Purine transport and metabolism in man: the effect of exercise on concentrations of purine bases, nucleosides and nucleotides in plasma, urine, leucocytes and erythrocytes

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Summary
1. After decreasing muscle ATP by a 2 min period of intense exercise, we have studied purine metabolism by using high-pressure liquid chromatography.
2. A major increase in hypoxanthine concentration in plasma and urine was found with increases in xanthine and, in plasma, inosine. Erythrocyte hypoxanthine rose with the level in plasma, but there was no corresponding rise in IMP, the first intracellular metabolite of hypoxanthine. No rises in uridine or urate were found in plasma.
3. Plasma adenosine did not rise and fall significantly after exercise, but a small rise and fall in adenine nucleotide concentrations in plasma was found.
4. Running, swimming and games, which tended to be at the weekend, were associated with a rise in hypoxanthine and xanthine excretion; exercise was probably the cause of the higher excretion during the day than at night. Such activities do not produce changes in concentrations of ATP in muscle, although turnover must rise.
5. The results are consistent with widespread purine exchange between tissues and a ‘circulating hypoxanthine pool’.

Key words: erythrocytes, exercise, leucocytes, metabolism, plasma, purines, transport, urine.

Introduction
A decrease in the energy currency of cells, ATP, is a central feature of the metabolic damage following severe hypoxia and/or ischaemia; this process is a feature of many diseases. Since serial tissue samples are rarely available from man, it is necessary to monitor the intracellular concentrations of ATP indirectly by the release of the principal extracellular metabolite, hypoxanthine \[1\], in order to study ATP breakdown in man.

The metabolic damage caused by hypoxia can be quantitatively related to the output of hypoxanthine \[2\]. The duration and extent of hypoxanthine release after actively decreasing muscle ATP by severe exercise has been studied in seven male volunteers, in order to interpret changes found in pathological hypoxia. Such studies can be based mainly on the fall in ATP concentrations in muscle produced by severe exercise \[3, 4\] and on exercise physiology \[5–8\].

More extensive studies of purine metabolism are now possible, owing to the development of specific, sensitive and precise methods of analysis based on high-pressure liquid chromatography \[9\]. We demonstrate the effects of a 2 min period of severe exercise in producing a ‘pulse’ of hypoxanthine and significant changes in xanthine and inosine. It has also been possible to determine renal excretion and concentrations of hypoxanthine and its metabolite inosine monophosphate (IMP) in the erythrocyte. Our results suggest that the widely used phrase purine
transport should perhaps be modified to purine exchange with a 'circulating hypoxanthine pool'.

Methods

Physiological

The duration and extent of exercise on a bicycle ergometer (Monark Crescent AB, Varberg, Sweden) was adjusted by preliminary tests so that the seven untrained male volunteers (25–49 years) were at or near their limits of endurance at the end of a standard period of work. This was also similar to conditions used by Sutton et al. [4]. At 50 rev./min, equivalent to 18 km/h, resistance was set at 2.5 kp for 1 min, followed by 5.0 kp for another 1 min (the kilopond, kp, is the force acting on a mass of 1 kg at normal acceleration of gravity; conversions into other units are listed in [10]). After this period of work the mean (±sd) pulse rate was 150 ± 13 (n = 6), excluding the 'heavily built' subject 4, whose pulse rate was 120/min and who showed other evidence of much less 'exhaustion'. Maximal oxygen uptake can be derived from work load and heart rate by published tables [lo].

Urine was collected from waking about 07.00 until 11.30 hours when the 2 min exercise was performed. Thereafter hourly urine collections were made until 16.30 or 17.30 hours. The evening collections were finished just before retiring to bed about 23.00 hours. Fluid intakes of 250–500 ml/h from about 11.00 until 16.30 hours ensured urine volumes adequate to minimize the effect of any urinary tract dead space.

In four subjects serial samples of venous blood were taken generally by an indwelling needle maintained open by heparinized 0.9% (w/v) sodium chloride (saline). EDTA was used as an anticoagulant for the plasma sample, since it also inhibits ATP breakdown. No samples showed visible haemolysis, which was therefore less than about 0.5%. In another series of plasma samples from which larger volumes were available, mean (±sd) haemolysis was estimated as 0.1 ± 0.035% (n = 9) from the absorption at 410 nm by using a methaemoglobin standard. Blood was immediately centrifuged to separate erythrocytes and plasma. Polymorphonuclear neutrophil leucocytes (PMN) and lymphocytes were separated from a heparinized sample of blood by sedimentation in a dextran–Ficoll based method as outlined below.

