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Hyperammonaemia in relation to high-intensity exercise duration in man

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Abstract. Adenine nucleotide (AN) degradation has been shown to occur during intense exercise in man and in the horse, at or close to the point of fatigue. The aim of the study was to compare plasma ammonia concentration ([NH_3]) as a result of intense exercise with plasma [lactate]. Plasma glutamine concentration ([Gln]) was also measured pre- and post-exercise. On separate occasions, nine healthy subjects (two females) exercised on a motorised treadmill for periods of between 30 s and 210 s, at 5.6 $m \cdot s^{-1}$ (0% incline). On one occasion, running at the same speed, two subjects ran at +4% incline whilst one other subject ran at +7% incline. Blood samples were taken and plasma was analysed for [lactate], [NH_3] and [Gln]. Subjects showed varying degrees of AN degradation as indicated by plasma [NH_3]. A comparison of plasma [NH_3] with that of plasma [lactate] indicated a marked increase in AN degradation, corresponding to a [lactate] of around 14 $mmol \cdot l^{-1}$ in plasma. The data further support the hypothesis that there is a critical intramuscular pH below which there is a stimulus to AN degradation during intense exercise, possibly as a result of a substantial reduction in the kinetics of adenosine diphosphate (ADP) rephosphorylation provided by phosphocreatine, resulting in an increase in [ADP].

Key words: Adenine nucleotide – Ammonia – Purine nucleotide catabolism – Human – Exercise

Introduction

Intense exercise in both man and the horse is frequently associated with a decrease in the muscle adenine nucleotide (AN) content, which is manifest mainly as a fall in adenosine triphosphate concentration ([ATP]). AN degradation occurs as a result of deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP), with release of ammona (NH_3). Decreases of ATP of the order of 50% have been observed in the horse after repeated gallops or treadmill exercise (Snow et al. 1985; Harris et al. 1987, 1991; Sewell and Harris 1992) and as a result of flat racing over distances ranging from 1400 m to 2400 m (Sewell et al. 1992). Decrease in [ATP] in these studies was stoichiometrically matched by the change in muscle [IMP]. Stoichiometric changes in muscle [ATP] and [IMP] (and also in [NH_3]) have previously been reported in stimulated rat muscle and in human muscle after brief intense exercise (Meyer and Terjung 1979, 1980; Sahlin et al. 1978). Reported losses of muscle ATP in the exercising thoroughbred horse have generally been greater than have been found in dynamic exercise studies of man (Sahlin and Katz 1988).

Previous work in the horse investigated muscle ATP loss as a result of increasing exercise intensity (Harris et al. 1991). Recent work in the thoroughbred horse (Sewell and Harris 1992) suggests that the degradation of AN during intense exercise in the horse does not begin until there is an accumulation of adenosine diphoshate (ADP; as indicated by muscle [IMP]) and a significant degree of intracellular acidosis (as indicated by muscle and blood [lactate]). Exercise intensities which result in exhaustion at around 150 s result in significant hyperammonaemia. The present study was undertaken in order to compare the changes in plasma [NH_3] with plasma [lactate] as a result of high-intensity treadmill exercise, for durations up to and including exhaustion, in man.

Methods

Subjects. Nine healthy, recreationally active but not highly trained subjects (two female) volunteered to take part in the study, which was approved by the Coventry Research Ethics Committee. Physical characteristics of each subject are shown in Table 1. Subjects had abstained from strenuous activity and drinking alcohol for 24 h before each session, as alcohol consumption can cause purine nucleotide degradation (Faller and Fox 1982). No food was taken for 3 h prior to each exercise sessions.

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Table 1. Physical characteristics of subjects

Subject	Age (years)	Sex	Body mass (kg)	Height (m)	Maximum exercise duration at 20 $km \cdot h^{-1}$ (s; 0% incline unless stated)
A	21	M	82	1.82	150
B	22	M	78	1.85	210 (and 79 at 7% incline)
C	36	M	64	1.72	55
D	47	M	67	1.66	133
E	19	M	76	1.80	150 (and 120 at 4% incline)
F	30	M	80	1.73	150
G	31	M	65	1.73	150 (and 93 at 4% incline)
Mean (SD)	29 (10)		73 (8)	1.77 (0.07)	
H	19	F	66	1.70	88
J	19	F	56	1.63	90
Mean	19		61	1.67	

