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HEAT STRESS INCREASES AMMONIA ACCUMULATION DURING EXERCISE IN HUMANS

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SUMMARY

Seven men were studied during 40 min of exercise at 70% $\dot{V}_{O_2}$ peak, in an environmental chamber maintained at either 20 or 40 °C, to examine the effect of heat stress on ammonia metabolism during exercise. Heart rate and rectal and muscle temperatures were higher during exercise in the heat, while no differences were observed in pulmonary oxygen uptake or respiratory exchange ratio. Plasma ammonia levels and muscle ammonia accumulation were higher during exercise at 40 °C compared with 20 °C. Such metabolic alterations may be associated with reduced performance during exercise in the heat.

INTRODUCTION

Previous studies in humans have observed increased rates of muscle glycogenolysis (Fink, Costill & Van Handel, 1975) and muscle and blood lactate accumulation (Fink *et al.* 1975; Young, Sawka, Levine, Cadarette & Pandolf, 1985) during exercise in the heat. Similar results have been obtained in dogs who become hyperthermic during prolonged exercise (Kozlowski, Brzezinska, Kruk, Kaciuba-Uscilko, Greenleaf & Nazar, 1985). In the latter study, an increased muscle adenine nucleotide degradation, resulting in higher muscle ADP and AMP levels, was also observed during hyperthermic exercise. An increase in free ADP and AMP will activate AMP deaminase, resulting in the formation of inosine monophosphate (IMP) and ammonia, although these metabolites were not measured in the study by Kozlowski *et al.* (1985). To our knowledge, no studies have previously examined the effect of heat stress on ammonia metabolism during exercise in humans. Thus, the present study was undertaken.

METHODS

Seven healthy, active but untrained, male subjects, aged 23·6±1·9 years (mean±S.E.M.), with a mean body weight of 70·1±1·3 kg, agreed in writing to take part in this study after being informed of all risks and stresses associated with participation. The study was approved by the Victoria University of Technology Human Experimentation Committee. Peak pulmonary oxygen uptake ($\dot{V}_{O_2}$ peak), measured during cycling exercise to volitional fatigue, averaged 3·6±0·1 l min^{-1} (mean±S.E.M.). None of the subjects was specifically heat acclimated and the study was conducted between July and December, with the daily maximum temperature averaging 19·4±1·9 °C. Subjects reported to the laboratory in the morning after an overnight fast and having abstained from strenuous exercise, tobacco, alcohol and caffeine for at least 24 h. A catheter was positioned in a forearm vein for blood sampling and was kept patent by periodic flushing with 0·9% saline containing heparin (10 U ml^{-1}). Exercise was performed for 40 min on an electrically-

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braked bicycle ergometer, at a workload estimated to require 70% $\dot{V}_{O_2}$ peak (175±5 W), in an environmental chamber maintained at either 20 or 40 °C. Relative humidity was approximately 20% in both trials and there was no wind motion within the chamber. The order of the trials was randomized and they were conducted at least 1 week apart. A venous blood sample was obtained after 5–10 min of rest and after 10 and 40 min of exercise. Five millilitres of blood, placed in a lithium heparin tube, were spun and the plasma was frozen in liquid N_2 for subsequent ammonia determination using flow injection analysis (Svensson & Anfält, 1982). The coefficient of variation for repeated determinations in our laboratory is 5·7%. Immediately before and after exercise, muscle samples were obtained from the vastus lateralis using a percutaneous needle biopsy technique, with suction. These samples were immediately frozen in liquid N_2 for later ammonia analysis. Briefly, 5–12 mg of wet muscle were extracted as described by Katz, Broberg, Sahlin & Wahren, (1986). The sample was placed in a pre-cooled mixture of 0·6 M perchloric acid (PCA) and 30% methanol for 30 min at –20 °C. Ice-cold 0·6 M PCA was added and the sample placed on ice for 15 min, with frequent vortexing. After addition of ice-cold 1·8 M KOH, the sample was vortexed and spun at 50000 *g* for 2 min at 2 °C. The supernatant was analysed within 1 h of extraction by flow injection analysis (Katz *et al.* 1986). Within 10 s of obtaining the post-exercise muscle biopsy, muscle temperature was measured at a depth of 4 cm using a 25 g needle thermistor (YSI 524, Yellow Springs, OH, USA), inserted through the incision made for the muscle biopsy. Slightly more time was allowed for the measurement of the pre-exercise muscle temperature, but it was always measured within 30 s of the pre-exercise biopsy. Rectal temperature was monitored continuously during exercise by a thermistor (YSI 401, Yellow Springs, OH, USA) inserted to a depth of 10 cm beyond the anal sphincter. Expired gas samples were collected in Douglas bags at 10 min intervals during exercise. These samples were analysed for oxygen and carbon dioxide (Applied Electrochemistry S-3A and CD-3A analysers, Ametek, PA, USA) and the volume was measured using a Parkinson-Cowan gas meter, calibrated against a Tissot spirometer. Heart rate was monitored continuously by electrocardiography. No fluids were ingested during exercise in either trial. The data from the two trials were compared using analysis of variance for repeated measures, with significance accepted at the 0·05 level. Specific differences between means were located using the Student-Newman-Keuls *post hoc* test. All data are reported as means±S.E.M.

