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DOI

[10.1016/S0009-8981\(97\)00070-3](https://doi.org/10.1016/S0009-8981(97)00070-3)

Publication date

1997

Published in

Clinica chimica acta

[Link to publication](#)

Citation for published version (APA):

Brand, H. S., Jorning, G. G. A., Chamuleau, R. A. F. M., & Abraham-Inpijn, L. (1997). Effect of a proteinrich meal on urinary and salivary free amino acid concentration in human subjects. *Clinica chimica acta*, 264, 37-47. [https://doi.org/10.1016/S0009-8981\(97\)00070-3](https://doi.org/10.1016/S0009-8981(97)00070-3)

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Clinica Chimica Acta 264 (1997) 37–47



Effect of a protein-rich meal on urinary and salivary free amino acid concentrations in human subjects

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Received 28 October 1996; received in revised form 1 April 1997; accepted 11 April 1997

Abstract

The aim of the present study was to investigate whether in healthy volunteers acute changes in plasma free amino acid composition after a protein-rich test meal are reflected in the urinary and salivary concentrations of the corresponding amino acids. The ingestion of a protein-rich meal elicited a significant increase of plasma and urine amino acid concentrations. The postprandial salivary amino acid excretion showed only minor changes. For several amino acids (alanine, arginine, asparagine, glycine, threonine and valine) significant relations were observed between the increase in concentration of these amino acids in venous plasma and urine. In whole saliva, only threonine and valine showed a significant relationship with the corresponding plasma concentration. Our data suggest that the urinary amino acid excretion of several amino acids has the potential for estimating short-term changes in plasma concentrations. Determination of salivary amino acid concentrations seems less appropriate for this purpose. © 1997 Elsevier Science B.V.

Keywords: Plasma; Saliva; Threonine; Urine; Valine

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PII S0009-8981(97)00070-3

1. Introduction

Plasma concentrations of free amino acids exhibit considerable variation generated by the cyclic ingestion of food and the variation in meal consumption [1]. Consumption of a protein-rich meal will result in an increase of plasma amino acid levels within 1 to 3 h [2–4]. Several studies have shown that there is a relationship between the amount of amino acids in the fed protein and the magnitude of the increase in plasma levels of free amino acids [5], although this relationship may be restricted to the essential amino acids [3,4,6,7].

In the kidneys, more than 95% of the filtered plasma load of amino acids is absorbed by the renal tubule. Ingestion of protein causes an increase in renal excretion of free amino acids [8]. The majority of the free amino acid concentrations in urine seems to be related to protein intake [8–10]. This suggests that the alteration in urinary excretion is principally a reflection of the changes which occur in plasma free amino acid composition [8]. This suggestion is in agreement with the observation that the long-term changes in amino acid concentration of plasma and urine, induced by feeding protein-enriched milk to very low birth weight infants for 28 days, showed a strong correlation for most amino acids [10]. After parenteral nutrition, a positive relation was observed between the postabsorptive plasma taurine concentration and the 24-h urine excretion [11]. In healthy adults, good correlations between most 24-h average plasma levels of free amino acids and 24-h urinary free amino acid excretion have also been reported [12]. Exceptions of these correlations have been reported for alanine [12] and taurine [13]. No relation was observed for arginine, in very low birth weight children [14].

Caffeine, which has a molecular weight of 192.2, shows an excellent correlation between salivary and serum concentrations, indicating the potential use of saliva for monitoring the circulating level of small molecules [15]. There is general agreement that in both whole and parotid saliva relatively low concentrations of free amino acids are present [16–21]. Whether salivary free amino acid concentrations are related to plasma concentrations is not clear. In man, a raised serum level of free amino acids is accompanied by their raised concentration in saliva [22]. In addition, the saliva levels of free amino acids rose when the corresponding amino acid was given orally [23]. In children, however, no significant relationship was observed between free amino acids in total saliva and in serum [18]. In dogs, unequivocal results were obtained on the possible relation between the concentrations of amino acids in stimulated parotid saliva and the plasma levels [24,25].

