The influence of a high dietary intake of purine-rich animal protein on urinary urate excretion and supersaturation in renal stone disease

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Summary

1. Eight recurrent renal stone-forming patients were housed in a metabolic ward and fed on a low (LPD) and a high (HPD) animal protein diet, which were isoenergetic. Metabolic studies were made after 2 weeks on each diet.

2. There was a 90% increase in urinary urate on HPD compared with LPD, whereas serum urate did not change consistently.

3. The urinary acid excretion increased by 200%, including a 100% increase in ammonium ion excretion. A fall in urine pH by 0.9 unit was also seen.

4. The calculated ion activities of the urines revealed a profound increase in the uric acid supersaturation, from undersaturation to supersaturation, and in some cases even surpassing the formation product ratio. The ammonium urate supersaturation also increased. The sodium urate supersaturation was unchanged, despite an induced natriuresis.

5. The risk of forming uric acid or ammonium urate crystals or stones in the urine was increased on a high protein diet, whereas the risk of forming sodium urate crystals was no greater than on a low protein diet.

6. As uric acid and ammonium urate crystals under certain conditions may adsorb a macromolecular fraction of the human urine, which inhibits calcium oxalate crystal growth, it is proposed that this mechanism, along with a decrease in urine pH, may also interfere with the inhibitory activity of calcium oxalate crystal growth and aggregation.

Key words: dietary protein, purine, supersaturation, urate, urolithiasis.

Abbreviations: FP, formation product; HPD, high protein diet; LPD, low protein diet; SP, solubility product.

Introduction

An increased consumption of dietary animal protein has been proposed as a reason for the increased incidence of urolithiasis in the industrialized world [1, 2]. The urinary excretion of both calcium and oxalate, the main constituents of the most common type of stone, has been reported to increase with dietary animal protein [3, 4] and so has the urinary excretion of urate [3, 5]. Both serum [6] and urinary [7] urate have been claimed to be risk factors in renal calcium stone disease. Two principal mechanisms have been suggested, by which urate may promote the formation of calcium oxalate crystals or stones. Micromorphs of sodium urate may possibly induce an epitaxial growth of calcium oxalate [8, 9] or interfere with urinary glycosaminoglycans, inhibitors of calcium oxalate crystallization [10, 11]. Under certain conditions, uric acid [12] or ammonium urate [13] crystals may also adsorb naturally occurring macromolecular substances of human urine, which are able to retard calcium oxalate crystal growth and aggregation.

The aim of the present investigation was to study the effects of dietary protein on urate metabolism. As the possible mechanisms for
promoting calcium oxalate crystallization seem to require the existence of urate microcrystals, we were particularly anxious to follow the urinary supersaturation with respect to various urate salts.

Material and methods

Patients

Eight calcium stone-forming patients were admitted to a metabolic ward for 4 weeks. There were seven males and one postmenopausal female, aged 36–65 years. Their body weight ranged between 59 and 98 kg. Altogether they had passed 78 stones in 85 patient-years. According to our out-patient metabolic evaluation four patients had hypercalciuria (> 7.5 mmol/24 h), one had hyperuricosuria (6-3 mmol/24 h) and one had an incomplete proximal renal tubular acidification defect (ipRTA) [14]. They were fed on a low protein diet (LPD) for 2 weeks and on a high protein diet (HPD) for 2 weeks. Four patients began with LPD followed by HPD and the other patients received the diets in the reverse order. Two patients had received stone prophylactic medication before the study but the medication was suspended some variations from one day to another. The two diets were isoenergetic and the nutrient contents obtained from food tables [15]. They contained equal amounts of fat and the difference in protein calories was balanced by carbohydrates. The energy content of the LPD was 12% protein and 48% carbohydrates. This is close to the average Swedish protein intake, which was 60 g of animal and 27 g of vegetable protein, in an average 2870 kcal diet in 1981 [16]. In the HPD the energy distribution was 29% protein and 31% carbohydrates. The excess of protein in HPD was mainly through animal protein. The two diets contained equal amounts of calcium but differed somewhat in other nutrients (Table 1). Supplements were not given to compensate for these differences.

