DIETARY INFLUENCE ON THE URINARY EXCRETION OF POLYAMINES

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VPLYV DIÉTY NA VYLUČOVANIE POLYAMÍNOV MOČOM

The amino groups of amino acids, the constituents of proteins, are catabolized in the urea cycle. One intermediate of this cycle, ornithine, is a precursor molecule of polyamines. The influence of dietary protein intake on the production and excretion of polyamines in the urine is yet unclear. The aim of this study was to ivestigate the excretion of polyamines in the urine following three days of creatine-free, creatinine-free and low-polyamine diet in four persons. On the fourth day they were loaded with creatine-free,creatinine-free and low-polyamine high-protein diet (80 g/70 kg body weight). High-protein diet resulted in no increase of urinary polyamine excretion. The low-polyamine diet caused a non-significant decrease in urinary polyamine excretion (by 15 %). ***(Tab. 1, Ref. 30.)***

Key words: polyamines, urine excretion, proteins diet.

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Aminokyseliny sú základné stavebné jednotky bielkovín. Ich aminoskupina sa odbúrava v cykle syntézy močoviny. Jedným z medziproduktov toho cyklu je ornitín, ktorý je zároveň východiskovou látkou pri syntéze polyamínov. Zatiaľ nie je objasnené, ako ovplyvňuje príjem bielkovín produkciu a vylučovanie polyamínov v moči. Cieľom tejto práce bolo sledovanie vylučovania polyamínov v moči po diéte bohatej na bielkoviny u dobrovoľníkov. Dobrovoľníkom počas troch dní sa podávala kaloricky vypočítaná strava, ktorá neobsahovala kreatín a kreatinín a prakticky ani polyamíny. Na štvrtý deň sa podala strava bohatá na bielkoviny (80 g/70 kg telesnej hmotnosti), znovu bez kreatínu, kreatinínu a polyamínov. Merania vylučovania polyamínov uskutočnené deň pred podaním stravy bohatej na bielkoviny a ďalšie dva dni po ňom slúžili ako kontrolné merania. Zistilo sa, že zaťaženie stravou bohatou na bielkoviny nezapríčinilo zvýšenie vylučovania polyamínov v moči. Počas stravy chudobnej na polyamíny kleslo ich vylučovanie močom nesignifikantne o 15 %. ***(Tab. 1, Ref. 30.)***

Kľúčové slová: polyamíny, vylučovanie močom, proteínyová diéta.

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The nutritional content of food can influence the quantity of a substance in the body in two ways, namely it can be absorbed if the substance concerned is present in the food, or the availability and absorption of its precursors is important for its synthesis. Both may also influence the body pool and hence the excretion of a number of substances including purines, creatine and polyamines. The creatine pathway begins with arginine, that of polyamine with ornithine. These two amino acids are intermediate products in the urea synthesis cycle, the function of which is to degrade amino acids following the ingestion of proteins. It would be possible that loading with proteins through an accumulation of intermediate products in the urea synthesis may in turn result in increased synthesis and excretion of creatinine (the degradation product of creatine) and polyamines. As for creatinine, it is already known that this is not the case (10). As regards polyamines, it is not yet known. In our study of the role of diet in the pathogenesis of gout it was found that loading with purine-free proteins (22) was followed by increased production of purines (16), an area in which previous findings were contradictory (8, 28) for several reasons. The aim of this study was the investigation of polyamine excretion after the same load of proteins (16). Basically, polyamines are synthesized in the body (2). However, when requirements increase they may be also absorbed from the intestines, in which case the polyamines produced by bacteria might play a far more important role than those supplied by nutrition intake (1, 7, 9, 18, 19, 20, 25). There is as yet no answer to the question as to how the excretion of polyamines is influenced by the ingestion of their precursors, i.e. the amino acids.

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Methods

Four subjects (I — female healthy subject 1965, II — male subject 1941, Omeprazol 20 mg/day for reflux oesophagitis; III — male healthy subject 1934, IV — male subject 1937, Allopurinol 300 mg/day for gout, stopped 10 days before the test), were fed for six days a purine-, pyrimidine-, creatine- and creatinine-

free (22) and low-polyamine (3) diet, calculated according to body weight (13, 22). The ratio of proteins, carbohydrates and fats in g was 2:10:4 and the caloric content 19005 kJ (4540 kcal)/70 kg body weight/day (22) in order to avoid catabolism. The meals comprised milk, cream, corn starch, glidine and vanilla pudding with artificial flavouring. They were equivalent in composition as well as in caloric value and were taken daily at 08.00, 12.00, 16.00 and 20.00. At 08.00 on day 4 there was an additional loading with 80 g/70 kg body weight of purine-, pyrimidine-, creatine- and creatinine-free and low-polyamine (3) proteins (22) in the form of glidine (16), approximating 400 g of meat. This was found to be sufficient to reach the upper limit of urea synthesis capacity (22), and indeed an averagely nourished person consumes no more proteins daily. Urine was collected between 08.00 and 8.00 on days 0, 3,4,5 and 6 of the diet regimen. Immediately after miction it was stored in a refrigerator at 4 ˚C to 8 ˚C. Before freezing to -20 ˚C the urine samples were heated for 30 minutes at 55 ˚C (for other purposes of the study) to redissolve any crystallised uric acid. This was repeated before the analysis. Preliminary tests proved that heating had no influence on the quantities of creatinine and polyamines. The quantitatively most important polyamines in human urine (14, 15) putrescin and spermidin and their acetyl matabolites N1-acetylspermidine and N8-acetylspermidine and acetylputrescine were measured in the urine only (14, 15), because the amounts of polyamines in plasma are at the limits of detectability (7, 14). The limit of sensitivity of the method was 0.5 pmol, while the recovery rate of polyamines amounted to 98 % (14). Excretion was calculated at 1.73 m^2/day. Statistical analysis was carried out using the Friedmann test for repeated measurements involving the same subject. Subsequently the Dunn test was used to compare the data for days 3—6 with those for control day 0. $p<0.05$ was taken as statistically significant.