The aim of the present study was two-fold. Firstly, to investigate whether short-term changes induced in free amino acid composition of plasma are reflected in the urinary excretion of the corresponding amino acids. Secondly, to explore the possible relationship between free amino acid composition of plasma and saliva.

2. Materials and methods

2.1. Subjects

The studies were performed in 18 healthy adults (eleven males, seven females). Individuals with diseases for which variations in amino acid metabolism have been described [8] were excluded. The mean age of the subjects was 37 ± 3 years, height was 177 ± 2 cm and weight was 72.5 ± 2.6 kg. We also excluded volunteers with medication interacting with amino acid excretion. All subjects gave their informed consent prior to the experiments.

The subjects were instructed to abstain from smoking, eating, and drinking anything but water for 10 h prior to admission. In addition, all volunteers were asked to abstain from tooth brushing to prevent minimal gingival bleeding.

2.2. Sample collection

After admission to the study center at 8:00 am the subjects were asked to empty their bladder. These urine samples were discarded. The bladder was again emptied at 9:00 am and in these samples urinary amino acid excretion was determined. Between 9:00 am and 9:10 am saliva samples were collected, immediately followed by collection of blood samples. Next, the subjects consumed a protein-rich meal consisting of 300 g roast beef in 20 ± 3 min, together with 300 ml of water.

At 10:00 am the bladder was emptied again and this urine sample was discarded. At 11:00 am second urine specimens were collected, immediately followed by collection of saliva and blood samples.

On a separate occasion, we investigated possible changes in plasma, urinary and salivary amino acid composition in the absence of feeding. Therefore, seven of the volunteers provided blood, urine and saliva samples at 9.00 am and 11.00 am without consumption of a protein-rich meal.

2.3. Preparation and storage of samples

Unstimulated whole saliva was collected according to the spitting method [26,27]. Tap water was used to rinse the mouth. The period between rinsing and saliva sampling was 5 min. Saliva was collected in preweighed, ice-chilled tubes. The saliva collection period was 10 min.

One ml aliquots of saliva and urine were deproteinized with 50 mg sulfosalicylic acid (Sigma, St. Louis, USA) and centrifuged for 10 min at 4000 g at 4°C. The supernatants were stored at -70°C until further analysis, together with a non-acidified aliquot of urine for the creatinine assay.

Four millilitres of blood was drawn from the antecubital vein into lithium heparin-containing tubes, placed on ice and centrifuged (4000 g, 10 min, 4°C).

100 μ l plasma was deproteinized with 5 mg sulfosalicylic acid and stored at -70°C until further analysis.

2.4. Analysis of samples

The amino acid concentrations in plasma, urine and saliva were determined using a gradient reversed-phase HPLC system with precolumn derivatization with *o*-phthaldehyde (Pierce, Rockford, USA) and 3-mercaptopropionic acid (Fluka, Buchs, Switzerland) and fluorescence detection [28]. Norvaline was added as an internal standard.

The urinary creatinine concentration was measured with a Hitachi BM 717 automatic analyzer using the appropriate kit (Boehringer Diagnostics, Mannheim, Germany). Urine amino acid concentrations were expressed per mmol of creatinine.

2.5. Statistical methods

Data are expressed as mean \pm SEM. The effect of the protein-rich meal on the concentrations of amino acids was analyzed by applying a paired *t* test. The relationship between changes in amino acid concentrations was explored by means of the Spearman rank correlation coefficient. The statistical analyses were performed using the SPSS/PC + Statistical Software Package version 5.0 (SPSS Inc, Chicago, USA). Levels of significance were set at $P < 0.05$.

