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Effect of L-arginine dietary supplementation on salivary urea concentration and pH in physically active individuals

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ABSTRACT

Background: The aim of this study was to assess if the consumption of 3 g of a commercially available L-arginine dietary supplement causes a postabsorptive rise in urea concentration or pH of unstimulated saliva in a group of physically active individuals.
Methods: Salivary urea and pH were determined for 117 participants in a randomized double-blinded placebo-controlled study. Samples were collected by 'spitting' method in fasting conditions. One hour prior to their second visit, participants consumed three tablets of L-arginine or placebo.
Results: Urea concentration was significantly lower at second measurement for both the study and control group. The magnitude of the change was not significant between the groups. pH was higher for both groups at second measurement, but only significant for the study group. The magnitude of the change was significant between the groups. Participants who intermittently ingested protein dietary supplements and those with a Body Mass Index (BMI) higher than 25 had significantly higher basal urea concentration.
Conclusions: The results of this study did not confirm the hypothesis. Further studies are needed to determine the effects of different doses of L-arginine supplements on the biochemical composition of saliva and the influence of their long-term consumption on the risk of developing dental diseases.

Keywords: L-arginine, oral health promotion, salivary pH, salivary urea, unstimulated saliva.

Abbreviations and acronyms: BMI = Body Mass Index; L-arg = L-arginine; SaU = salivary urea concentration.

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INTRODUCTION

Saliva is the principal biological protective factor against tooth demineralization. Both its quantitative and qualitative properties are important determinants of the risk of developing dental diseases.[1] One of the key factors in the pathogenesis of caries is dental plaque pH.[2,3] Saliva modifies plaque pH in a number of different ways, one of which is enhancement of alkali production. The formation of bases counteracts the effect of acids produced in dental plaque on plaque pH value and its microbial composition. Therefore, provision of alkali-producing substrates such as L-arginine (L-arg) and urea which can be metabolized by plaque bacteria to yield base (principally ammonia) could contribute to caries prevention by favouring higher local pH values. In such conditions tooth demineralization and the overgrowth of acidogenic/aciduric bacteria is less likely to occur.[4,5]

Sportspeople are common users of commercially available dietary supplements containing L-arg. Previous studies showed that oral supplementation of L-arg increases serum urea levels.[6–9] This could be followed by a rise of salivary urea concentration (SaU) because urea is excreted through salivary glands and positively correlates with serum urea levels.[10] A number of studies confirmed that urea can induce a concentration dependent rise of dental plaque pH.[11–15] Its hydrolysis by bacterial ureases might also influence salivary pH. The expected rise of SaU and/or salivary pH upon consumption of L-arg supplements can be considered a positive 'side effect' of these products as it augments the protective role of saliva in oral health preservation.

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This could be beneficial, especially for sportspeople, because they are considered to be under a particular risk of acid-mediated pathological tooth wear due to their active life and nutritive habits.[16] Because SaU correlates with serum urea levels, its rise could be especially valuable for the overall protective capacity of saliva during the night when salivary flow rate is reduced. Taking L-arg at bedtime is not unexpected because product labels often state this as a recommendation.

The purpose of this study was to assess if the consumption of a commercially available L-arg dietary supplement in a dose recommended by the manufacturer affects urea concentration and pH value of unstimulated saliva in a group of physically active young individuals.

SUBJECTS AND METHODS

Ethics approval

The study was approved by the Ethics Committee of the School of Dental Medicine and all participants signed informed consent forms. All experimental procedures were conducted in accordance with the Declaration of Helsinki.

Subjects

Students of the Faculty of Kinesiology, University of Zagreb, Croatia, were invited to voluntarily participate in the study. All volunteers completed a questionnaire regarding their general and oral health, medicine usage, nutritive habits, possible allergic reactions to drugs and nutrients, oral hygiene, smoking and usage of commercially available food supplements. Students with chronic general diseases, fixed orthodontic appliances, poor oral hygiene, active caries lesions, broken tooth fillings, gingival or periodontal inflammatory lesions and those undergoing dental bleaching were excluded from participation in the study.

Study design

The study was conducted as a randomized double-blinded placebo controlled study.

The total number of volunteers who met the inclusion criteria was 191. Seventy-four volunteers dropped out in the course of the study, primarily due to a lack of motivation. A total of 117 students completed the study; 59 in the study group (44 males and 15 females) and 58 in the control group (44 males and 14 females).