Venous blood (5 ml) was placed into a tube containing equivalent to 50 units of heparin/ml.

Erythrocytes were sedimented with 1% Dextran 500 (Sigma), and the neutrophils and lymphocytes separated from the leucocyte-rich plasma supernatant by density centrifugation [11], freed of residual erythrocytes by hypotonic lysis and resuspended in a known volume of iso-osmotic saline for cell counting. The cell suspensions were then centrifuged and the pellets extracted with 14% (w/v) trichloroacetic acid for analyses by high-pressure liquid chromatography.

In order to study the effects of the exercise involved in a working week on urinary oxypurine excretion, separate day and night collections were made for one week by five subjects, three men and two women, aged 25–49 years. Detailed diaries of daily activities and exercise were kept.

Analytical

Trichloroacetic acid extracts of plasma and cells 'back extracted' with water-saturated diethyl ether were prepared for analysis [9]. Hypoxanthine and xanthine were extracted from urine by ion exchange chromatography on Zerolit 225 (BDH, Poole, U.K.). Nucleosides in trichloroacetic acid extracts of plasma and in unconcentrated urine were quantitatively recovered by the method of Davis et al. [12], by using a boronate affinity column. Nucleoside solutions derived from plasma were concentrated tenfold by freeze-drying before analysis by high-pressure liquid chromatography.

Purine bases, uridine, nucleosides and nucleotides in extracts were determined by reversed-phase high-pressure liquid chromatography by using the methods of Simmonds & Harkness [9] or modifications thereof. IMP in cellular extracts was resolved from the major components ATP, ADP and AMP by using the same reversed-phase columns previously reported (ODS-Hypersil; Shandon Ltd, U.K.), and a mobile phase (pH 6.0) of KH,PO₄ (0.001 mol/l) and tetrabutylammonium hydroxide (0.0015 mol/l) with 20% (v/v) methanol (temperature 50°C). Adenosine in plasma and urine was estimated by using a similar column with a 3 μm particle size and a linear 20 min gradient of 1–20% (v/v) methanol in KH,PO₄ (0.004 mol/l), pH 6.0 at 30°C. Protein was determined with the Folin–phenol reagent [13] and urinary creatinine with the Sigma (London, U.K.) kit.

The trends in plasma concentrations with time were studied after logarithmic transformation of the data by fitting linear regressions to the data from the peaks at 10–15 min to about 3 h after exercise and calculating the correlation coefficient of concentrations with time.
Results

Blood

The effects of short but intense exercise on plasma and erythrocyte concentrations of purines, nucleosides and nucleotides are shown in Figs. 1–4 and quantitatively compared in Table 1. In plasma the concentrations of hypoxanthine, xanthine and inosine were raised, whereas those of adenosine, uridine and urate were not altered significantly. The most marked increase was in the concentration of hypoxanthine in plasma; this rose to peak values about 10 times resting values about 10–15 min after exercise (Fig. 1).

Concentrations of hypoxanthine in erythrocytes were also raised about 2–4-fold (Fig. 2). Similar peak concentrations were obtained by repeating the exercise in two individuals (open and closed symbols, Figs. 1 and 2). In contrast, the concentrations of the metabolic product of hypoxanthine in erythrocytes, IMP, were unaltered after exercise (Table 1). No systematic alteration in the concentrations of the major intracellular nucleotides ATP, ADP and AMP were detected in erythrocytes.

After exercise there was a significant fall to control values for plasma concentrations of inosine (Fig. 3) and xanthine (Table 1), although both trends were less marked than those for hypoxanthine. There was no significant trend with time in adenosine concentrations in plasma (Table 1), but there may have been a slight early rise. Control or resting concentrations were also low, about 0·05–0·1 μmol/l.

There were no definite precursor–product relationships in the concentrations in plasma (Figs. 1–4), despite the close metabolic relationships of the compounds studied. Overall concentrations were comparable except for subject 4, who showed a smaller rise in hypoxanthine after exercise; this was consistent with his lower pulse rate, 120/min, at the end of exercise. The regression for hypoxanthine in Figs. 1 and 2 and Table 1 therefore do not include data from subject 4, which are, however, shown in these Figures.

