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The effect of molybdenum levels in sorghum (*Sorghum vulgare* Pers.) on uric acid and copper excretion in man

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1. The effect of various dietary levels of molybdenum on uric acid and copper excretion was studied in experiments with four adult men given diets based on two sorghum varieties (*Sorghum vulgare* Pers.) differing widely in Mo content.

2. With a Mo intake of 160, 540 or 1540 μg/d the urinary excretion of uric acid was unaltered.

3. The excretion of Cu in urine increased with increasing Mo intake.

4. Cu-balance studied with high- and low-Mo diets showed that with a high-Mo diet urinary Cu excretion increased but faecal Cu was unaffected. This indicates that dietary Mo had no effect on Cu absorption.

5. The high serum concentration of Cu with diets high in Mo indicates that Mo either mobilizes tissue Cu or inhibits Cu uptake, or both.

In populations subsisting on plant foods, the principle sources of dietary molybdenum are cereals, millets and pulses. Mo is an integral part of xanthine oxidase (Xanthine: oxygen oxidoreductase; *EC* 1.2.3.2), an enzyme concerned in uric acid synthesis. Tissue concentrations of this enzyme in experimental animals are related to the dietary intake of Mo (Hart & Bray, 1967; Underwood, 1971). In regions with soil rich in Mo, prevalence of gout is reported to be high (Kovalsky & Vorotnitskaya, 1970) and uric acid production is shown to be affected by very high intakes of Mo (Kovalsky, Yarovaya & Shmavonyan, 1961). In certain parts of India, urolithiasis is common in millet-eating populations (Patwardhan, 1961). The millet sorghum (*Sorghum vulgare* Pers.), one of the important staple foods in these regions, is relatively rich in Mo when compared with rice (Deosthale, unpublished). However, there is no direct evidence of the role of dietary Mo in uric acid synthesis in man, especially in relation to renal-stone formation.

Dietary Mo also affects copper metabolism in various animal species. High intake of Mo apparently hastens Cu deficiency, especially in cattle and sheep; other species are apparently less affected (Underwood, 1971). The mechanism underlying the effect of high Mo intake on Cu deficiency is unknown.

Two sorghum grain samples differing widely in their Mo content were available in large quantities. We are able, therefore, to study the effect of diets based on the high- and low-Mo varieties of sorghum on uric acid and Cu excretion in human subjects.

EXPERIMENTAL

Expt 1

Two samples of sorghum grain, containing 0·21 and 1·39 μg Mo/g were used to make up diets low and high in Mo content. The diets supplied daily 11·92 MJ (2·850 Mcal), 50 g protein and 7 g total minerals; total sulphur intake was 350 mg/d. Apart from sorghum, the diets consisted of rice, vegetable oil, butter, and vegetables such as tomato, onion, potato, cabbage, cauliflower, cucumber and palak. Milk was given with tea or coffee. Four adult male volunteers were given the diet based on the low-Mo variety of sorghum for 10 d followed by the diet based on the high-Mo variety for a further 10 d period. Total Mo intake during the first period was 166 μg/d and during the second period it was 540 μg/d. The contribution to total dietary Mo from foodstuffs other than sorghum grain was constant and was about 100 μg/d for both diets. Daily urine samples were collected during the last 3 d of each period. The subjects then continued on the high-Mo sorghum diet and were given a supplement of ammonium molybdate so that the total Mo intake was raised to 1540 μg/d. This diet was given for 7 d and urine samples were collected on the last 3 d.

Expt 2

Cu balances of four adult male subjects on the low- and high-Mo sorghum diets, as in the first experiment, were measured. The Cu intakes from cooked diets were 2·400 mg/d and 2·417 mg/d on the low- and high-Mo sorghum diets respectively. During the last 3 d of each dietary period 24 h samples of urine and faeces were collected for estimation of Cu. Blood samples were taken on the last day of each dietary period from all the four subjects and analysed for serum Cu.

Analytical methods

The 24 h urine and faeces samples were collected by the procedure described by Vasantgadkar & Tulpule (1963). Total nitrogen, creatinine and uric acid in urine were estimated by the methods described by Vasantgadkar, Venkatachalam & Tulpule (1963). Urine samples were analysed for calcium, phosphorus and inorganic S (Hawk, Oser & Summerson, 1954). Mo was estimated in sorghum and other foodstuffs by a thiocyanate method (Sandell, 1959). Cu was estimated spectrophotometrically in serum and urine (Glorgio, Cartwright & Wintrobe, 1964), and in faeces and foodstuffs (Gubler, Lahey, Ashenbrucker, Cartwright & Wintrobe, 1952).

