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Elevated erythrocyte phosphoribosylpyrophosphate and ATP concentrations in Japanese sumo wrestlers

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1. Japanese sumo wrestlers have a diet rich in energy, which results in marked obesity. Their plasma urate and triglyceride levels were significantly elevated.

2. Erythrocyte phosphoribosylpyrophosphate (PRPP) and ATP concentrations in sumo wrestlers were significantly elevated when compared to the levels in control subjects.

3. There were no significant differences in erythrocyte PRPP synthetase (*EC* 2.7.6.1), purine nucleoside phosphorylase (*EC* 2.4.2.1) and hypoxanthine guanine phosphoribosyl transferase (*EC* 2.4.2.8) activities between sumo wrestlers and control subjects.

4. Erythrocyte adenosine kinase (*EC* 2.7.1.20), adenosine deaminase (*EC* 3.5.4.4) and adenine phosphoribosyl transferase (*EC* 2.4.2.7) activities in sumo wrestlers were significantly elevated.

5. It seems that sumo wrestlers have an increased turnover of adenine nucleotides which may contribute to hyperuricaemia.

Sumo is a national sport in Japan and there are approximately 800 professional sumo wrestlers. They enter the wrestlers' society at the age of 15 years and carry out hard training for 5 h daily. In the fighting match, overweight is a big advantage for the strong wrestler. Their high-energy diet is rich in protein and averages 23100 J/d (396 g protein, 98 g fat, 780 g carbohydrate). Meals are taken twice daily and under these conditions their body-weight increases significantly. The wrestlers show significantly elevated serum urate and triglyceride levels. Increased 24 h urinary urate excretions, mean value approximately 800 mg, were found in these subjects (Hayashi, 1971).

Many epidemiological and clinical studies have shown a strong correlation between body-weight and plasma urate levels (Rose & Prior, 1963; Healey *et al.* 1966; O'Brien, *et al.* 1966; Prior *et al.* 1966; Hall *et al.* 1967). Reports have also shown that weight loss causes a fall in serum urate levels (Nicholls & Scott, 1972; Emmerson 1973). However, the pathogenesis of hyperuricaemia caused by excess energy in the diet is still uncertain. The intracellular concentration of phosphoribosylpyrophosphate (PRPP) has been emphasized as an important factor in several routes of purine nucleotide synthesis (Fox & Kelley, 1971). A rise in serum urate levels due to increased degradation of ATP is well established (Bode *et al.* 1973; Edwards *et al.* 1979).

In the present study, we measured erythrocyte PRPP and ATP concentrations in healthy male controls and sumo wrestlers. In experiments with chickens, Katunuma *et al.* (1970) reported increased activities of enzymes involved in purine metabolism, such as PRPP amidotransferase (*EC* 2.4.2.14), following increased protein intake. Therefore, enzymic activities related to purine metabolism in erythrocytes of sumo wrestlers and controls were also measured. The pathogenesis of hyperuricaemia in obese subjects is discussed.

MATERIALS AND METHODS

Blood samples were collected from sixty sumo wrestlers and from thirty healthy control subjects matched for age and sex. All blood specimens were collected on the same day by

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venepuncture into heparinized tubes after an overnight fast. Our study was performed as part of the health examination of the Japanese sumo wrestlers and was done during a resting period with no training. Blood samples were immediately centrifuged at 1900 **g** for 15 min at 4° and erythrocytes were separated from the plasma. Erythrocytes were washed twice with 4 vols cold physiological saline (9 g sodium chloride/l). The concentrations of PRPP and ATP, and the activity of adenosine deaminase (*EC* 3.5.4.4; ADA) were estimated immediately. PRPP synthetase (*EC* 2.7.6.1), adenosine kinase (*EC* 2.7.1.20), purine nucleoside phosphorylase (*EC* 2.4.2.1; PNP), adenine phosphoribosyl transferase (*EC* 2.4.2.7; APRTase) and hypoxanthine guanine phosphoribosyl transferase (*EC* 2.4.2.8; HGPRTase) activities were measured in 7 d using packed cells stored at −70°. Plasma was analysed for uric acid and triglycerides.