Supplementary salt intake was allowed within certain limits, but was registered and not appreciably different on the two diets. Male patients were held on 147 kJ (35 kcal) and female patients on 126 kJ (30 kcal) per kg body weight in order to maintain a steady weight. Small deviations from this could occur and were registered.

### Table 1. Average content of nutrients per 8.4 MJ (2000 kcal) in the two diets based on a 1 week menu

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Low protein diet</th>
<th>High protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g)</td>
<td>57</td>
<td>142</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>232</td>
<td>150</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Calcium (mmol)</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Magnesium (mmol)</td>
<td>3.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Phosphate (mmol)</td>
<td>34</td>
<td>59</td>
</tr>
<tr>
<td>Oxalate (mmol)</td>
<td>0.41</td>
<td>0.45</td>
</tr>
<tr>
<td>Purine nitrogen (mmol)</td>
<td>4.6</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Study protocol

On each diet the following order of study was pursued.

On 2 consecutive days by the end of each period serum samples were taken in the morning and collections of urine were made during the day (08.00–20.00 hours) and the night (20.00–08.00 hours) for analysis of electrolytes, organic acids and nitrogen compounds. Urine pH was measured on freshly voided urine at 08.00 and 20.00 hours on the same days. The mean values of these 2 consecutive days are presented below, unless stated otherwise.

Laboratory methods and calculations

Urate was analysed with an enzymatic (uricase) method [17].

Glomerular filtration rate (GFR) was estimated from the endogenous creatinine clearance based on both 24 h and 2 h collections of urine.

Nitrogen balance was calculated from the main nitrogen-containing substances in the urine: urea, creatinine, urate and ammonia. The average nitrogen content in the diet was assumed to be 11 mmol/g of protein.

Thermodynamic calculations of urinary complexes, ionic activities and supersaturations were made through an iterative, ab initio procedure (EQUIL) [18]. The limits of the metastable ionic activity products, the solubility products (SP) and the formation products (FP) were adopted from Marshall & Robertson [19].

Student's t-test (paired values) was used to compare differences between mean values.

Results

There was no change in the mean values for serum urate (Table 2) whereas all patients increased their urinary urate excretion (Fig. 1) on HPD compared with LPD. The renal clearance of urate was almost doubled. The urate excretion
Table 2. Serum and urinary values on the low protein (LPD) and the high protein (HPD) diets

Mean values ± 1 SD are shown. N.S., Not significant.

<table>
<thead>
<tr>
<th></th>
<th>LPD</th>
<th>HPD</th>
<th>Δ (HPD - LPD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urate (μmol/l)</td>
<td>371 ± 46</td>
<td>359 ± 45</td>
<td>-12 ± 14</td>
<td>N.S.</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>86 ± 18</td>
<td>83 ± 12</td>
<td>-3 ± 7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>5.0 ± 1.0</td>
<td>9.0 ± 1.8</td>
<td>+4.0 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Standard bicarbonate (mmol/l)</td>
<td>25.4 ± 1.0</td>
<td>24.3 ± 0.6</td>
<td>-1.1 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>24 h urinary excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>1717 ± 597</td>
<td>1973 ± 459</td>
<td>+256 ± 558</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urate (mmol)</td>
<td>3.5 ± 0.4</td>
<td>6.6 ± 1.0</td>
<td>+3.1 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mmol)</td>
<td>14.3 ± 2.9</td>
<td>17.5 ± 3.3</td>
<td>+3.3 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea (mmol)</td>
<td>238 ± 50</td>
<td>740 ± 127</td>
<td>+502 ± 90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nitrogen (mmol)</td>
<td>556 ± 110</td>
<td>1610 ± 277</td>
<td>+1054 ± 195</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Titratable acid (mmol)</td>
<td>5 ± 7</td>
<td>28 ± 12</td>
<td>+23 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ammonium ion (mmol)</td>
<td>26 ± 6</td>
<td>52 ± 14</td>
<td>+27 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net acid (mmol)</td>
<td>28 ± 10</td>
<td>81 ± 21</td>
<td>+53 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium (mmol)</td>
<td>161 ± 30</td>
<td>204 ± 35</td>
<td>+42 ± 30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urine pH</td>
<td>6.7 ± 0.5</td>
<td>5.8 ± 0.7</td>
<td>-0.9 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Clearance values (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>116 ± 19</td>
<td>150 ± 19</td>
<td>+34 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urate clearance</td>
<td>6.6 ± 0.9</td>
<td>12.9 ± 2.0</td>
<td>+6.3 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 1. Serum (a) and urinary (b) excretion of urate on low protein (LPD) and high protein (HPD) diets.

