm 0$ . Similarly, HR increased ( $P < 0.05$ ) to



**Fig. 1** Core (circles) and muscle temperature (triangles) in CON (closed) and EHS (open) during the experimental protocol ( $n=8$ ). \* Denoted a significant difference in core temperature between trials. # Denotes a significant difference in muscle temperature between CON and EHS ( $P<0.05$ )

173 ± 3 bpm in EHS as compared to 156 ± 3 bpm after 40 min of exercise in CON.

#### Sprint performance

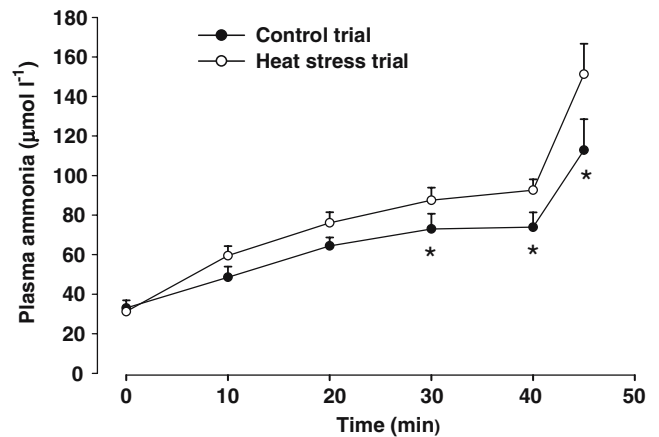
Power output was similar across trials during the first sprint, but then declined to a larger extent during EHS than control. Consequently, the average power output over the five sprints was lower ( $P<0.05$ ) in EHS (558 ± 14 W) compared to CON (618 ± 12 W) (for details see Drust et al. 2005).

#### Plasma ammonia and potassium

The plasma  $\text{NH}_3$  concentration increased ( $P<0.05$ ) gradually during the first 40 min of exercise in both trials, but the accumulation of  $\text{NH}_3$  in the circulation was augmented by heat stress and consequently plasma  $\text{NH}_3$  was higher after 40 min of exercise in EHS compared to CON ( $P<0.05$ ). Plasma  $\text{NH}_3$  was further elevated by the maximal sprints and remained ~ 30% higher ( $P<0.05$ ) in EHS than CON (Fig. 2). The plasma  $\text{K}^+$  was 3.9 ± 0.1 mM at rest and increased to a similar extent during the first 40 min of exercise in CON (4.7 ± 0.1 mM) and EHS (4.9 ± 0.1 mM). However, following the maximal sprints plasma  $\text{K}^+$  became elevated to 6.2 ± 0.3 mM in CON, which was higher ( $P<0.05$ ) than in EHS (5.2 ± 0.3 mM; Fig. 3).

#### Muscle metabolites

ATP and IMP levels did not differ across the environmental settings, but in both trials the ATP content was lower ( $P<0.05$ ) following the maximal sprints compared to the resting and submaximal exercise values. IMP

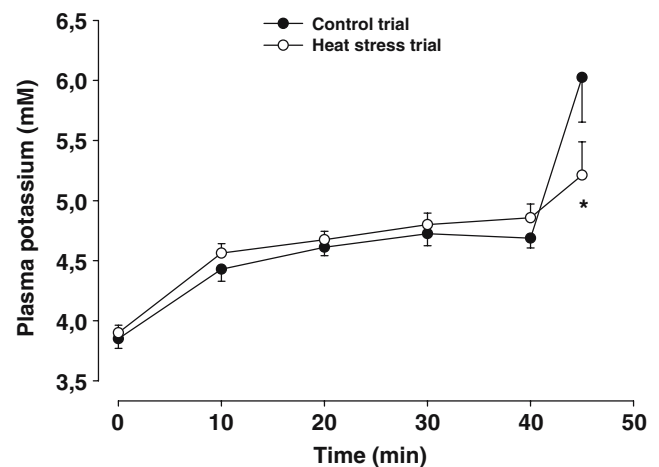


**Fig. 2** Venous  $\text{NH}_3$  concentrations during the entire exercise protocol in CON (closed circles) and EHS (open circles;  $n=8$ ). Data are presented in mean ± SEM. \* Denotes a significant difference between CON and EHS ( $P<0.05$ )

content increased ( $P<0.05$ ) during the intermittent exercise and became further elevated during the maximal sprints (Table 1). The CP concentration declined ( $P<0.05$ ) gradually during the exercise protocol to reach similar levels in CON and EHS by the end of the maximal sprints. Similarly, muscle glycogen decreased ( $P<0.05$ ) in both trials to reach similar levels by the end of the sprints (CON; 177 ± 18 and EHS; 227 ± 36 mmol  $\text{kg}^{-1}$  dw; Fig. 4). Muscle pH declined to a lesser extent in EHS (6.96 ± 0.02) compared to CON (6.81 ± 0.02;  $P<0.05$ ).