Tab. 1. Polyamine excretion in urine (µM/day/1,73 m^2) of 4 persons. Day 0 with usual meals, day 3—6 under special creatine-, creatinine- and polyamine free diet from day 1. On day 4 (*) at 08.00 a.m. load of 80 g creatine-, creatinine- and polyamine — free proteins/70 kg body weight (see Method).
Tab. 1. Vylučovanie polyamínov močom (µM/deň/1,73 m^2) u 4 osôb. Deň 0 s bežným príjmom potravy, dni 3—6 so špeciálnou diétou bez obsahu kreatínu, kreatinínu a polyamínov od 1 dňa. 4. deň (*) o 8. h podanie 80 g proteínov obsahujúcich kreatín, kreatinín a polyamíny na 70 kg hmotnosti (pozri metódy).

	Person Osoby					
Day Deň	I	II	III	IV	Mean Priemer	S.D.
0	11,2	23,4	21,7	23,0	19,3	5,8
3	10,7	23,8	19,7	16,8	17,7	5,6
4*	11,6	18,3	–	18,7	16,1	4.1
5	10,0	18,4	20,0	20.3	17,2	4,9
6	10,5	15,2	–	22,3	19,0	7,0

* — in two urines of polyamine analysis was impossibble from unknown reason.
* — v dvoch vzorkách bola polyamínová analýza z neznámych príčin nemožná.

Results

Compared with normal nutrition (day 0), the purine-, pyrimidine- creatine- and creatinine-free and low-polyamine diet leads per se to a 15 % not significant ($p=0.525$) decrease in the excretion of polyamines (Table 1). A single loading with 80 g protein/70 kg body weight on the morning of day 4 had no influence on the polyamine excretion (Table 1). Only the amounts of total polyamines are specified, because no differences could be established in the behaviour of the determined polyamines.

Discussion

The advantage of the method used (22) is the comparability of the results under load with its own control values within the same experiment and thus the evaluation of each subject separately. Compliance with the diet was controlled by means of urea values in the plasma and the urine and with the collection of urine by means of daily creatinine excretion (both not published). The results support the reliability of the protocol in this respect. Due to methodological factors (complex and exacting compliance with dietary conditions, urine storage in a refrigerator after each collection) a greater number of persons cannot be investigated and possible changes detectable only by multiple analyses could not be found or their statistical significance ascertained. This applies possibly also to the influence of low-polyamine diet on polyamine excretion in the urine (Table 1). It is not clear whether this decrease could be due to a reduced absorption of the polyamines present in the diet (3) or whether the changes in the physical composition of the fibre-free meals influenced polyamine synthesis in the intestines (30). The increase in the excretion of polyamines in urine after pharmacological doses of polyamines (5, 27) were not detectable with physiological amounts (5). Pöyhönen et al. (21, 29) investigated the influence of various conditions on the excretion of polyamines in human urine. They found an increased excretion of total polyamines during the first week of a low-calorie diet (2100—3350 kJ), but this returned again to normal during the next two weeks (29). In one subject norrmal diet, the excretion of acetylised polyamines was almost identical during the subsequent six days (24). Our results (Table 1) support the findings that under normal conditions the polyamines present in the nutrition play only a minor role in quantitative terms in the formation of the endogenous pool (9, 19, 24). A low-polyamine diet is also most unnatural (2) and can be normally attained only by total fasting (3). There would be no benefit if the amount of polyamines, which are biologically active substances (2), depended on normal variations in the daily diet. Loading with pure (purine-, pyrimidine-, creatine-, creatinine-free and low- polyamine) proteins on the fourth day did not result in increased excretion of polyamines in the urine (Table 1). It may be expected that after overloading the urea cycle its intermediate products, i.e. ornithine too, will accumulate. The activity of ornithine-decarboxylase — the first enzyme which regulates polyamine synthesis — depends on the quantity of ornithine available (2, 20). Nevertheless no increased polyamine excretion in the urine was observed either in our experiments or after parenteral infusion of amino acids in multiple trauma victims (11). The polyamines in the blood were generally not measured for methodological reasons (7, 14). Therefore our statement can regard only the excretion of polyamines in the urine. The conclusions about

the body pool of polyamines from their excretion in urine must be taken with caution also because of the possible accumulation of new synthetised polyamines in the tissues (30). Measuring the urine excretion of a substance in 24-hour urine offers the possibility of estimating the glomerular filtration rate, which influences polyamine excretion (4), as well as the advantage of calculating urine excretion/1.73 m^2 body surface in comparison with that in the specimen of morning urine (6, 17, 26). This is probably the reason why our three male subjects (II, III, IV) show small interindividual rates of polyamine excretion in urine (Table 1) in contrast to the findings of Seiler and Knögden (24). The same was found as regards the estimation of uric acid excretion (13, 16). However in contrast to the excretion of uric acid (16), the only female subject (I) had a substantially lower excretion of polyamines in her urine (Table 1). This may be due to the known influence of sex hormones on the synthesis of polyamines (12, 23). It appears that in the synthesis, turnover and excretion of polyamines under physiological conditions, sex plays a more important role than nutrition. This finding has to be confirmed in further experiments.

The following conclusions may be drawn from the results:

A single loading with proteins in the nutrition does not result in increased excretion of polyamines in the urine.

A trend to a not significant decrease of about 15 % in the excretion of polyamines in the urine was observed on a low- polyamine nutrition.*

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PREDSTAVUJEME NOVÉ KNIHY

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