Exercise protocol. At least 2 weeks prior to commencing the experiment, subjects were familiarised with the laboratory environment, treadmill and running at the test speed. On separate occasions subjects reported to the laboratory and were rested (sitting) for 5 min before a pre-exercise blood sample was taken by venepuncture from an antecubital vein. Shortly after, subjects exercised at 5.6 $m \cdot s^{-1}$ (20 $km \cdot h^{-1}$) on a level motorised treadmill (Sport Engineering, UK) for as many test durations as possible. Test durations were designed to be 30, 60, 90, 120 and 150 s or to exhaustion. One subject completed 30 and 55 s, one subject 30–88 s, one subject 30–90 s, one subject 30–133 s and four subjects 30–150 s. One subject found the protocol relatively easy and was exercised from 90 to 210 s. Three of the subjects who were able to complete at least 150 s took part in a supplementary session. Two were exercised at a 4% incline, one at a 7% incline. Exercise sessions were separated by 1 week and the order of the test durations was randomised. A warm-up consisting of 1 min at 2.8 $m \cdot s^{-1}$, 3 min at 3.3 $m \cdot s^{-1}$, 1 min standing/stretching rest and 1 min at 2.8 $m \cdot s^{-1}$ preceded the exercise. After each test duration was completed the treadmill was stopped, the subject walked to a chair and was seated, whereupon immediate post-exercise and 5-min post-exercise blood samples were taken from an antecubital vein. Environmental conditions were maintained at 15–20° C and 55–70% relative humidity.

A test speed of at least 5.6 $m \cdot s^{-1}$ was chosen for this study in order to elicit a time to maximal effort for most subjects of between 60 and 180 s. Also, at this speed, subjects have begun to release lactate from the working muscles, even with the shortest duration of exercise (30 s) undertaken. Heart rates were monitored and recorded using a telemetric heart rate system (Sport Tester PE4000, Polar Electro, Finland; chest belt transmitter and wrist watch receiver) averaging heart rate over 5-s intervals. Information was subsequently downloaded using an interface to a standard personal computer and analysed using commercially available software.

Blood sampling and analysis. Ten millilitres of venous blood was taken by venepuncture at rest prior to exercise, immediately post-exercise and at 5 min post-exercise. At the exercise intensities used in this study, peaks in blood [lactate] and plasma [NH_3] occur around 5 min into recovery (Itoh and Ohkuwa 1990; Harris et al. 1991). One aliquot of blood was placed into a 2.5 ml "fluoride oxalate" tube (for subsequent plasma lactate analysis) and another into a 5.0 ml lithium heparin tube [for NH_3 and glutamine (Gln) analysis]. Both samples were placed on ice and centrifuged within 30 min of sampling. Plasma was removed and immediately frozen in liquid nitrogen, particularly to prevent significant increases in NH_3 concentration (Fonseca-Wollheim 1990).

Plasma lactate was analysed enzymatically using lactate dehydrogenase on perchloric acid extracts. Analysis of plasma NH_3 concentration (sum of ammonia and ammonium ions) was carried out within 24 h of sampling using a micro method of a commercially available kit (Sigma, Poole, UK). Plasma glutamate was determined by a microfluorimetric enzymatic method involving measurement of reduced nicotinamide-adenine dinucleotide formed by oxidative deamination of glutamate by glutamate dehydrogenase (bovine liver; Boehringer Mannheim, Lewes, UK). Plasma Gln was hydrolysed by the enzyme glutaminase (*E. coli*; Sigma, Poole, UK) to glutamate, and the glutamate measured as described above.

Statistics. Values in tables and text refer to mean (SD). Linear regression analysis, when used, has been based on a simple model describing a straight line: $y = a + bx$. The significance of any changes observed was assessed using Student's *t*-test for paired data where appropriate. The accepted level of significance was $P < 0.05$.

Results

Blood lactate and ammonia responses

The rise in plasma [NH_3] in relation to increasing exercise duration can be seen in Fig. 1. Three subjects (C, H and J) appeared to have a lower exercise capacity to the rest of the subjects and showed a larger rise in both 5-min post-exercise plasma NH_3 (Fig. 1) and plasma lactate (Fig. 2) in relation to exercise duration. In contrast, subject B had a higher exercise capacity to the rest of the subjects and was capable of running for at least 210 s. Increases in plasma [NH_3] to above 150 $\mu mol \cdot l^{-1}$ coincided with exhaustion in subjects B, C, E, H and J. Two subjects (A and F) ran for durations up to and including 150 s without plasma [NH_3] rising above 105 $\mu mol \cdot l^{-1}$ and were not tested further; however, two subjects (D and G) exercised to exhaustion without plasma [NH_3] rising above 100 $\mu mol \cdot l^{-1}$.