3. Results

The ingestion of a protein-rich meal elicited in healthy volunteers an increase 12–266 $\mu\text{mol/l}$ (14–149%) above the preprandial baseline values of virtually all free plasma amino acids within 2 h (Table 1). Relatively, the largest increase was observed for the amino acids glutamate, isoleucine, leucine, lysine and methionine. In absolute amounts, the largest increases in plasma concentration were observed for alanine, leucine, lysine, threonine and valine. In the absence of feeding, no significant increases in plasma amino acids were observed (data not shown).

Thirteen of the 18 amino acids studied were detected in the urine samples of all volunteers. The excretion of 12 of these 13 amino acids into urine showed a significant increase postprandially (Table 2). No significant changes of urinary amino acid concentrations were observed in the absence of feeding (data not shown).

The largest postprandial increases in urinary excretion were observed for alanine, glycine, taurine, and threonine, both in absolute amounts as well as

Table 1

Effect of a protein-rich test meal consisting of 300 g roast beef on plasma amino acid concentrations ($\mu\text{mol/l}$) in 18 human subjects (means \pm SEM)

	Before test meal	2 h after test meal	% of initial value
Ala	413 \pm 19	580 \pm 26 ^a	(141%)
Arg	113 \pm 2	226 \pm 7 ^a	(201%)
Asn	55 \pm 2	91 \pm 5 ^a	(166%)
Cit	39 \pm 2	51 \pm 3 ^a	(130%)
Gln	661 \pm 20	756 \pm 25 ^a	(114%)
Glu	28 \pm 3	67 \pm 10 ^a	(241%)
Gly	254 \pm 16	323 \pm 21 ^a	(128%)
Ile	72 \pm 4	179 \pm 6 ^a	(249%)
Leu	134 \pm 5	294 \pm 9 ^a	(220%)
Lys	207 \pm 5	473 \pm 19 ^a	(228%)
Met	27 \pm 1	58 \pm 2 ^a	(214%)
Phe	57 \pm 2	82 \pm 2 ^a	(144%)
Ser	125 \pm 8	177 \pm 9 ^a	(141%)
Tau	42 \pm 1	71 \pm 3 ^a	(168%)
Thr	208 \pm 13	348 \pm 23 ^a	(167%)
Trp	52 \pm 2	73 \pm 3 ^a	(141%)
Tyr	69 \pm 3	110 \pm 4 ^a	(159%)
Val	256 \pm 9	430 \pm 13 ^a	(168%)

Statistical significance: ^a $P < 0.0005$ (paired t test).

Table 2

Effect of a protein-rich test meal consisting of 300 g roast beef on urinary amino acid concentrations ($\mu\text{mol}/\text{mmol}$ creatinine) in 18 human subjects (means \pm SEM)

	Before test meal	2 h after test meal	% of initial value
Ala	20.01 \pm 2.81	67.14 \pm 12.97 ^a	(240%)
Arg	2.43 \pm 0.27	3.79 \pm 0.49 ^c	(156%)
Asn	9.69 \pm 0.74	19.51 \pm 3.51 ^b	(201%)
Cit	21.68 \pm 0.82	34.15 \pm 1.78 ^e	(158%)
Gln	38.64 \pm 2.94	64.26 \pm 5.56 ^e	(166%)
Glu	2.95 \pm 0.22	3.02 \pm 0.43	(102%)
Gly	112.94 \pm 17.78	290.07 \pm 42.26 ^e	(257%)
Met	1.38 \pm 0.11	2.28 \pm 0.22 ^e	(165%)
Ser	27.46 \pm 1.97	55.84 \pm 5.73 ^e	(203%)
Tau	34.90 \pm 9.60	216.78 \pm 31.55 ^c	(621%)
Thr	53.05 \pm 9.51	150.01 \pm 28.05 ^e	(283%)
Tyr	9.77 \pm 1.45	16.58 \pm 1.77 ^e	(170%)
Val	3.99 \pm 0.27	6.55 \pm 0.53 ^e	(164%)

Statistical significance: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.005$, ^d $P < 0.001$, ^e $P < 0.0005$ (paired t test). The concentrations of Ile, Leu, Lys, Phe and Trp were below the detection limit in urine specimens of one or more of the volunteers and are therefore not included.

relatively. The urinary postprandial excretion of glutamate did not differ significantly from the preprandial baseline values.