The pH value of unstimulated saliva and SaU were determined for each participant on two separate visits with 1–7 days interval between the visits. Both visits were scheduled at approximately the same time. Saliva specimens were collected by a 'spitting' method[17] between 7.30 am and 9.30 am under fasting conditions and avoiding morning toothbrushing. Smokers were asked to refrain from smoking prior to saliva sampling and students who consumed dietary supplements (mostly whey proteins and vitamin and mineral supplements at periods of intensive training) were asked not to use them at least one day prior to the defined day of measurements. The participants were instructed to drink at least one glass of water before going to sleep to assure adequate hydration. Collection time was 10–15 minutes and was the same at both visits for individual participants. The only difference between the visits was that the participants consumed three tablets of L-arg or placebo with water one hour prior to their second visit. The tablets were given to them at their first visit with written instructions. L-arg used in this study was L-arginine 3000 mg (Natrol Inc., Chatsworth, California, USA). Placebo tablets were fabricated to match L-arg tablets in size, shape and colour. They were provided by Belupo d.d. from Koprivnica, Croatia.

L-arginine dosage and safety

Commercially available L-arg is recommended in a single daily dose that ranges from 500 mg to 5 g. The most probable reason for this diversity in dosage is the fact that the recommended dietary allowance for L-arg is not determined. Shao and Hathcock[18] suggest a daily dose of 20 g as the observed safe level for healthy adults based on the available published human clinical trial data and the evidence for the absence of adverse effects.

Our study assessed postabsorptive salivary effects of a single dose of 3 g because of the following reasons: (1) it is a single daily dose recommended by many manufacturers (including Natrol Inc., USA); (2) it is approximately an average single daily dose recommended by different manufacturers; (3) it does not exclude the possibility of taking higher total daily doses. It can be assumed that an average user of dietary supplements would follow the directions on the product label and take the recommended dose. However, exceeding daily doses recommended by the manufacturer should not be unexpected because L-arg is a normal dietary constituent and as such likely to be considered harmless if taken in a somewhat higher dose.

Saliva specimen processing

Immediately after the completion of the collection period, the cups were weighed in order to determine

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salivary flow rate. The volume of saliva required for determining SaU (0.3 ml) was transferred from collection cups to plastic tubes (Terumo Europe NV, Leuven, Belgium) and kept on ice before freezing. The rest of the saliva sample was used to measure salivary pH. pH was determined with a hand-held pH meter (Piccolo Plus ATC pH meter, Hanna Instruments, Kehl am Rhein, Germany). These measurements were performed at the Department of Sport and Exercise Medicine, Faculty of Kinesiology, University of Zagreb. The saliva samples used for measurement of SaU were kept frozen (–20 °C) until the sialochemical analysis was possible. Samples were thawed at room temperature and centrifuged for 10 minutes at 3000 rpm (relative centrifugal force = 2000 g) before analysis. SaU was determined by UV enzymatic urease-glutamate dehydrogenase method on an Olympus AU 2700 immunochemistry analyser (Beckman Coulter Inc., Brea, California, USA) with appropriate reagents, calibrators and controls purchased from the same manufacturer. Measurement of SaU was performed at the Department of Laboratory Diagnostics, University Hospital Centre Zagreb.

Statistical analysis

Kolmogorov–Smirnov test and Levene's test were used to check the assumptions of normality and homogeneity of variance. Since the data were not normally distributed and none of the transformations was successful in transforming the data for all variables, non-parametric statistical analysis was performed. Thus, median was used as an average value and the interquartile range (IQR) as a measure of data variability. The Wilcoxon test was used for paired comparisons made on the same individual, and Mann–Whitney test was used for between-group comparison. Effect size was calculated using the formula $r = z/\sqrt{N}$.[19] Fisher's exact test was used to assess the difference of proportions for gender and dietary supplements usage between the groups and Chi-square test was used for comparison of difference for body weight categories and body mass index (BMI) categories between the study and control group. Correlation was tested using Spearman's rank correlation coefficient. Data were analysed using commercial software SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA), with significance preset at $\alpha < 0.05$ for a two-sided test.