The relationship between plasma [NH_3] and plasma [lactate] (Fig. 3) is similar to that which might be expected to be seen between muscle [IMP] and muscle [lactate]. Regression analysis of values where plasma [lactate] exceeded 13 $mmol \cdot l^{-1}$ ($n = 10$) demonstrated

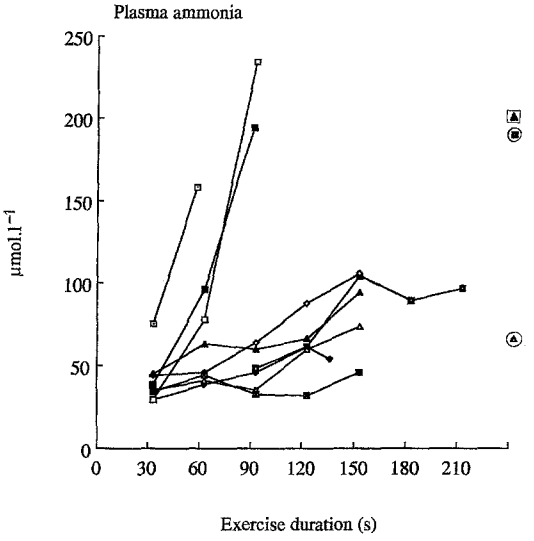

Fig. 1. Concentration of plasma ammonia (NH_3) of samples taken 5 min after treadmill exercise of 30–210 s duration at a velocity of 5.6 $m \cdot s^{-1}$. Data points inside a circle or square indicate exercise to exhaustion at a 4% or 7% incline, respectively. —⊡—, C; —◆—, D; —■—, B; —◇—, A; —■—, H; —□—, J; —▲—, E; —△—, G; —⊠—, F

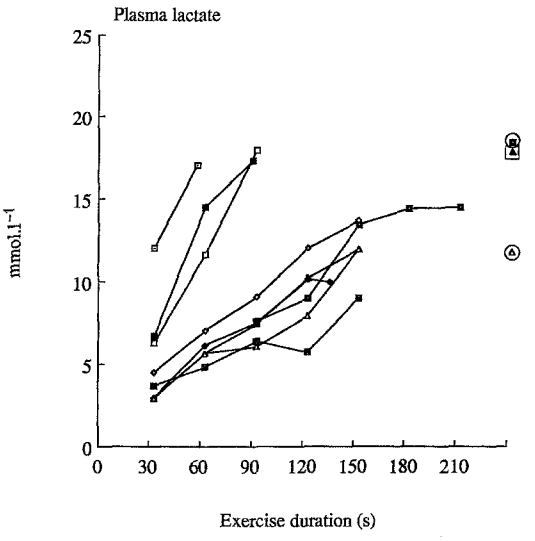

Fig. 2. Concentration of plasma lactate of samples taken 5 min after treadmill exercise of 30–210 s duration at a velocity of 5.6 $m \cdot s^{-1}$. See Fig. 1 legend for key to subject plot symbols

a linear increase in $[NH_3]$ above this concentration ($r=0.93$, $P<0.001$; Fig. 3). These data are consistent with a threshold of AN degradation corresponding to the accumulation in plasma of approximately 14 mmol lactate $\cdot l^{-1}$.

Plasma glutamine responses

Mean plasma [Gln] was higher immediately post-exercise as a consequence of running at 5.6 $m \cdot s^{-1}$ (see Table 2). The change from rest was weakly significant for exercise durations of 60 and 90 s and as a result of exhaustive exercise durations and inclines.

Discussion

The present study sought to examine exercise hyperammonaemia using an exercise model in which the rate of dynamic muscle contraction remained fairly constant and the intensity of exercise was such that a high proportion of type II muscle fibres was recruited. Of the five subjects who were able to complete 150 s of the original protocol, four felt that this was the maximum or very close to the maximum they could achieve. Three subjects volunteered to undertake one further test of greater metabolic severity, at the same velocity but at an intensity (using an incline) which was estimated would exhaust subjects in less than 150 s.