<table>
<thead>
<tr>
<th>Table 1. Alterations of ATP and metabolites in plasma and erythrocytes after severe exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-pressure-liquid-chromatographic methods used are described in the text. The intercept of the regression line at zero time is an estimate of the mean maximum rise in concentration produced by exercise. Correlation coefficients were calculated from log-transformed data after the 'peak' concentration at 10–15 min and before a return to 'resting' values at 150–180 min after exercise.</td>
</tr>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Plasma hypoxanthine</td>
</tr>
<tr>
<td>Erythrocyte hypoxanthine</td>
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<tr>
<td>Plasma xanthine</td>
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<tr>
<td>Plasma inosine</td>
</tr>
<tr>
<td>Plasma adenosine</td>
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<tr>
<td>Plasma ATP + ADP + AMP</td>
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<tr>
<td>Erythrocyte IMP</td>
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</tbody>
</table>
After exercise increases in cytoplasmic enzymes in plasma [4] and in ATP have been shown [14]. Adenine nucleotides were therefore measured in plasma. Concentrations were surprisingly high, about 15 μmol/l (Fig. 3), especially relative to adenosine and inosine (Fig. 4), about 50 nmol/l. Resting mean (±SEM) plasma concentrations of ATP, ADP and AMP were 10·9 (±4·4), 4·9 (±1·5), 1·2 (±0·13) μmol/l respectively in four normal men. The mean (±SEM) energy charge in plasma was 0·74 (±0·03), which is similar to but lower than the value in active cells. In plasma the energy charge showed no systematic variation with time after exercise, so total adenine nucleotide concentrations after exercise are shown in Fig. 5. There was a significant negative linear correlation with time (P < 0·05).

Urate, the product of the catabolism of hypoxanthine and xanthine, did not increase after this short period of exercise; mean (±SD) plasma concentrations before and after exercise were 148 (±36) and 134 (±15) μmol/l respectively. More prolonged exercise is known to increase urate concentrations [4]. Uridine was studied because this pyrimidine nucleoside occupies a similar central position in pyrimidine metabolism to that of the purine base hypoxanthine in purine metabolism. In addition, plasma and amniotic-fluid concentrations of uridine increase after ATP breakdown in infants during intrapartum hypoxia [15, 16]. Uridine concentrations in plasma were also unaltered by the short period of intense exercise; mean (±SD) concentrations before and after exercise were 4·6 (±1·3) and 4·4 (±1·3) μmol/l.

Urine

The 20-fold increase in excretion of hypoxanthine and a limited 2-fold increase in xanthine excretion after exercise is shown in Table 2. Creatinine excretion was unaltered by exercise. Concentrations of hypoxanthine relative to creatinine therefore increased (Table 2). After the first hour hypoxanthine excretion fell to baseline values in the fourth and subsequent hours after exercise. Xanthine excretion rose in the first and in the second hour after exercise, falling in the third hour to return to baseline values in the fourth and subsequent hours. There is therefore a precursor–product relationship between hypoxanthine and xanthine in urine after exercise.

The mean morning excretion of hypoxanthine and xanthine was slightly higher than late-afternoon and evening values, but all are similar to
TABLE 2. Increased excretion of hypoxanthine and xanthine after severe exercise by seven normal untrained males

Methods used are described in the text. All results are corrected for body weight. In early studies 'evening' urine collections were divided but showed no changes, and therefore ten samples were grouped together.

<table>
<thead>
<tr>
<th>Time after exercise (h)</th>
<th>Morning</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxanthine excretion rate (nmol/h per kg)</td>
<td>53-4</td>
<td>1105</td>
<td>544</td>
<td>134</td>
<td>46</td>
<td>46</td>
<td>33-9</td>
</tr>
<tr>
<td>Mean</td>
<td>5-6</td>
<td>226</td>
<td>60</td>
<td>22</td>
<td>6-7</td>
<td>9-4</td>
<td>3-2</td>
</tr>
<tr>
<td>Xanthine excretion rate (nmol/h per kg)</td>
<td>31-2</td>
<td>43-7</td>
<td>61-8</td>
<td>43-4</td>
<td>27-9</td>
<td>34-4</td>
<td>22-3</td>
</tr>
<tr>
<td>Mean</td>
<td>5-4</td>
<td>6-3</td>
<td>14</td>
<td>1-9</td>
<td>6-1</td>
<td>4-8</td>
<td>2-1</td>
</tr>
<tr>
<td>Hypoxanthine (μmol/l)/creatinine (mmol/l) ratio</td>
<td>6-2</td>
<td>101</td>
<td>50-7</td>
<td>13-8</td>
<td>4-6</td>
<td>4-0</td>
<td>4-1</td>
</tr>
<tr>
<td>Mean</td>
<td>0-9</td>
<td>11</td>
<td>3-5</td>
<td>2-3</td>
<td>0-8</td>
<td>0-6</td>
<td>0-7</td>
</tr>
</tbody>
</table>

![Fig. 6. Hypoxanthine excretion for a week by an adult female, 1B (wife of 1A). The stippled bars show excretion at night. SW indicates swimming.](image)

other values obtained over a working day and are consistent with our findings (Figs. 5 and 6) that moderate exercise is associated with small changes in the excretion of hypoxanthine and xanthine.