The volume of unstimulated saliva produced before consumption of the protein-rich meal (0.34 ± 0.06 ml/min) was identical to the volume of saliva produced postprandially (0.35 ± 0.06 ml/min). Eleven amino acids studied were detected in the salivary samples of all volunteers. After the consumption of the high-protein meal, the salivary excretion of glycine showed a large increase (Table 3). In contrast, the salivary concentration of taurine postprandial showed a limited, but significant, decrease compared to the preprandial baseline value. The consumption of the protein-rich meal had no significant effect on the saliva concentrations of the other free amino acids. In the absence of feeding, no significant changes in the saliva amino acid concentrations were observed (data not shown).

For 6 of the 13 amino acids which were present in urine, we found a significant relation between the increase in plasma concentration and the increase in urinary amino acid excretion induced by consumption of a high-protein meal (Table 4). The r values for alanine, arginine, asparagine, glycine, threonine and valine varied between 0.46 and 0.77. For two of these amino acids (threonine and valine), we also observed a significant relationship between the increase in plasma concentration and the postprandial changes in saliva amino acid concentration.

Table 3

Effect of a protein-rich test meal consisting of 300 g roast beef on the amino acid concentrations in whole saliva ($\mu\text{mol/l}$) of 18 human subjects (means \pm SEM)

	Before test meal	2 h after test meal	% of initial value
Ala	12.43 ± 1.46	12.98 ± 1.24	(104%)
Arg	6.07 ± 0.78	8.24 ± 1.69	(136%)
Cit	3.97 ± 0.81	4.61 ± 0.79	(116%)
Gln	7.04 ± 0.93	7.47 ± 1.33	(106%)
Glu	12.94 ± 3.50	8.94 ± 1.15	(69%)
Gly	42.69 ± 7.15	93.69 ± 19.81^a	(219%)
Ser	8.77 ± 1.94	9.15 ± 1.37	(104%)
Tau	59.71 ± 7.02	50.39 ± 4.71^a	(84%)
Thr	6.53 ± 0.67	8.33 ± 1.74	(128%)
Tyr	56.49 ± 10.81	79.64 ± 21.11	(114%)
Val	5.84 ± 0.76	5.09 ± 0.70	(87%)

Statistical significance ^a $P < 0.05$ (paired t test). The concentrations of Asn, Ile, Leu, Lys, Met, Phe and Trp were below the detection limit in saliva specimen from one or more of the volunteers and are therefore not included.

Table 4

Concordance in reactivity between the changes in amino acid concentration in plasma, urine and saliva induced by a protein-rich test meal ($n = 18$)

	Plasma versus urine	Plasma versus saliva
Ala	0.515 ^a	0.190
Arg	0.457 ^a	-0.123
Asn	0.476 ^a	—
Cit	-0.189	-0.195
Gln	0.386	0.161
Glu	-0.342	0.193
Gly	0.462 ^a	0.294
Met	0.303	—
Ser	0.005	0.174
Tau	-0.235	-0.026
Thr	0.770 ^d	0.659 ^c
Tyr	0.247	0.082
Val	0.524 ^a	0.509 ^a

Statistical significance: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.005$, ^d $P < 0.001$.

The concentrations of Asn, Ile, Leu, Lys, Met, Phe and Trp were below the detection limit in both urine and/or salivary specimens in one or more of the volunteers and are therefore not included.

4. Discussion

A large number of studies have shown that consumption of an imbalanced protein-containing meal significantly affects the level and pattern of the plasma free amino acid concentrations [4–7,29,30]. The aim of the present study was to investigate whether acute postprandial changes in plasma free amino acid composition after a protein-rich test meal are reflected in the urinary and salivary concentration of the corresponding amino acids.