Erythrocyte PRPP concentrations were determined by the method of Henderson & Khoo (1965) with minor modifications. Erythrocyte lysate (25 μl) together with 275 μl 50 mM-Tris hydrochloric acid buffer (pH 7·4) containing 1 mM-ethylene diamine tetra-acetic acid (EDTA), was heated at 100° for 1 min and then chilled in an ice-cold water-bath. The tube was then centrifuged at 3000 **g** for 10 min at 4°. A portion of the supernatant fraction (100 μl) was incubated for 30 min at 37° in 100 μl 100 mM-Tris HCl buffer (pH 7·4) containing 10 mM-magnesium chloride, 100 μM-[^{14}C]adenine (10 μCi/μmol; Radiochemical Centre, Amersham, England) and 10 μl highly purified APRTase solution. The reaction mixture (25 μl) was spotted onto thin-layer chromatography plates (Eastman Kodak, New York) and developed with butanol:methanol:water:ammonium hydroxide (250 ml/l) (60:20:20:1, by vol.). Purines were located on the chromatogram under u.v. light. AMP spots were scraped off, suspended in Aquasol II manufacturer's solution and radioactivity measured using a Packard Tri-Carb liquid-scintillation counter. APRTase was prepared from human erythrocytes by the method of Thomas *et al.* (1973). The specific activity of purified APRTase was 0·1 i.u./mg protein. Erythrocyte ATP concentrations were determined by the enzymic method described by Adam (1972) using an APT kit (Boehringer Mannheim Corp., Mannheim, West Germany).

Erythrocyte PRPP synthetase activity was determined by the method of Hershko *et al.* (1969) with minor modifications. Packed erythrocytes (0·5 ml) were added to 3·0 ml 1 mM-EDTA and were dialysed in 8 mM-sodium phosphate buffer, containing 1 mM-EDTA and 5 mM-glutathione, for 24 h at 4°. Dialysed erythrocytes (50 μl) were incubated with 1·0 ml of 100 mM-Tris HCl buffer (pH 7·4) containing 0·35 mM-ribose-5-phosphate, 0·5 mM-ATP, 1 mM-EDTA, 25 mM-reduced glutathione and 25 mM-$MgCl_2$. After 20 min incubation at 37°, the reaction mixtures was heated at 100° for 1 min and then chilled in an ice-cold water-bath. Cold charcoal (100 g/l, 0·25 ml) was added to the reaction mixture. The tube was centrifuged at 3000 **g** for 10 min at 4°. PRPP concentrations in 100 μl of the supernatant fractions were measured by the method described previously.

HGPRTase activity was assayed by measuring the production of [^{14}C]IMP from [^{14}C]hypoxanthine (Arnold *et al.* 1972). The assay mixture (total volume of 100 μl) contained 800 μM-[^{14}C]hypoxanthine (20·7 mCi/mmol; Radiochemical Centre), 2 mM-PRPP and 10 mM-$MgCl_2$ in 55 mM-Tris HCl buffer (pH 7·4). The reaction was started by the addition of 50 μl of the erythrocyte lysate to the assay mixture at 37° for 30 min and terminated by the addition of 20 μl 100 mM-EDTA. A portion (25 μl) was spotted onto thin-layer chromatography plates and developed as described previously. The radioactivity content of labelled IMP was measured as described for PRPP.

APRTase activity was assayed by measuring the production of [^{14}C]AMP from [^{14}C]adenine. The reaction mixture containing 55 mM-Tris HCl buffer (pH 7·4), 2 mM-PRPP, 10 mM-$MgCl_2$, 800 μM-[^{14}C] adenine and 50 μl haemolysate was incubated for 30 min at 37°. After the addition of 20 μl 100 mM-EDTA, 25 μl of the reaction mixture was spotted on

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Table 1. *Mean body-weight, plasma uric acid and plasma triglyceride levels, erythrocyte APT and erythrocyte phosphoribosylpyrophosphate (PRPP) concentrations and erythrocyte enzyme activities in sumo wrestlers and control subjects*

(Mean values with their standard deviations)

	Sumo wrestlers		Control	
	Mean	SD	Mean	SD
Body-wt (kg)	106	16	61	7**
Plasma (mg/l)				
Uric acid	54	16	46	8**
Triglyceride	1520	600	1170	300**
Erythrocyte concentration (nmol/ml packed cell)				
PRPP	12·2	3·2	8·7	1·6*
ATP	1714	334	1224	214**
Erythrocyte enzyme activity (nmol/mg protein per h)				
PRPP synthetase	34·6	4·9	35·4	5·4
Purine nucleoside phosphorylase	1092	174	1062	192
HGPRTase	118	18	121	23
Adenosine kinase	12·11	2·32	6·93	2·14**
Adenosine deaminase	25·4	6·2	19·4	7·4**
APRTase	22·6	5·2	16·8	4·0*

* $P < 0.05$, ** $P < 0.01$.

thin-layer chromatography plates and developed as described previously. The radioactivity in the labelled AMP was measured as described previously.

Adenosine kinase activity was measured by the method of Meyskens & Williams (1970) with minor modifications. The reaction mixture containing 5 mM-ATP, 10 mM-$MgCl_2$, 200 μM-[^{14}C]adenosine (58 mCi/mmol; Radiochemical Centre), coformycin (1 μg/ml) (Meiji Seika Corp., Tokyo) in 50 mM-acetate buffer (pH 5·7) and haemolysates were incubated for 20 min at 37°. The reaction was terminated by the addition of 20 μl 100 mM-EDTA. Portions of the reaction mixture (25 μl) were applied to thin-layer plates and developed as described for AMP.