#### Discussion

Intermittent exercise with environmental heat stress was associated with a reduced capacity for repeated sprinting and an augmented accumulation of plasma  $\text{NH}_3$ . The



**Fig. 3** Venous  $\text{K}^+$  concentrations during the entire exercise protocol in CON (closed circles) and EHS (open circles;  $n=8$ ). Data are presented in mean ± SEM. \* Denotes a significant difference between CON and EHS ( $P<0.05$ )

**Table 1** Muscle ATP, IMP, CP and pH before and after the intermittent exercise (INT) and the repeated sprint trial (RST) in CON and EHS ( $n=8$ )

	ATP (mmol kg dw)	SE	IMP (mmol kg dw)	SE	CP (mmol kg dw)	SE	pH (-log H <sup>+</sup> )	SE
Rest								
CON	23.53	0.73	0.05	0.01	84.0	5.6	7.14	0.01
EHS	23.80	0.68	0.06	0.01	82.4	2.2	7.13	0.01
After INT								
CON	24.20	0.67	0.17	0.03	51.6	5.9	7.10	0.01
EHS	23.72	0.81	0.22	0.07	56.6	6.3	7.06	0.01
After RST								
CON	18.58	0.92	5.23	0.98	36.1	5.7	6.81*	0.02
EHS	21.49	1.14	2.63	0.83	31.4	4.0	6.96	0.02

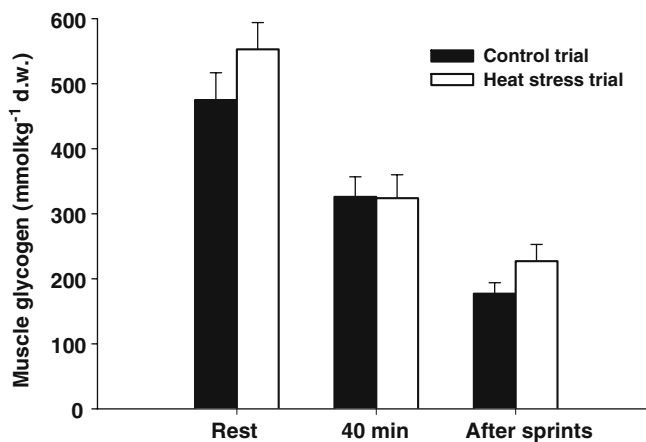
\*A significant difference between CON and EHS ( $P < 0.05$ )

elevated  $\text{NH}_3$  levels did not relate to differences in muscle ATP, IMP or CP, and the heat stress induced hyperammonemia persisted throughout the protocol, despite the accomplishment of less work during the sprints in the heat compared to control. The accumulation of “peripheral fatiguing agents” such as extracellular  $\text{K}^+$  and muscle  $\text{H}^+$  were lowered rather than elevated following the environmental heat stress trial, and this appears to be the consequence of rather than the explanation for the reduced exercise capacity. Since  $\text{NH}_3$  potentially may influence both central and peripheral fatigue (Banister and Cameron 1990; Graham 1994; Nybo and Secher 2004), the impaired performance in the hot trial could relate to the higher levels of systemic  $\text{NH}_3$ . A central effect of the hyperammonemia may be supported by the observation that maximal handgrip strength performed during the submaximal bicycling protocol was significantly impaired during heat stress compared to the control trial (see Drust et al. 2005 for details).

Heat stress has been demonstrated to accelerate muscle glycogenolysis and lactate production (Drust et al. 2005; Febbraio 2000, 2001). Therefore muscle glycogen could decline more rapidly when exercise is carried out in the heat, and as the glycogen level in the exercising muscles becomes low it may compromise oxidative phosphorylation and increase the turnover of

purine nucleotides, elevating  $\text{NH}_3$  production and reducing the total pool of adenine nucleotides (Baldwin et al. 2003). However, in the present study no difference was observed between muscle glycogen concentrations between the two trials after the intermittent or the maximal sprint trial. Moreover, muscle glycogen utilisation was not correlated to the systemic  $\text{NH}_3$  concentrations. Additionally, ATP and IMP levels were similar across trials, indicating that the degradation of purines was unaffected by heat stress. However, it should be mentioned that the systemic  $\text{NH}_3$  levels were correlated ( $r=0.68$ ;  $P < 0.05$ ) to muscle IMP concentrations following the sprints in the hot trial, suggesting some influence from the metabolism of nucleotides on the hyperammonic state.  $\text{NH}_3$  was measured in blood from an arm vein as an indication of the circulating plasma level, but since  $\text{NH}_3$  during cycling exercise is produced primarily in the muscles of the lower limbs, the arterial concentrations may have been slightly higher. However, ammonia clearance in the forearm is expected to be minor and the sampling site should not have any effect on the observed differences between trials. In contrast, the systemic plasma level of  $\text{NH}_3$  is indeed influenced by clearance in the liver and kidneys, and it is likely that part of the heat stress induced hyperammonemia relates to reduced removal of  $\text{NH}_3$  from the circulation, since heat stress impairs the perfusion of both the liver and kidneys (Rowell et al. 1965). Furthermore, the exercise-induced hyperammonemia may relate to metabolism of branched chained amino acids (Katz et al. 1986), though it is not known to what extent heat stress influences this process.

