Dietary Intake and Urinary Excretion of Maillard Reaction Products (MRPs)

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Abstract: The aim of our study was to investigate the influence of nutrition on the urinary excretion of Amadori products, pyrraline and pentosidine in a dietary study involving 18 healthy volunteers. Starting with day two, participants had to avoid Maillard product containing food for a period of 7 days, followed by day nine without dietary restrictions. Samples of 24 h-urine were collected and analysed for free furosine, pyrraline and pentosidine using dedicated chromatographic methods. For all MRPs, a significant decrease in the amount excreted with urine was observed due to the MRP-free diet. Urinary excretion of free pyrraline and fructoselysine, which was calculated from furosine analysis, were lowered about 90% from 3.9 ± 1.4 mg/d to 0.4 ± 0.3 mg/d and 7.2 ± 4.1 mg/d to 0.9 ± 0.2 mg/d, respectively. Urinary excretion of free pentosidine was only in the μg/d range and its decrease added up to 50% from 7.3 ± 3.7 μg/d to 3.4 ± 1.1 μg/d. These results indicate that renal excretion of MRPs is directly affected by dietary intake of those. With respect to the daily intake via heated foods, mainly as protein-bound derivatives, pyrraline seems to be of better bioavailability than the Amadori product and pentosidine. This points to different metabolic pathways. Whereas metabolic transformation of AGEs may quantitatively be of little importance, the major part of ingested Amadori products seems to be degraded *in vivo*.

Keywords: diet; urinary excretion; Maillard reaction products; AGEs

INTRODUCTION

Maillard reaction products (MRPs), often referred to as advanced glycation endproducts (AGEs), are known to be elevated in human tissues, plasma and urine in case of metabolic and vascular disorders like diabetes mellitus, artheriosclerosis and renal diseases [1–3]. Depending on the glycaemic status Portero-Otin *et al*. [4] reported elevated amounts of free pyrraline in the urine of type 1 diabetics compared to healthy subjects (1.80 ± 0.7 to 2.51 ± 0.8 vs. 1.68 ± 0.5 μg/mg creatinine). Similar results were obtained by Yoshihara *et al*. [5] and Tsukahara *et al*. [6], whereas Tsukahara [7] quantified higher values (diabetics: 10.01 ± 8.5 μg/mg creatinine; healthy subjects: 8.36 ± 6.9 μg/mg creatinine). Yoshihara *et al*. also studied the urinary excretion of pentosidin. Excreted amounts were by one order of magnitude lower than those of pyrraline and were found to increase in relation to age. Slightly increased values of urinary pentosidine of diabetics compared to healthy subjects have been quantified by Tsukahara *et al*. [6] (12.43 ± 5.2 vs. 8.72 ± 2.8 ng/mg creatinine). Knecht *et al*. [8] studied the urinary excretion of fructoselysine and carboxymethyllysine (CML) also in healthy and diabetic subjects. Amounts of CML were only slightly increased in diabetics (1.0 ± 0.3 vs. 1.2 ± 0.5 μg/mg creatinine) again, whereas urinary excretion of fructoselysine was strongly elevated from 4.0 ± 2.8 to 9.2 ± 6.5 μg/mg creatinine. Elevated amounts of excreted MRPs are generally discussed to be due to increased formation *in vivo* correlating with blood glucose in diabetics, or declined renal clearance in case of renal diseases, resulting in the accumulation of those glycation products. As considerable amounts of MRPs are formed in foods due to thermal processing, recent papers discuss the role of dietary AGEs as possible "glycotoxins", which may represent a risk factor for the development and progression of metabolic disorders like diabetes mellitus and uremia [3]. To date, however, only limited information concerning resorption, biodistribution and elimination of individual food-derived AGEs is available. The

purpose of our study was to investigate whether urinary excretion of pyrraline, pentosidine and Amadori products is affected by diet, thus indicating bioavailability of dietary MRPs. 18 healthy volunteers were asked to collect 24 h urine samples for 9 days. From the second to the eight day, foods containing Maillard compounds had to be avoided. Urinary excretion of free Amadori products, pyrraline and pentosidine were measured using chromatographic techniques.

EXPERIMENTAL

Dietary study. A dietary study with 18 healthy volunteers aged between 22 and 42 years was performed. Participants had to collect 24 h urine samples for 9 days. There were no dietary restrictions on the first and the last day. Starting with day two, a diet virtually free of Maillard reaction products (MRP) (i.e. no cooked or roasted foods, coffee etc.) was supplied until day eight. Samples of 24 h-urine were collected and stored at –18°C until analysis.

MATERIAL AND METHODS

Pyrraline was determined in the urine samples via reverse-phase HPLC with UV-detection at λ = 297 nm using a modified method according to Portero-Otin *et al*. [4]. Column temperature was 37°C, solvent A was 0.02 M ammonia acetate. The determination of free furosine was carried out after ultra filtration (cutoff 5 kD) and acid hydrolysis using reverse-phase HPLC with UV-detection at λ = 280 nm [9]. For free pentosidine, urine samples were concentrated in vacuum and analysed via ion exchange chromatography with fluorescence-detection at 335 nm excitation and 385 nm emission wavelength [10].