Erythrocyte ADA activity was measured by the method of Hopkinson *et al.* (1969). The assay mixture contained 1 mM-adenosine, 0·025 i.u. PNP (Boehringer Mannheim Corp.) and 20 μl xanthine oxidase (8 i.u./ml; PL Biochemicals, Milwaukee, Wisconsin) in 3·0 ml 0·05 M-phosphate buffer (pH 7·4). After addition of 100 μl 1:10 diluted erythrocyte lysate, the increase in absorbance at 292·5 nm was recorded. On determining the erythrocyte PNP activity, 50 μl 1:100 diluted haemolysate were added to 3·0 ml 0·05 M-phosphate buffer (pH 7·4) containing 1 mM-inosine and 20 μl xanthine oxidase. The increase in absorbance at 292·5 nm was recorded.

Uric acid was measured by the enzymic methods of Liddle *et al.* (1959), triglyceride by the method of Sugiura *et al.* (1977) and protein content by the method of Lowry *et al.* (1951). Statistical analysis was performed using the *F*-test.

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RESULTS

As shown in Table 1, the mean body-weight of sumo wrestlers was elevated significantly compared to those of controls. There was a significant difference between the mean (±SD) values of plasma triglyceride of sumo wrestlers and controls. The mean plasma urate of sixty sumo wrestlers was significantly higher than the average level of the matched controls ($P < 0{\cdot}01$). Hyperuricaemia defined as a plasma urate concentration in excess of 70 mg/l was present in 15% of wrestlers and in none of the controls. In wrestlers, body-weight had a significant positive correlation with plasma urate level (r 0·509). The mean erythrocyte PRPP content of sumo wrestlers was significantly higher than the mean content of the controls ($P < 0{\cdot}05$). However, there was no significant relationship between erythrocyte PRPP concentration and plasma urate level in sumo wrestlers (r 0·151). Mean erythrocyte ATP concentration in sumo wrestlers was significantly higher than that of controls ($P < 0{\cdot}01$).

The results of enzymic activities in erythrocytes are shown in Table 1. There were no significant differences in mean erythrocyte PRPP synthetase, PNP and HGPRTase activities between sumo wrestlers and controls. However, the mean adenosine kinase, ADA and APRTase activities in the sumo wrestlers were significantly higher than those of controls.

DISCUSSION

In the present study, Japanese sumo wrestlers showed significantly increased body-weight and elevated serum urate and triglyceride levels. In addition, a significant positive correlation between body-weight and plasma urate level was observed in sumo wrestlers. These results are in agreement with previous findings of Healey *et al.* (1966).

However, the pathogenesis of hyperuricaemia in obese subjects remains uncertain. Accelerated purine biosynthesis or impaired renal handling of uric acid or both are known to be involved in hyperuricaemia. In chicks, administration of a high-protein diet resulted in increased urate excretion (Itoh & Tsushima 1972; Hevia & Clifford, 1977). Increased urinary urate excretion after a high-protein intake in humans was also reported by Bowering *et al.* (1970). However, Emmerson (1973) showed that weight loss in an obese gouty patient caused a rise in the renal urate clearance and a slight reduction in the rate of urate production. After weight loss on a low-energy diet, a fall in plasma urate levels was observed by Nicholls & Scott (1972) and urinary uric acid decreased after weight loss only in subjects with the highest initial urinary urate levels. These findings suggested that obese subjects have decreased renal urate clearance. In the present study, the PRPP concentrations in erythrocytes of sumo wrestlers were significantly elevated when compared with controls. However, there was no significant positive correlation between erythrocyte PRPP concentration and plasma urate in the sumo wrestler. It seems that many factors contribute to hyperuricaemia in obese subjects.

In animal experiments, such as with chicks and rats, there were increased activities of enzymes involved in purine metabolism following increased protein administration. Arnold *et al.* (1972) found that dietary purine restriction increases HGPRTase activity in erythrocytes of patients with Lesch–Nyhan syndrome. Contrary to expectations, there were no significant differences in erythrocyte PRPP synthetase, PNP and HGPRTase activities between sumo wrestlers and control subjects. However, significantly increased activities of adenosine kinase, ADA and APRTase were found in erythrocytes of sumo wrestlers. In addition, erythrocyte ATP concentrations were also significantly elevated in sumo wrestlers. A rise in serum urate levels due to increased degradation of ATP is well established (Bode *et al.* 1973; Edwards *et al.* 1979). These results suggest that both increased formation and

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turnover of adenine nucleotides in sumo wrestlers may exist and may induce production of uric acid, leading to hyperuricaemia.

The possibility of increased purine nucleotide degradation in obese subjects and induction of enzymes such as APRTase by high protein intake warrants further study.

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