RESULTS AND DISCUSSION

Expt 1

Effect on urinary uric acid excretion. The excretion pattern of various constituents of urine at different intake levels of Mo is presented in Table 1. The dietary intakes of protein and energy were the same on low- and high-Mo sorghum diets. The excretion of total N and creatinine in urine was therefore the same in the three dietary periods.

Table 1. *Effect of different levels of dietary molybdenum on 24 h urinary excretion in four male human subjects*

Urine measurements (mean values)	Urinary excretion on Mo intake of: 160 μg/d	540 μg/d	1540 μg/d
Volume (ml)	1599	1495	1552
pH	6·54	6·62	6·40
Total N (g)	4·54	4·30	4·27
Creatinine (mg)	634	623	641
Uric acid (mg)	503	457	488
Calcium (mg)	27·7	27·0	32·8
Phosphorus (mg)	535	523	506
Inorganic Sulphur (mg)	201	192	186
Copper (μg)	24	42	77

Table 2. *Effect of diets containing low- or high-molybdenum sorghum on urinary, faecal and serum copper in four male human subjects*

	Low-Mo diet			High-Mo diet		
	Urine (μg/d)	Faeces (μg/d)	Serum (μg/l)	Urine (μg/d)	Faeces (μg/d)	Serum (μg/l)
Mean	25·4	1831	805	71·2	1828	1130
SE	5·39	74	111	3·30	49	83

The mean uric acid excretion in urine was 503 mg/d with the low-Mo diet; it was 457 mg/d with the high-Mo supplemented diet. No significant change was observed in the total volume of urine in the three dietary periods and therefore the concentration of uric acid remained constant. The pH of the various urine samples was similar, which suggested that the uric acid solubility as well as the uric acid-holding capacity of the urine did not change significantly with different Mo intakes. The suggested daily requirement for Mo is about 120 μg (Schroeder, Balassa & Tipton, 1970). In the present study, a twelve- to thirteen-fold increase in dietary intake in excess of this suggested requirement failed to result in any appreciable change in uric acid excretion in urine. At much higher intakes, 10–15 mg Mo/d, uric acid production in man is increased (Kovalsky *et al.* 1961). This suggests that uric acid metabolism in man is altered only at very high intakes of Mo.

The excretion of Ca, P and inorganic S was unchanged in all three dietary periods.

In marked contrast, the excretion of Cu was significantly increased in all subjects with increasing levels of Mo in sorghum diets. The mean Cu excretion with the low-Mo diet was 24 μg/d, which increased to 42 μg/d with the high-Mo diet. A further increase in dietary Mo of 1000 μg/d increased the Cu excretion in urine, the mean value being 77 μg Cu/d. These differences in Cu excretion could not be ascribed to the differences in the dietary intake of Cu, as the Cu content of the two sorghum samples was similar. Cu deficiency due to excess dietary intake of Mo has been attributed to altered absorption of Cu (Dick, 1956) and also a change in tissue metabolism (Dowdy

& Matrone, 1968*a*, *b*; Dowdy, Kunz & Sauberlich, 1969). In view of these reports and the present finding of increased Cu excretion in urine with increased levels of dietary Mo, a second experiment was done to obtain results for Cu balance.

Expt 2

Copper excretion. The results are shown in Table 2.

An increase in Mo intake significantly increased Cu excretion in urine, confirming the observations made in Expt 1. The mean urinary Cu content increased from 25·5 μg/d on the low-Mo diet to 71·2 μg/d on the high-Mo diet. However, the amount of Cu excreted in faeces in the two dietary periods was unchanged, suggesting that absorption of Cu was not affected by the level of Mo intake. The increase in urinary Cu with a high intake of Mo was probably not due to increased absorption in the gut.

The serum Cu concentrations were higher in all four subjects when they were given the diet containing sorghum with a higher content of Mo. The increase ranged from 110 to 520 μg/l ($P < 0{\cdot}01$). The higher serum concentrations of Cu following increased Mo intake suggest mobilization of tissue Cu and increased urinary excretion. On the other hand, in vitro studies have shown that Mo inhibits Cu uptake by tissues (Dowdy & Matrone, 1968*a*, *b*; Marcilese, Ammerman, Valsecchi, Dunavant & Davis, 1969). This may explain the high serum concentrations of Cu and the increased urinary excretion.

The important finding is that levels of Mo in varieties of sorghum can bring about increased urinary excretion of Cu. The effect of this continued excretion of Cu in populations eating these sorghum varieties as part of their staple diet requires study. Cu deficiency in animals has been associated with anaemia and osteoporesis (Davis, 1950). Careful investigations among populations eating high-Mo varieties of sorghum may indicate clinical symptoms resulting from a deficiency of Cu.

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