RESULTS AND DISCUSSION

A significant decrease in the amounts of free pyrraline, pentosidine and Amadori products (measured as furosine) excreted with the urine was observed within the first 48 h after starting the MRP-free diet (Figure 1). Urinary excretion of pyrraline was lowered from 3.9 ± 1.4 mg/d (day 1) to 0.8 ± 0.2 mg/d (day 3) followed by a further decrease to 0.4 ± 0.3 mg/d on the eight day. Fructoselysine, which was calculated from furosine measurements according to [11], followed the same pattern, starting at 7.2 ± 4.1 mg/d (day 1) lowering to 1.2 ± 0.7 mg/d on day three and finishing with 0.9 ± 0.2 mg/d on day eight. The excreted amounts of free pentosidine were by one order of magnitude lower than those of pyrraline and fructoselysine, showing a less pronounced influence of the MRP-free diet. Excretion of pentosidine was 7.3 ± 3.7 μg/d on the first day and decreased to a minimum of 3.4 ± 1.1 μg/d on day eight. For all three of the amino acid derivatives, the excreted amounts increased with the return to conventional nutrition on day nine. These results indicate that diet directly affects the urinary excretion of Amadori products and AGEs. Based on data given by Henle [12] the estimated daily supply of the amino acid derivatives ranges about 1–6 mg/d for pyrraline, 75–100 mg/d for fructoselysine and 200–300 μg/d for pentosidine. Comparing the excreted amounts

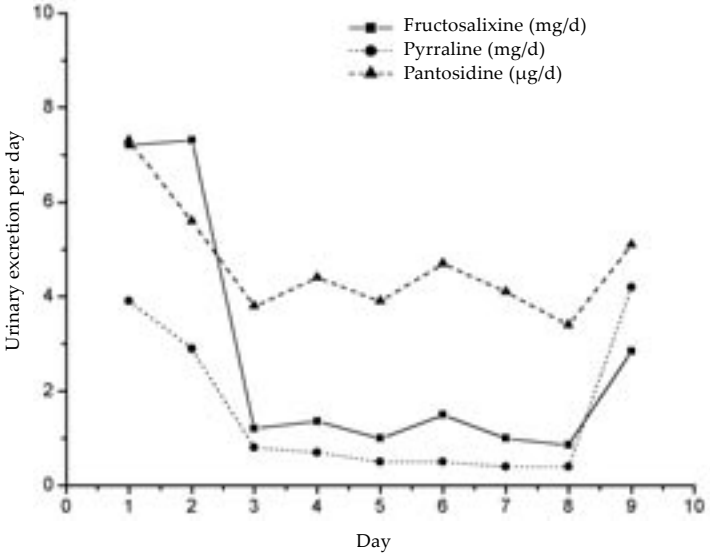

Figure 1. Urinary excretion of free Amadori product, pyrraline and pentosidine as influenced by a diet virtually free of foods containing Maillard products

at day one with those estimated intakes, we found similar values in case of pyrraline, whereas for the Amadori product and pentosidine significant lower recovery rates were obtained. This comparison indicates that peptide-bound pyrraline is completely bioavailable, which means that it is proteolysed and resorbed during digestion, followed by a rapid elimination. Compared to this, the low recovery of Amadori products and pentosidine points to impaired proteolytic breakdown and possible metabolic degradation.

Slightly elevated urinary pyrraline levels in diabetics compared to healthy subjects as reported by Portero-Otin *et al*. [4] and others, therefore seem to be mainly caused by dietary habits, which is in accord to higher urinary levels if the glycaemic status is poorly controlled. For fructoselysine and pentosidine other metabolic pathways than renal clearance of the intact derivatives are conceivable. Concerning to the Amadori product enzymatic deglycation via fructosamine kinases, either by microorganisms in the gut as well as after resorption, are under debate [13]. Referring to pentosidine, Miyata *et al*. [14] demonstrated that about 16% of the radiolabeled derivative intravenously injected to rats were excreted unmetabolised via the kidneys, whereas further 64% were eliminated after modification by currently unknown metabolic mechanisms. With respect to the decline of urinary excretion of the amino acid derivatives in consequence of MRP-free diet, free pyrraline and Amadori products seem to be nearly completely of dietary origin. In contrast to this, however, other sources in addition to the diet must account for free urinary pentosidine, as the amounts of this crosslinking amino acid only decreased by 50%, with the remaining excretion being due to formation *in vivo*. Sell and Monnier [15] proved the accumulation of pentosidine in human tissues like collagen. Thus catabolic processes within regular protein turnover, leading to decomposition of proteins, may account for the formation of free pentosidine, which is continuously cleared via the kidney. Further studies must show whether there is a direct relation between urinary pentosidine excretion and protein turnover.

CONCLUSIONS

Urinary excretion of Amadori compounds as well as of the AGEs pyrraline and pentosidine is directly affected by dietary intake. Whereas Amadori products and pyrraline in urine are nearly exclusively of dietary origin, about half of the daily pentosidine excretion results from food intake. We conclude that the food-derived MRPs are efficiently cleared by the kidney, but dietary intake has to be taken into account within the discussion of possible physiological effects caused by individual MRPs.

Acknowledgement: We want to thank all participants of the dietary study. Special thanks are due to Mrs. K. Schlosser for pentosidine analyses.

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