was positively correlated to the purine intake \( r = 0.93, P < 0.001 \). The slope was 1.75 mmol/10 mmol of purine nitrogen in the diet. The intercept for this relation corresponded to an urinary excretion of 2.36 mmol/24 h on a purine free diet. Morning urine pH decreased in all but one patient while on HPD (Table 2). The same pattern was revealed for afternoon urine pH but the difference between LPD and HPD was not as pronounced as for the morning values. All patients increased their excretion of titratable
acid, ammonium ions and net acid (Table 2). The patient with ipRTA did not excrete less acids than the others on either diet. There was also a decrease of standard bicarbonate, within the normal range.

There was a 29% increase in the 24 h excretion of creatinine, whereas the morning serum creatinine values were unchanged (Table 2). Thus renal clearance of creatinine based on 24 h urinary excretion increased to the same amount. When calculated from a 2 h excretion in the morning, after 15 h fast, there was no difference in creatinine excretion and clearance on the two diets.

Urea, the main nitrogen compound in the urine, increased over 200% (Table 2). Even serum urea increased despite a normal GFR in all patients.

Urine volume and sodium excretion were also higher on HPD than on LPD.

Calculation of the thermodynamic equilibrium showed an increase in the ion activity product (AP) of uric acid and in six of the eight patients the urines were supersaturated with respect to uric acid on HPD but not on LPD (Fig. 2). The calculated amount of undissociated uric acid also increased. The AP of sodium urate increased from the contribution of sodium and urate but this change was counteracted by the effects of pH and urine volume (Fig. 3). The AP of ammonium urate was increased on HPD compared with LPD, despite the decreases in pH and increases in urine volume (Fig. 4).

**Fig. 2.** Cumulative changes in the ion activity of uric acid by stepwise addition of changes in urinary urate, pH and volume on a high protein (HPD) compared with a low protein (LPD) diet. FP, Formation product; SP, solubility product. **P** < 0.001.

**Fig. 3.** Cumulative changes in the ion activity of sodium urate by stepwise addition of changes in urinary sodium, urate, pH and volume on a high protein (HPD) compared with a low protein (LPD) diet. FP, Formation product; SP, solubility product. *P* < 0.05; ***P* < 0.001.

**Fig. 4.** Cumulative changes in the ion activity product of ammonium urate by stepwise addition of changes in urinary ammonium, urate, pH and volume on a high protein (HPD) compared with a low protein (LPD) diet. FP, Formation product; SP, solubility product. *P* < 0.05; ***P* < 0.001.
Discussion
In the present investigation it was found that the content of animal protein in the diet had profound effects on urate excretion and on the acidity of the urine. The high protein diet certainly contained an excess of animal protein compared with the low protein diet, which was close to the average Swedish intake of protein [16]. Judged by the nitrogen balance, the overall patient compliance was good. Some 83–85% of the estimated nitrogen intake was recovered in the urine, if it is assumed that 2 g of the ingested nitrogen was eliminated via the intestine or the skin [20], and that the nitrogen content of urea, creatinine, ammonia and urate in the urine is close to the total urinary nitrogen.