The effect of everyday work and exercise on hypoxanthine and xanthine excretion was studied in three men and two women continuously for one week. The excretion of hypoxanthine was significantly higher during the day than at night (sign test \( P < 0.002 \)). Xanthine excretion was also greater during the day (sign test \( P < 0.011 \)). The day–night difference was not an inherent rhythm, since the difference could be abolished by taking a 3 km bicycle ride or other exercise during the night in another series of similar studies. The effect of various types of exercise, running, squash, badminton and swimming was also to increase hypoxanthine excretion (e.g. Figs. 5 and 6) (copies of the data from the other three subjects can be obtained on request to R. A. Harkness). Excretion by the three men during the day was about 40-80 nmol/h per kg, increasing within this range with the amount of bench work. Running produced the highest excretion rates, 160–170 nmol/h per kg. Excretion by the two women during the day varied from about 20 to 50 nmol/h per kg, with the maximum rise on swimming shown in Fig. 6. The exercise effect was more marked in the three men and was concentrated at the week-ends. The effect of some bed rest during the day is shown in Fig. 5 by the decreased hypoxanthine excretion during an influenza-like illness.

Leucocytes

Initially, leucocytes were studied because they can take up preformed purines [17]. Surprisingly, there was a fall in polymorphonuclear leucocytes and lymphocytes in 11 of 12 pairs of ATP, ADP and AMP concentrations (sign test, \( P = 0.003 \)) after exercise. The pattern of fall and rise was consistent and has therefore been simplified as a series of means ± SEM in Fig. 7. The relative concentrations of these adenine nucleotides, expressed as the energy charge, (([ATP] + \( \frac{1}{2} \) [ADP])/([ATP] + [ADP] + [AMP]), was relatively constant at all times after exercise, ranging from 0.815 to 0.893 in the time intervals shown in Fig. 7 (mean ± SD 0.847 ± 0.028 for the six time intervals). These consistent energy charges suggest that sample preparation was consistent. In view of the consistency of relative adenine nucleotide concentrations it was justifiable to use total concentrations in Fig. 7.

In order to distinguish a disturbance caused by a pulse of hypoxanthine, the concentration of this compound was measured in leucocytes. There were no consistent changes in leucocyte hypoxanthine concentrations, which ranged from 0.3 to 8.8 (mean 2.8) μmol/l cell volume (n = 14). Guanine, the related base and co-substrate for the recycling enzyme hypoxanthine/guanine phosphoribosyltransferase, was present in somewhat higher concentrations, ranging from 0.4 to
Purine salvage

There is a variety of evidence consistent with extensive recycling [1, 22–24]. The majority of the 30% fall in total adenine nucleotide concentration in muscle [4] can be accounted for by a rise in IMP [3], but a small loss of purine nucleotide of about 0.4% of the total adenine nucleotides in muscle can be derived from these data. From this value, despite the large errors, the purine nucleotide ‘loss’ from muscle may be estimated as about 200 times greater than the amount of purine base recovered from urine. There is therefore recycling of purine base.

The relationship between changes in plasma and those in urine can also be considered. The mean total increase of hypoxanthine in plasma and erythrocytes of our four subjects was 61.4 pmol; the mean increase in their urinary excretion was 123 pmol. This value agrees with that derived by Sutton et al. [41 using comparable conditions. About half the excess excretion has been located in the plasma and erythrocytes. Since hypoxanthine readily crosses membranes and the extracellular fluid is about 3 times the blood volume, an unexpectedly large proportion of the hypoxanthine excreted is in the plasma and could therefore be described as ‘circulating’.

Transport of purines

Overall the recent results do not fit easily into the hypothesis of permanent purine-exporting organs. Activities of the first enzyme in the de novo biosynthetic pathway, amidophosphoribosyltransferase (EC 2.4.2.14), are similar in a wide variety of tissues [25], with considerable variations in the activity of the salvage enzyme hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8) [26]. Human brain can also synthesize purine de novo [27]. Our results show that a pulse of hypoxanthine from muscle reaches the kidney and the urine. It is also detectable in erythrocytes, but not in leucocytes. However, IMP concentration in erythrocytes is unaffected, despite extensive evidence of incorporation of [14C]hypoxanthine into nucleotides and their turnover in erythrocytes [28].