RESULTS

A total of 117 students completed the study. Due to the low salivary flow (Q), salivary pH could not be determined for two participants and high viscosity of saliva disenabled SaU measurement for nine participants. Students reported no adverse effects. A couple of students declared the tablets were rather large and therefore difficult to swallow.

There were no statistically significant differences between the groups with regard to the participants' age, gender, body weight, BMI and consumption of dietary supplements (Table 1).

SaU was significantly lower at second measurement for both the study and control group with small or moderate effect size ($p = 0.013$, $r = -0.242$; $p < 0.001$, $r = -0.389$, respectively). However, the magnitude of the change in SaU was non-significant between the groups. Salivary pH was higher for both groups at second measurement, but the difference was statistically significant only for the study group ($p < 0.001$, $r = -0.372$). The magnitude of the pH change was significantly higher for the study than the control group ($p = 0.025$, $r = -0.209$). The study group had a significantly higher basal Q than the placebo group with small effect size ($p = 0.039$, $r = -0.193$). The magnitude of the salivary flow change (ΔQ) was significantly higher for the control than the study group ($p = 0.002$, $r = -0.280$). These results are shown in Table 2.

The results of Spearman correlation analysis performed to assess the degree of association between basal values of measured salivary variables and between changes in measured salivary variables were statistically significant but pointed out small or moderate association for the basal values of Q and pH (rho = 0.325, $p < 0.001$), for the basal values of Q and SaU (rho = –0.330, $p = 0.001$), for the ΔQ and ΔpH (rho = 0.262, $p = 0.049$) and for the ΔQ and ΔSaU (rho = –0.392, $p = 0.004$).

Male students had significantly higher basal values of both SaU and pH with small effect size ($p = 0.002$, $r = -0.300$; $p = 0.014$, $r = -0.229$, respectively).

Out of the total number of participants, 27 of them had BMI >25 thus making a subgroup of overweight participants. Comparison of basal values of measured salivary variables between the group with BMI >25 and participants with BMI 18.5–24.9 (normal body weight) showed that the basal pH was not significantly different between the groups, but SaU was significantly higher in the overweight group with small effect size (6.4 (IQR 5.7–9.1) vs 5.6 (IQR 4.9–6.6), respectively; $p = 0.005$, $r = -0.272$). There were no statistically significant differences in the magnitude of pH and SaU change due to L-arg consumption between the weight groups.

Out of the total number of participants, 17 of them had, during the course of the study, intermittently consumed commercially available dietary supplements. Comparison of basal values of measured salivary variables showed that the basal pH was not significantly different between the groups, but SaU was

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Table 1. Descriptive statistics of the sample

			Group			
			Placebo (n = 58)	L-arginine (n = 59)	Total (n = 117)	p
Age†	Mean ± SD		21.5 ± 1.84	21.9 ± 1.7	21.7 ± 1.8	
	Median (IQR)		21.5 (20.0–23.0)	22.0 (20.0–23.0)	20.0 (20.0–23.0)	0.223
Gender‡	Male	N	44	44	88	
		%	75.90	74.60	75.20	
	Female	N	14	15	29	
		%	24.10	25.40	24.80	1.000
Dietary Suppl‡	No	N	50	50	100	
		%	86.20	84.70	85.50	
	Yes	N	8	9	17	
		%	13.80	15.30	14.50	1.000
Body weight† (kg)	Mean ± SD		73.6 ± 10.9	74.3 ± 11.1	74.0 ± 10.9	
	Median (IQR)		73.0 (66.0–82.0)	74.0 (68.0–82.5)	74.0 (67.0–82.0)	0.758
Body weight categ§(kg)	<60	N	8	9	17	
		%	13.80	15.30	14.50	
	61–68	N	9	7	16	
		%	15.50	11.90	13.70	
	69–75	N	18	17	35	
		%	31.00	28.80	29.90	
	76–84	N	13	15	28	
		%	22.40	25.40	23.90	
	>85	N	10	11	21	
		%	17.20	18.60	17.90	0.972
BMI† (kg/m^2)	Mean ± SD		23.1 ± 2.0	23.6 ± 2.2	23.4 ± 2.1	
	Median (IQR)		23.1 (21.5–24.5)	23.6 (22.2–24.9)	23.3 (21.7–24.7)	0.266
BMI categ§ (kg/m^2)	<18.4	N	0	1	1	
		%	0	1.7	0.9	
	18.5–24.9	N	46	43	89	
		%	79.3	72.9	76.1	
	>25	N	12	15	27	
		%	20.7	25.4	23.1	0.490

N indicates sample size; SD = standard deviation; IQR = interquartile range; categ = category; BMI = Body Mass Index; †Mann–Whitney test; ‡Fisher's exact test; §Chi-square test.