AN degradation, as indicated by plasma NH_3 appearance, varied considerably between subjects with exercise duration. Plasma NH_3 concentrations greater than 100 $\mu mol \cdot l^{-1}$ were seen in three subjects (C, H, J) at or near to the point of fatigue as a result of the

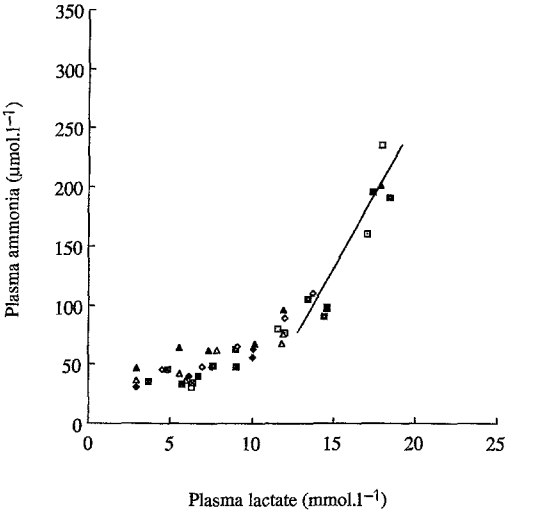

Fig. 3. Comparison of concentration of plasma ammonia $[NH_3]$ with the concentration of plasma lactate in samples taken 5 min after exercise of 30–210 s duration at a velocity of 5.6 $m \cdot s^{-1}$. See Fig. 1 for key to subject plot symbols. For data with a plasma [lactate] > 13 mmol $\cdot l^{-1}$, $n=10$. $[NH_3]$ ($\mu mol \cdot l^{-1}$) = −257 + 25.7 [plasma lactate] (mmol $\cdot l^{-1}$). $r=0.93$; $P<0.001$

original protocol. One subject was easily capable of sustaining the original exercise protocol for longer than 150 s. By using an incline to make the exercise more intense it was possible to induce a similar plasma NH_3 response in this and one other subject at the point of volitional exhaustion but not in the third subject.

Plasma lactate responses to increasing exercise duration also demonstrated great variation. Three subjects (C, H, J) who were unable to exercise at the required intensity for longer than 90 s can be clearly distinguished from the rest of the subjects (Fig. 2). Despite the considerable difference in NH_3, lactate and exercise duration responses between individual sub-

Table 2. Plasma glutamine ($mmol \cdot l^{-1}$) in subjects as a result of running for different durations on a flat treadmill at a speed of 20 $km \cdot h^{-1}$ (except[a]where three subjects exercised at an incline)

	30 s	60 s	90 s	120 s	150 s	[a]Exhaustion
Pre-exercise	677 (96)	668 (49)	651 (102)	656 (88)	613 (134)	662 (130)
		*	*			*
Immediately post-exercise	716 (142)	703 (62)	704 (111)	678 (101)	832 (123)	757 (142)
5 min post-exercise	657 (118)	629 (74)	609 (129)	625 (70)	635 (106)	687 (124)
n	8	9	7	6	2	9
Immediately post-exercise compared with pre-exercise	NS 39 (62)	$P<0.05$ 35 (42)	$P<0.05$ 53 (51)	NS 22 (113)	NS 219 (208)	$P<0.05$ 95 (121)
5 min post-exercise compared with pre-exercise	NS −20 (53)	NS −39 (68)	NS −42 (76)	NS −31 (70)	NS 22 (130)	NS 25 (126)

Values given as mean (SD) NS = no significant difference
* Significantly different from pre-exercise value ($P<0.05$)

jects, the relationship between plasma NH_3 and lactate (Fig. 3) appears to follow a distinct pattern. In previous studies using electrical stimulation in man (Harris and Hultman 1985) and using the thoroughbred horse (Sewell and Harris 1992), the existence of a threshold for AN loss in relation to lactate increase was suggested. A curvilinear upturn in the accumulation of plasma NH_3 above a plasma [lactate] of approximately 14 $mmol \cdot l^{-1}$ in the present study provides further evidence to suggest a sharp increase in the activity of AMP-deaminase.

Mechanisms which have previously been proposed to account for AN degradation in exercising muscle include pH activation of AMP deaminase, a lowering of the K_m of AMP-deaminase for AMP and an increase in the availability of the substrate AMP. These mechanisms have been discussed previously (Sewell and Harris 1992).

Increases in muscle [AMP] occurring above a plasma [lactate] of 14 $mmol \cdot l^{-1}$ will in turn reflect much greater increases in muscle [ADP]. Whilst some increase in [ADP] will inevitably occur with the start of ATP hydrolysis during contraction, it is likely that this initially remains below the critical threshold for NH_3 formation. For combinations of exercise intensity and duration that result in postexercise blood [lactate] in excess of 14 $mmol \cdot l^{-1}$, the kinetics of ADP removal appear to undergo a radical change, resulting in its accumulation.