The main mechanism involved in hypoxanthine incorporation into nucleotides is probably energy-charge-dependent synthesis of 5-phosphoribosyl 1-pyrophosphate [26, 29]. It is possible that purine-depleted tissues will automatically refill themselves from the hypoxanthine ‘pool’ when their energy charge has been restored after a period of high ATP consumption.

Our data on mild exercise and day–night
differences suggest that ATP turnover may be associated with slight hypoxanthine 'leakage'. This hypoxanthine leakage and the known recycling of the hypoxanthine pool could explain the extensive evidence for the exchange of 14C-labelled purines [28].

Putting the above evidence on transport and exchange together, it appears justifiable to attempt to define purine 'transport' more precisely. Regular transport in the erythrocyte comparable with the lung-oxygen–haemoglobin system is probably not operative, since no one tissue may dominate biosynthesis de novo. All tissues can and probably do contribute to the hypoxanthine pool, and their weight as well as their biochemical activities and capacities will affect the contribution made. Nucleotide-depleted tissues will, after the restoration of their energy charge, show net uptake. However, all tissues will have ATP turnover, which may be associated with small amounts of hypoxanthine leakage and re-uptake. If our suggestions are correct, tissues with a high ATP turnover such as brain should be heavily dependent on the salvage pathway; the functional cerebral damage in the Lesch–Nyhan syndrome due to hypoxanthine/guanine phosphoribosyltransferase deficiency [24] is thus consistent with our model of organ co-operation through the large and extensively recycled circulating hypoxanthine.

Inosine, adenosine and the adenine nucleotides

The adenine-based compounds, adenosine, AMP, ADP and ATP, should be considered separately. Our failure to detect a significant fall in the already low adenosine concentration contrasts with the significant fall in its metabolic product inosine. No precursor–product relationship between inosine and hypoxanthine was detected, despite purine nucleoside phosphorylase activity of 36 nmol/h per mg of protein being found in plasma. These results are consistent with previous work; inosine is released from the heart during angina [30], but adenosine is found only after 10 min, not 5 min, intensive exercise of perfused dog muscle [31]. Tissue concentrations of adenosine are higher than in plasma and approximate to the apparent Michaelis constants of the metabolizing enzymes [31, 32]. Adenosine is therefore rapidly inactivated by conversion into inosine.

The concentrations of ATP in plasma are similar to those obtained by M. J. Harber (personal communication), but somewhat higher than those found by Jabs et al. [33], who used luminescence methods. Thus the concentrations of adenine nucleotides in plasma are higher than those of adenosine and are thus consistent with the suggestion of Burnstock [34] that ATP may occupy the receptor sites which have been identified by Schrader et al. [35] on the external membranes of cells.

The significant fall in total adenine nucleotide concentrations in plasma after exercise is consistent with other evidence for the release of ATP by muscle after exercise [14, 36], although Bockman et al. [37] only detected adenosine. However, much of the ATP that we detected in plasma may not have come from muscle, since the energy charge in plasma was high at 0.74 and nucleotide breakdown is rapid [36]. Local sources are therefore probable. Although a small contribution from haemolysis estimated at about 0.1% is possible, this was not large enough to account for the results obtained. Since both heparinized and EDTA-treated plasma samples contain ATP and platelets contain mainly ADP, other cellular sources must contribute. Such local sources should include the erythrocytes, leucocytes and endothelium.

Leucocytes

Our studies of leucocytes were only exploratory. The results suggest that the leucocytes released by exercise, which are assumed to have been adherent to endothelial surfaces [38], are depleted of adenine nucleotides. Polymorphonuclear leucocytes which have been adherent to filters or to each other in a pellet show losses of adenine nucleotides [39].

Choice of samples for diagnosis

Significant increases in hypoxanthine excretion are concentrated into the daytime and at weekends. Similar changes in hormone excretion linked with behaviour also occur [40]. Resting or night excretion is relatively constant and is therefore preferable for diagnostic studies, for example after metabolically damaging hypoxia [2] and in defects such as xanthine oxidase deficiency.

These results are consistent with much previous work, but not with some assumptions. It may be more accurate to use the description 'purine exchange with a circulating hypoxanthine pool' to describe the mechanisms of organ co-operation in purine metabolism, rather than purine transport.

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References