Table 2. Between and within group comparison of measured salivary variables

	Placebo				L-arginine				p
	N	Mean ± SD	Median	IQR	N	Mean ± SD	Median	IQR	
[Urea1](mmol/l)	54	6.34 ± 2.40	5.90	5.00–7.50	54	6.16 ± 2.41	5.75	4.80–7.20	0.660
[Urea2](mmol/l)	54	5.22 ± 1.86	4.70**	3.90–6.40	54	5.36 ± 1.96	5.40**	4.20–6.60	0.513
Δ[Urea](mmol/l)	54	−1.12 ± 1.82	−1.10	−2.20–(−0.40)	54	−0.81 ± 2.25	−0.70	−1.40–0.30	0.135
pH1	58	6.92 ± 0.31	6.95	6.70–7.15	57	6.92 ± 0.28	6.98	6.75–7.07	0.987
pH2	58	6.97 ± 0.30	6.97	6.78–7.15	57	7.05 ± 0.21	7.07**	6.90–7.17	0.111
ΔpH	58	0.05 ± 0.20	0.02	−0.08–0.10	57	0.13 ± 0.22	0.10	−0.04–0.25	0.025*
Q1	58	0.37 ± 0.24	0.29	0.21–0.44	57	0.46 ± 0.27	0.42	0.25–0.61	0.039*
Q2	58	0.44 ± 0.27	0.39**	0.24–0.49	57	0.49 ± 0.29	0.45	0.26–0.61	0.224
ΔQ	58	0.07 ± 0.14	0.03	−0.03–0.17	57	0.03 ± 0.18	0.02	−0.09–0.11	0.126

N indicates sample size; SD = standard deviation; IQR = interquartile range; Q = resting salivary flow; Δ = the difference between the second and the first measurement; *statistically significant for Mann–Whitney test; **statistically significant for Wilcoxon test.

significantly higher in the group that consumed dietary supplements compared with the group that did not with small effect size (7.8 (IQR 5.7–10.9) vs 5.7 (IQR 4.9–6.7), respectively; p = 0.007, r = –0.259). Intermittent consumption of dietary supplements did not have an effect on the differences in the magnitude of pH and SaU change due to L-arg consumption between the study and control group.

DISCUSSION

The aim of this study was to assess if the consumption of a commercially available L-arg dietary supplement taken in a single dose of 3 g causes a postabsorptive rise in SaU or pH value of unstimulated saliva in a group of physically active healthy young individuals. The results of our study did not confirm this. If they

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had, it could be assumed that individuals who regularly consume these products might have higher protective ability of unstimulated saliva for a certain period of time after consumption. In the long-term, these biochemical changes could be associated with a reduced risk of tooth demineralization.

There are several possible reasons which could explain, at least in part, why our results showed a decrease in SaU for both groups instead of the expected increase in SaU in the study group.

Physiological range of SaU is relatively wide and depends on serum urea level which varies depending on the nutritive habits and metabolic state of an individual. Ensuring equal conditions for all participants with regard to qualitative and quantitative food intake and the extent of physical activity would probably contribute to lowering the inter- and intra-individual variability in SaU. Lack of control of these two factors, as well as a lack of strict control over the adherence of the participants to the protocol can be considered as drawbacks of this study. Thus, based on our results, a postabsorptive effect of 3 g L-arg on SaU cannot be excluded but could be smaller than expected. It is important to emphasize that the composition of L-arg tablets was not tested because testing of amino acids is not a routine procedure in Croatian laboratories that perform food analysis and quality control.