The association between AN degradation and lactate accumulation suggests an explanation based on a reduction in the effectiveness of phosphocreatine (PCr) to buffer the rise in [ADP] (for recent discussion see Sewell and Harris 1992). One question is whether there is a critical pH below which there is a substantial reduction in the buffering provided by PCr sufficient to account for a marked increase in [ADP]. Reduced muscle IMP accumulation during exercise as a result of induced metabolic alkalosis has been demonstrated in the horse (Greenhaff et al. 1991) supporting the concept that there is a pH effect on AN degradation as a result of intense exercise. Increasing substrate availability (e.g. by elevating muscle total creatine content) would also appear to reduce AN degradation (Balsom et al. 1993).

The present results and previous studies in the horse (Harris et al. 1991; Sewell and Harris 1992) differ from data of Buono et al. (1984) in humans which demonstrated a linear correlation between plasma [NH_3] and [lactate]. However, most early studies in humans were based on exercise of relatively low intensity (Wilkerson et al. 1977; Buono et al. 1984; Eriksson et al. 1985) where the degradation of AN was probably much less than in the present study and the contribution of NH_3 from amino acid catabolism much greater. In a recent human study, a linear relationship between blood [NH_3] and [lactate] was demonstrated as a result of subjects performing cycle ergometer exercise of submaximal, maximal and supramaximal intensity (Itoh and Ohkuwa 1990). This exercise protocol did not, however, result in blood [lactate] exceeding 14 $mmol \cdot l^{-1}$, which is below the threshold implicated by the present results. In a further study of Itoh and Ohkuwa (1991), the relationship between blood [NH_3] and [lactate] was determined as a result of short-term cycle ergometer sprint exercise. Linear relationships were noted as a result of maximal cycle sprint exercise of three different durations (15 s, 30 s, and 45 s). In contrast to our results, blood NH_3 concentrations of greater than 150 $\mu mol \cdot l^{-1}$ were achieved below the lactate threshold we suggest. This may arise because muscle contraction frequency is not constant during maximal sprints and the fibre recruitment pattern of the muscles used during each exercise duration will be different. In such exercise there may be a greater dependence upon PCr and less upon glycolysis compared with the model used in the present study. Intracellular [AMP] will be raised resulting in AN degradation but with relatively less lactate accumulation.

Ammonia produced in skeletal muscle is rapidly lost into the circulation. Plasma [NH_3] therefore represents a balance between its production by skeletal mus-

cle and its uptake mainly by skeletal muscle and liver (Dudley et al. 1983). Although de novo formation of Gln from glutamate and NH_3 could offset the accumulation of free NH_3 and allow the transport of NH_3 to the kidneys in the form of Gln, it is generally accepted that an increase in plasma [Gln] [as seen in the present and in previous studies (Babij et al. 1983; Katz et al. 1986)] is due to Gln release from muscle. In the present study, after the longer exercise durations, plasma [Gln] increased immediately post-exercise, but unlike [lactate] did not continue to rise in the following 5-min recovery period. This would suggest that there is little de novo synthesis of Gln and subsequent release in the recovery period.

In addition of NH_3 from AN degradation, in endurance exercise there is a contribution also as a consequence of the metabolism of amino acids (Broberg and Sahlin 1989). In such exercise, plasma [NH_3] is independent of lactate metabolism. As a result of high intensity exercise, plasma [NH_3] appears to bear a relationship with [lactate] (Babij et al. 1983; Banister et al. 1983). Also, the close association between plasma [NH_3] and muscle [IMP] in short-term, high-intensity exercise suggests that AN degradation is the primary source of the NH_3 (Harris et al. 1991; Sewell and Harris 1992). In the present study, NH_3 appearance as a result of amino acid catabolism was probably very small.

The present data are consistent with the existence of a sharp increase in the rate of AN degradation, in which falling intracellular pH may act as the major stimulus by slowing the rate of ADP removal. The concomitant alteration in cellular ATP/ADP contents may occur as a result of the inability to maintain adequate PCr concentrations. AN degradation provides a mechanism for displacement of the myokinase reaction and maintenance of a high ATP/ADP ratio. The ability to lose AN in this way may be essential for maintaining the contraction process and delaying the onset of fatigue.

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