Accumulation of interstitial  $\text{K}^+$  may provoke muscular fatigue due to electrical disturbances across the sarcolemma (Fitts 1994). Moreover, exercise induced  $\text{K}^+$  efflux from the muscle cells has been closely linked to the anaerobic metabolism (Nordsborg et al. 2003), since the opening probability of  $\text{K}_{\text{ATP}}$  channels is pH-sensitive and gradually increases with intramuscular acidification (Davies et al. 1991). In addition, training-induced increases in high intensity exercise performance is associated with the muscles ability to maintain the interstitial  $\text{K}^+$  homeostasis (Nielsen et al. 2004). Several studies have also shown that venous  $\text{K}^+$  reaches concentrations between 6 and 7 mM at exhaustion after intense exercise (Krustrup et al. 2003;



**Fig. 4** Muscle glycogen before and after the intermittent exercise and after the sprints in CON (white bars) and EHS (black bars)

Nielsen et al. 2004) and such plasma concentrations reflect interstitial  $K^+$  levels of 10–12 mM (Nielsen et al. 2004), which is potent enough to depolarise the membrane potential and impair force development markedly (Cairnes and Dulhunty 1995). In the control trial venous  $K^+$  rose above 6 mM after the maximal sprints, indicating that interstitial  $K^+$  accumulation may have detrimental effects on performance at least in the control trial. The lower extracellular  $K^+$  levels in the hot trial were accompanied by lower muscle  $H^+$  and lactate accumulation after the maximal sprints. Hence, a possible scenario may be that the lower degree of muscle acidification in the heat results in less activity in the  $K_{ATP}$  channels, which reduces the  $K^+$  efflux and thereby also the extracellular accumulation of  $K^+$ . One should of course always be aware that temporarily related phenomena may not necessarily be causally related, but the observation that the concentrations of these main markers of peripheral muscle fatigue were depressed under intense exhaustive exercise in the heat, could indicate possible alterations in the fatiguing process as a consequence of environmental heat stress.

The brain has no effective uric cycle, which makes removal of excessive  $NH_3$  dependent on glutamine synthesis from glutamate and  $NH_3$  (Suarez et al. 2002) and this may lower the cerebral levels of glutamate and also gamma-aminobutyric acid (GABA), since glutamate is precursor for the synthesis of GABA. Thus, excessive  $NH_3$  in the brain may create disturbances in neurotransmitter homeostasis, which results in cerebral dysfunction analogously to patients with liver failure (Butterworth et al. 1987). Supportive to this hypothesis exercise induced cerebral  $NH_3$  accumulation as well as changes in the levels of glutamate and GABA are observed in rats (Guezennec et al. 1998). In humans cerebral  $NH_3$  uptake has recently been demonstrated both during short-term maximal (Dalsgaard et al. 2004) as well as prolonged submaximal exercise (Nybo et al. 2005). The direct coupling between systemic  $NH_3$  infusion and central fatigue has not been investigated, but prolonged exercise will induce  $NH_3$  accumulation in the cerebrospinal fluid, suggesting that the capacity of the brain to clear  $NH_3$  becomes insufficient. In the present study submaximal intermittent exercise, which induced a higher degree of hyperammonemia in the hot trial, was completed prior to the sprints. This may have produced a continuous cerebral uptake of  $NH_3$ , which may have exhausted the ability of the brain to remove  $NH_3$  prior to the sprints. Thus, during the sprint test in the heat stressed state hyperammonemia may have hastened the development of central fatigue before the accumulation of peripheral fatigue factors was as large as during the control trial.

In summary, the present results demonstrate that circulating  $NH_3$  levels are higher during intermittent exercise performed under heat stress compared to a neutral thermal environment, and the heat stress induced hyperammonemia was not related to low muscle glycogen levels or increased adenine nucleotide degra-

ation. Thus, it may be suggested that factors like reduced hepatic blood flow or increased turnover of BCAA could in part explain the higher accumulation of  $NH_3$ . Repeated sprint performance was impaired during the heat stress trials, and this was not mediated by traditional peripheral fatiguing agents. Rather, the cause of fatigue during high intensity intermittent exercise in the heat may relate to central factors and hyperammonemia could be a component influencing the cerebral function.

**Acknowledgement** We would like to thank the subjects involved in the study and the technical assistance of Erik A. Richter, Ingelise Kring, Merethe Vannbye, Winnie Taagerup and Karina Olsen is highly appreciated. The study was supported by a grant from The British Royal Society and The Research Institute of Sport and Exercise Sciences.

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