The basis of this study were results of previous studies on the effects of L-arg which showed that oral supplementation of L-arg increases serum urea levels.[6–9] However, single doses of L-arg in listed studies were higher (mostly 7 g) and the consumption of L-arg lasted continuously between three days and four weeks. Schwedhelm *et al.*[20] who used L-arg immediate-release 1.0 g tid and L-arg sustained-release 1.6 g bid for seven days did not find significant changes in serum urea concentrations. It is probably necessary to reach a certain critical dose of L-arg taken *per os* in order to gain a statistically significant urea rise. Cardoso *et al.*[10] showed a significant correlation between serum and salivary urea. Because serum urea levels were not measured in our study, we assume, based on the reported correlation, that 3 g of L-arg could still be too low of a dose to cause a notable serum urea rise. Cardoso *et al.*[10] also reported that SaU was independent of salivary flow rate. Other reports[21] suggest that there is a relationship between these two variables: higher SaU was found at lower salivary flow rates. Our results also showed a negative correlation between basal salivary flow and SaU, and between a change in salivary flow and a change in SaU. All of these results suggest that a rise in SaU which, presumably, would follow a sufficiently high L-arg dose would be most beneficial for protective capacity of saliva during the night, when salivary flow rate is the lowest. Besides showing dose dependence, certain effects of L-arg supplementation could also be dependent on the duration of continuous consumption of the supplement due to the complex mechanisms of regulation of the expression and activity of enzymes of L-arg metabolism.[22]

Dietary habits and nutritional status of individuals do determine values of biochemical parameters. The results of our study also indicate this. They showed significantly higher SaU in a subgroup of participants who declared to be users of commercially available protein supplements. The subgroup with BMI >25 also had significantly higher basal SaU. However, it is likely that a high BMI does not denote a group of overweight participants but rather their higher percentage of muscular tissue because participants were young physically active adults. This assumption is supported by the fact that six participants with BMI >25 were also students who declared to be users of commercial protein supplements.

Relation between salivary pH and SaU is such that pH rises only if salivary urea disappears, if it is hydrolized. Ureolysis undoubtedly influences salivary pH. However, even though a higher pH was measured in the study group upon consumption of L-arg, it cannot be concluded that this pH change can be attributed primarily to a higher rate of ureolysis, i.e. other factors may have modified its influence on the magnitude of the pH change. A negative correlation shown between basal salivary flow and SaU suggests a flow dependent delivery of urea and substrate availability may be one of the determinants of the rate of ureolysis. Other flow related factors also influence salivary pH: it is known that salivary pH rises with the rise of salivary flow. Both groups had a higher salivary flow at second measurement and a positive correlation between salivary pH and salivary flow was confirmed. However, even though a significant rise in pH was shown for the study group at second measurement, the change in salivary flow was non-significant. On the other hand, the control group had a significant rise in salivary flow but the pH change in this group was non-significant. This suggests that many factors influence oral acid-base balance and that the contribution of a specific factor to the pH change is difficult to single out in an *in vivo* experiment. Incidentally, this study assessed the influence of L-arg supplementation on salivary pH but a potential rise in SaU following L-arg consumption could be more important in the regulation of dental plaque pH. Other studies showed that salivary urea rapidly enters dental plaque where its hydrolysis causes a significant pH rise[23,24] and that the pH change positively correlates with the concentration of urea.[12,14,15,25,26] In addition, urea serves oral bacteria not only to regulate intra- and extracellular pH but also as a source of nitrogen for the synthesis of different compounds.[27]

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This could also determine the impact of ureolysis on both dental plaque pH and salivary pH.

It must be accentuated that even though higher salivary pH and higher SaU can be considered beneficial for the overall protective capacity of saliva, their specific clinical significance, as well as the significance of any other individual salivary variable in caries and/or dental erosion reduction, will depend on a number of coexisting factors that determine the overall individual risk of dental diseases.

To our knowledge, this is the first study to assess salivary effects of L-arg dietary supplementation.

More research is necessary to gain insight into serum and salivary biochemical changes caused by L-arg supplements depending on their dosage and time of continuous consumption. These changes could further be modified by interindividual differences in metabolic utilization of L-arg due to the different metabolic needs of the body, individual's dietary habits and his or her general health. However, it is highly unlikely that sportspeople and other users will consume L-arg or other dietary supplements in order to gain positive 'oral effects'. Dosage and the time of continuous consumption of a specific product are regularly determined by other goals (indications). This is why it would be cost-effective if the oral/salivary effects of L-arg were studied as a part of the studies that seek scientific confirmation of the effects that the users of this product (or their therapists) want to achieve. Indeed, a number of investigators are interested in determining the effects of L-arg supplementation on different physiological processes as well as its preventive and therapeutic potential. Oral effects of L-arg could be assessed simultaneously as potential positive side effects of the supplement consumption.