RESULTS

No differences were observed between the two trials in oxygen uptake and respiratory

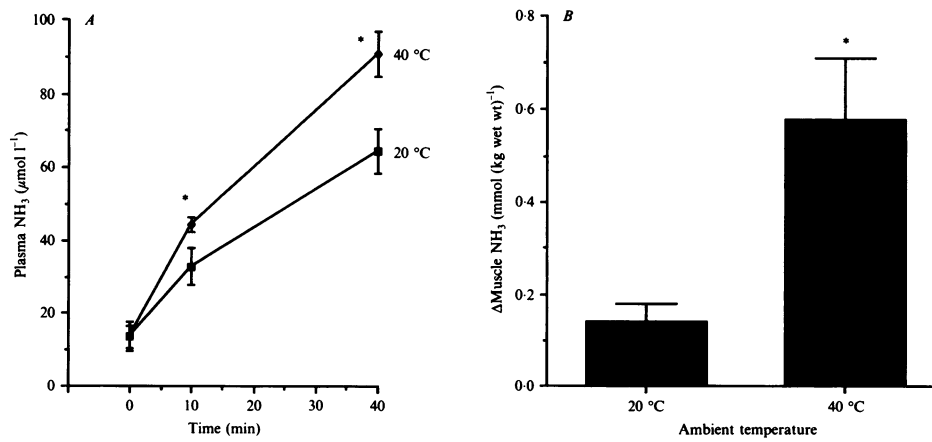

Fig. 1. *A*, plasma ammonia levels before and during exercise at 20 and 40 °C. *B*, increase (post *vs.* pre) in muscle ammonia during exercise at 20 and 40 °C. Values are means±S.E.M. (n = 7). * Significantly different from 20 °C value, $P < 0.05$.

exchange ratio, which averaged 2·42±0·10 and 2·47±0·12 l min^{-1} and 0·92±0·01 and 0·91±0·01 during exercise at 20 and 40 °C, respectively. In contrast, heart rate was higher ($P < 0·05$) throughout exercise in the heat, averaging 159±5 and 177±5 beats min^{-1} during exercise at 20 and 40 °C, respectively. No differences were observed between trials in either rectal or muscle temperature before exercise; however, 40 min of exercise at 40 °C resulted in higher ($P < 0·05$) rectal (38·7±0·1 *vs.* 38·1±0·2 °C) and muscle (39·5±0·2 *vs.* 38·3±0·2 °C) temperatures. No differences in resting plasma ammonia levels were observed between the two trials; however, plasma ammonia levels were higher ($P < 0·05$) during exercise at 40 °C (Fig. 1). Pre-exercise muscle ammonia levels were similar in both trials (0·48±0·10 mmol (kg wet wt)$^{-1}$. Muscle ammonia levels were higher ($P < 0·05$) after exercise at 40 °C (1·06±0·11) compared with 20 °C (0·62±0·09). The increase in muscle ammonia during exercise was higher ($P < 0·05$) in the hot environment (0·58±0·13 *vs.* 0·14±0·04 mmol (kg wet wt)$^{-1}$, Fig. 1).

DISCUSSION

The results of the present study indicate that heat stress increases plasma and muscle ammonia accumulation during exercise in humans (Fig. 1). The potential sources of ammonia production during exercise include AMP deamination and/or amino acid catabolism, although the relative importance of each of these mechanisms during submaximal exercise has not been clearly defined (Graham & MacLean, 1992). It is possible that amino acid catabolism could account for the higher plasma and muscle ammonia levels; however, it has been suggested that protein metabolism, as measured by urea excretion, is reduced during exercise in the heat (Dolny & Lemon, 1988). Increases in muscle adenine nucleotide degradation during contraction have been observed in humans (Edwards, Harris, Hultman, Kaijser, Koh & Nordesjö, 1972) and dogs (Kozlowski *et al.* 1985) when muscle temperature is elevated. The decrease in total adenine nucleotide concentration is likely to be due to activation of AMP deaminase, with production of IMP and ammonia. The increased muscle temperature following exercise at 40 °C in the present study, although relatively small ($\Delta = 1·2$ °C), may contribute, in part, to an increased ammonia production and the higher ammonia accumulation we have observed. Heat stress is also associated with increased adrenaline levels during exercise (Dolny & Lemon, 1988; Neilsen, Savard, Richter, Hargreaves & Saltin, 1990). Since adrenaline has been shown to increase IMP and ammonia levels in perfused rat muscle (Goodman & Lowenstein, 1977), an increase in circulating adrenaline, although not measured in the present study, could result in increased muscle and plasma ammonia. Finally, although it remains to be clearly established (Nielsen *et al.* 1990), a reduction in muscle blood flow during exercise in the heat may have influenced muscle ammonia metabolism. Reduced oxygen delivery could increase the reliance on anaerobic energy sources (Fink *et al.* 1975; Young *et al.* 1985), including the adenine nucleotides (Sahlin & Katz, 1989), resulting in increased ammonia production. Furthermore, a reduction in muscle blood flow may reduce the removal of ammonia from muscle, thereby resulting in increased intramuscular ammonia accumulation. While the most likely explanation for the increased plasma ammonia levels is increased ammonia production by, and release from, contracting skeletal muscle, decreased ammonia clearance is another possibility. Vasoconstriction in the splanchnic region and inactive muscle will occur during exercise

in the heat, although splanchnic ammonia uptake is not altered during exercise (Eriksson, Broberg, Bjorkman & Wahren, 1985).

In summary, we have observed higher plasma and muscle ammonia levels following exercise at 40 °C, compared with 20 °C, in untrained men. Such metabolic alterations could reflect increased muscle levels of free ADP and AMP, which may be associated with reduced performance during exercise in the heat.

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