The average increase was 90%. The main cause is probably dietary purines, which were calculated to be much higher in HPD. Variation in the bioavailability between purines makes it difficult to estimate the true load. Mononucleotides (AMP, GMP) are absorbed almost completely whereas ribonucleotides only to 50% and deoxyribonucleotides to 25% [21]. Because of a 30% intestinal elimination of uric acid [22] and the relatively high content of mononucleotides in meat, 40% of the purine nitrogen in meat can be expected to be recovered as urinary urate [21]. An exclusive purine load also increases serum urate [21]. A purine-free protein [23] or amino acid load [24] has been shown to cause an increase in urate excretion by 0.7–2.0 mg/g of protein, which is basically a renal effect, as serum urate decreased and urate clearance increased substantially (a decreased tubular reabsorption of urate). It is also possible that the synthesis de novo of purines from glycine or glutamine may play a role [25]. An increased xanthine oxidase activity (stimulates both xanthine and hypoxanthine degradation to urate) in man during protein load, analogous to the increased activity in the rat [26], could possibly contribute to an increased turnover rate of purines.

An attempt can be made to explain the partial contributions of purines and proteins to the raised urinary urate. If it is assumed that urinary urate increases by 1.5 (0.7–2.0) mg/g of purine-free protein this would account for 1 mmol of the increase in urinary urate. The nitrogen content of the remaining 2.1 mmol of the increase in urinary urate on HPD equals 45% of the average increase in purine nitrogen ingested (18.4 mmol), which is quite close to the 40% estimate by Zöllner [21]. This combined purine and protein load caused an increase in urate clearance by 96% whereas serum urate did not change.

The relation between urinary urate excretion and purine nitrogen ingested was very close (r = 0.93, P < 0.001). The slope was almost identical with that reported by Robertson et al., who added 35 g of protein to the diet [3]. From a dietary history estimation of purine nitrogen intake Coe et al. [5] calculated an urate excretion on a purine-free diet almost equal to ours (5.5 mg 24 h⁻¹ kg⁻¹ body weight = 2.45 mmol 24 h⁻¹ 75 kg⁻¹ body weight), but the slope was only 0.75 mmol/10 mmol of purine nitrogen intake.

Because of the high content of ash-acids in proteins an increase in titratable acid and ammonium ions could be predicted. There was a close correlation between the acid excretion and the amount of protein ingested. The calculated slope of 0.46 mmol 24 h⁻¹ g⁻¹ of protein is close to values reported previously [27]. Urine pH decreased (0.9 pH unit), which was not found in another study when 35 g of animal protein was added to the regular diet [3]. Despite an increase in the buffering capacity of the urine, there was a fall in urine pH.

The combined effect of a fall in urine pH and an increased urate excretion was that the urine became supersaturated with respect to uric acid. In two cases the ion activity product even surpassed the formation product, where uric acid could precipitate spontaneously. This was true even when changes in urine volume were taken into account (Fig. 2). There was no net change in the supersaturation of sodium urate, notwithstanding the increased excretion of both sodium and urate (Fig. 3). The changes in urine pH and volume lowered the urinary supersaturation of both sodium urate and ammonium urate. However, the ammonium excretion was raised enough to cause a net increase in the supersaturation of ammonium urate on HPD (Fig. 4). This suggests that the risk of forming uric acid or ammonium urate crystals or stones was increased on the high protein diet.

The propensity for crystal formation, growth and aggregation depends on the balance between urinary supersaturation and inhibitory activity [28, 29]. It is not known how the gross urinary inhibitory activity of calcium oxalate crystal growth and aggregation is influenced by the metabolic effects of a high intake of purine-rich animal protein. We saw a decrease in urinary citrate (not shown) and pH in this study, which would probably have a negative effect on the inhibitory activity of calcium oxalate stone formation [29, 30]. The increased acidity of the urine on the high protein diet would probably lower the inhibitory action of the urinary glycosaminoglycans [31], which are potent inhibitors of
calcium oxalate crystal growth and aggregation [10]. The ability of glycosaminoglycans to stabilize a calcium oxalate solution was shown to be attenuated by monosodium urate crystals [32] but not by ammonium urate or uric acid crystals [33]. Under certain conditions it seems that the action of macromolecular inhibitors of calcium oxalate crystal growth in human urine may be counteracted also by uric acid crystals [12]. Ammonium urate crystals could also be demonstrated to adsorb a macromolecular fraction of human urine [13]. Hence, the observed profound rise in uric acid and ammonium urate supersaturation, and risk of forming such crystals, is thought to contribute to an attenuation of the inhibition of calcium oxalate crystal growth on a high protein diet.

Acknowledgments

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References