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REFERENCES

1. Leone CW, Oppenheim FG. Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. J Dent Educ 2001;65:1054–1062.
2. Abelson DC, Mandel ID. The effect of saliva on plaque pH *in vivo*. J Dent Res 1981;60:1634–1638.
3. Margolis HC, Duckworth JH, Moreno EC. Composition of pooled resting plaque fluid from caries-free and caries-susceptible individuals. J Dent Res 1988;67:1468–1475.
4. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to *Streptococcus mutans* and the specific-plaque hypothesis. Crit Rev Oral Biol M 2002;13: 108–125.
5. Marsh PD, Bradshaw DJ. Physiological approaches to the control of oral biofilms. Adv Dent Res 1997;11:176–185.
6. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 1992;90:1168–1172.
7. Adams MR, Forsyth CJ, Jessup W, Robinson J, Celermajer DS. Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. J Am Coll Cardiol 1995;26:1054–1061.
8. Clarkson P, Adams MR, Powe AJ, *et al*. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. J Clin Invest 1996;97:1989–1994.
9. Bennett-Richards KJ, Kattenhorn M, Donald AE, *et al*. Oral L-arginine does not improve endothelial dysfunction in children with chronic renal failure. Kidney Int 2002;62:1372–1378.
10. Cardoso EML, Arregger AL, Tumilasci OR, Elbert A, Contreras LN. Assessment of salivary urea as a less invasive alternative to serum determinations. Scand J Clin Lab Invest 2009;69:330–334.
11. Stephan RM. The effect of urea in counteracting the influence of carbohydrates on the pH of dental plaques. J Dent Res 1943;22:63–71.
12. Peterson S, Woodhead J, Crall J. Caries resistance in children with chronic renal failure: plaque pH, salivary pH, and salivary composition. Pediatr Res 1985;19:796–799.
13. Sissons CH, Wong L, Hancock EM, Cutress TW. The pH response to urea and the effect of liquid flow in 'artificial mouth' microcosm plaques. Arch Oral Biol 1994;39:497–505.
14. Imfeld T, Birkhed D, Lingström P. Effect of urea in sugar-free chewing gums on pH recovery in human dental plaque evaluated with three different methods. Caries Res 1995;29: 172–180.
15. Fure S, Lingström P, Birkhed D. Effect of three months' frequent use of sugar-free chewing gum with and without urea on calculus formation. J Dent Res 1998;77:1630–1637.
16. van Loveren C, Scheper WA, Eijkman MA. Sports diets and oral health. Ned Tijdschr Tandheelkd 2005;112:136–140.
17. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72–77.
18. Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regul Toxicol Pharm 2008;50:376–399.
19. Field A. Discovering statistics using SPSS. 2nd edn. London: Sage Publications, 2005.
20. Schwedhelm E, Maas R, Freese R, *et al*. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. Br J Clin Pharmacol 2008;65:51–59.
21. Michels LFE. Sialometry and Sialochemistry. In: Graamans K, van den Akker HP, eds. Diagnosis of Salivary Gland Disorders. Dordrecht: Kluwer Academic Publishers, 1991: 147–148.

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22. Morris SM Jr. Enzymes of arginine metabolism. J Nutr 2004;134:2743S–2747S.

23. Shu M, Morou-Bermudez E, Suarez-Perez E, *et al*. The relationship between dental caries status and dental plaque urease activity. Oral Microbiol Imunol 2007;22:61–66.

24. Nascimento MM, Gordan VV, Garvan CW, Browngardt CM, Burne RA. Correlations of oral bacteria L-arginine and urea catabolism with caries experience. Oral Microbiol Immunol 2009;24:89–95.

25. Gopinath VK, Tandon S, Shirwaikar A. The effect of chewing gums on acidogenicity of plaque after a sucrose challenge. J Clin Pediatr Dent 1997;22:77–81.

26. Dibdin GH, Dawes C. A mathematical model of the influence of salivary urea on the pH of fasted dental plaque and on the changes occurring during a cariogenic challenge. Caries Res 1998;32:70–74.

27. Singer DL, Kleinberg I. Quantitative assessment of urea, glucose and ammonia changes in human dental plaque and saliva following rinsing with urea and glucose. Arch Oral Biol 1983;28: 923–929.

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