The mean preprandial plasma levels of all free amino acids in this study were within normal ranges published by other investigators [2,4,30]. The changes in plasma free amino acid concentrations 2 h after the consumption of 300 g roast beef were similar to the increases in free amino acid concentrations following the consumption of an identical amount of tenderloin steak [29] or 300 ml of a high-protein fluid [30]. The magnitude of the increase in plasma levels of essential free amino acids correlates directly with the amino acid content of the ingested protein [3,4].

The preprandial urinary excretion (between 8.00 am and 9.00 am) of almost all free amino acids was comparable with the corresponding mean 24-h urinary excretion in adults. For urinary threonine excretion, lower concentrations have

been reported [8,31], but in those studies the subjects differed with regard to age [8] and race [31]. The administration of the protein-rich meal increased the acute urinary excretion of all free amino acids significantly, with the exception of glutamate (Table 2). Glutamine synthase in the kidney, which converts glutamate into glutamine, may be responsible for this observation [32].

Positive correlations have been reported between long-term changes in amino acid concentration of plasma and urine [10–12]. Our data show that a positive relationship also exists between acute plasma changes and acute urinary excretion of several amino acids: alanine, arginine, asparagine, glycine, threonine and valine. These neutral and basic amino acids show, with the exception of asparagine, high absolute increases in plasma concentration postprandially. An increased plasma concentration of an amino acid will increase the tubular load. When the tubular load exceeds the rate of reabsorption of the amino acid, urinary excretion increases markedly and proportionally [33]. Despite a large postprandial increase in plasma glutamine concentration, no significant relationship with urinary excretion was observed. This may be related to extraction of glutamine by the kidneys in the postabsorptive state [34,35] which is consistent with the major role of glutamine in interorgan nitrogen transport [36]. Renal synthesis of serine [12] and conversion of phenylalanine to tyrosine [37] may obscure a possible relationship for these amino acids.

Urinary taurine excretion was altered in kittens and rats 7 days after changes in dietary intake of taurine [33,38,39]. A rapid change in rate of urinary taurine excretion in man is suggested by the disproportional high increase in urinary taurine concentration (+521%), compared to the modest increase in plasma concentration (+68%). This alteration occurs within 2 h.

There is general agreement that relatively low concentrations of free amino acids are found in whole saliva [16–21]. Since in animal studies intra-arterially injected radioactive amino acids appear unchanged in saliva, plasma free amino acids must contribute to the free amino acid content of saliva [24]. In man, a raised serum level of free amino acids is accompanied by their raised concentration in saliva [22]. In children, however, no significant relationship was observed between free amino acids in total saliva and in serum [18].

With the exception of tyrosine, we observed lower free salivary amino acid concentrations than previously reported [18,19,40]. However, these studies differed in saliva collection techniques, methods of amino acid analysis and/or age of the subjects. In our study, we analyzed unstimulated whole saliva from adults by a reversed-phase HPLC technique. In contrast, they analyzed either adult wax-stimulated whole saliva by gas chromatography [19,40] or unstimulated whole saliva from children with an amino acid analyzer [18].

Our data show a positive relationship in adults between acute plasma changes and acute salivary excretion of two amino acids: threonine and valine. These

neutral amino acids show very high absolute increases in plasma concentration postprandially.

Salivary glands are metabolically very active [21]. Selective metabolism of amino acids during passage from plasma to saliva may contribute to the differences in the relative concentrations of free amino acids in plasma and saliva [21,41,42].

In summary, our data suggest that in man the urinary amino acid excretion of several amino acids has the potential for estimating short-term changes in plasma concentrations, thereby making such investigations less invasive. Determination of salivary amino acid concentrations seems less appropriate for this purpose than urinary amino acid excretion.

Acknowledgments

The authors gratefully acknowledge G.M. van Woerkom (E.C. Slater Institute, Amsterdam) for performing amino acid analyses and Dr. O. Polsatsjova and C. Linthorst for technical assistance.

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