IJIRR

International Journal of Information Research and Review
Vol. 2, Issue, 02, pp. 382-387 February, 2015

Research Article

METABOLOMIC ANALYSIS OF SALIVA IN THE COURSE OF EXPERIMENT WITH TOPICAL APPLICATION OF GLUCOSE AND FLUORINE GEL APPLICATION

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ARTICLE INFO

Article History:
Received 27th November, 2014
Received in revised form
20th December, 2014
Accepted 30th January, 2015
Published online 28st February, 2015

Keywords:
Physiological,
Remineralizing,
Investigation,
Concentration.

ABSTRACT

Changes in the composition of saliva can be related to different physiological processes and various oral diseases. Examination of saliva as a diagnostic environment for various biomarkers is a modern method that has been used in dental medicine since last century. Aim of the study was in vivo investigation of topical fluorides and prophylactic remineralizing procedures' influence on the end products of fat and protein metabolism in the saliva. The results showed that aggressive carbohydrate attacks or stimulation of carbohydrate cariogenic metabolism in the dental plaque did not affect the metabolic profile of the fatty and amino acids in the saliva. Topical fluoride application during the course of oral prophylactic procedures in children also did not change the concentration of the fatty and amino acids.

INTRODUCTION

The oral cavity is a dynamic system that is influenced by many factors of local and general nature. Disturbed by external influences balance in the oral environment creates risky environments, which is determinative of the multifactorial etiology of varied oral pathology. On the other hand, changes in the saliva composition can be attributed to the processes of systemic nature or due to various oral diseases (Giannobile *et al*., 2011; Scannapieco 1994; Slavkin *et al*., 2011). The idea to use the saliva as a diagnostic environment for various biomarkers appears in the second half of 20-th century. Its main advantage is that it is an environment that can be examined non-invasively and thus diagnostic and prognostic monitoring of oral diseases and some systemic diseases can be made (Baum *et al*., 2011; Edwards *et al*., 2006; Poboży 2006; Giannobile *et al*., 2011; Lee *et al*., 2011). After the complete decoding of the human genome, a new era in the study of biological systems is evolved formulated in a scientific direction - "- omics" technology. High-tech "-omics" analysis is related to exploration of DNA sequences, levels of gene expression and protein synthesis or analysis of the final metabolites of cellular or tissue origin (Baum *et al*., 2011; Caporossi *et al*., 2010; Fabian *et al*., 2008; Slavkin *et al*., 2011; Vitorino *et al*., 2004; Wong, 2011). Metabolome is a collection of all the metabolites in the body, which are end products of gene expression in the organism. These are usually small molecule metabolites: metabolic agents, hormones, signaling molecules, secondary metabolites (Barnes *et al*., 2011; Edwards *et al*., 2006; Goncalves *et al*., 2010). Metabolomic analysis of saliva represents a study of the concentration changes of various metabolic products in the dynamically changing oral liquid media, which are associated with genetic, biological processes, or changes in the environment (Edwards *et al*., 2006).

Metabolomic analysis (metabolomics) is a major discipline in modern "systematic biology", with many advantages compared to other analytical methods. The metabolites are end products of the process of gene transcription and translation, and they are the final phenotypic expression of the studied individual processes. In the last 20 years metabolomic analysis develops quickly due to the possibilities that the combination of chromatography and electrophoresis for separation with high resolution as well as mass spectrometry (MS) for accurate identification of biological molecules offer.

Aim of the study: To investigate the influence of topical fluorides and prophylactic remineralizing procedures on the end

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products of fat and protein metabolism in the saliva (*in vivo* clinical trial).

Tasks

- Metabolic profile of the fatty acids in the saliva during the course of oral prophylactic procedures in children.
- Metabolic profile of the amino acids in the saliva in the oral prophylactic procedures in children.

MATERIALS AND METHODS

Object of the study

Twenty children (aged between 10-12 years; 12 boys and 8 girls) were included in this clinical trial, divided into two groups: 1) 10 children undergoing a program of topical fluoride and remineralizing prevention with MiPaste at home (1 week before the experiment); 2) 10 children – with no program - control group. In the course of the clinical trial in the dental office children of both groups were divided into 2 subgroups of 5 children:

-first subgroup- with local application of fluoride gel before the gargle with glucose;
-second subgroup without local application of fluoride gel before the gargle with glucose;

The study is authorized to be conducted by KENIMUS (Ethics Committee of Medical Research at the Medical University - Sofia). All the children parents presented informed consent to participate in the experiment.

Methods of clinical trial

Preparation of children

Before the experiment, the children's oral status was evaluated and registered, oral hygiene instructions were made, new brushes and pastes were given, professional oral hygiene was held. Instruction for rational nutrition was made. MiPaste for daily use at home for a week was dealt to 10 of the 20 children.

Clinical experiment

After the preliminary preparing in the dental surgery, all children were included in the following experiment:

- Registration of oral hygiene index of Green-Vermillion-simple at the time of the experiment;
- First sampling of saliva for metabolomic analysis;
- Local application of fluoride gel in 5 of the children in both groups;
- Gargle with 10% glucose for 1 minute in ½ hours;
- Second sampling of the saliva in ½ hours;
- Freezing of samples.

Sampling of saliva

Conditions for sampling of saliva were standardized. The samples were taken in the morning – between 9-10 am, at least 2 hours before eating and tooth brushing. Before the saliva sampling the oral cavity was washed with 20 ml distilled water. Samples of no stimulated saliva for 3 min in scaled sterile container were taken.
The saliva has been frozen The aim of the experiment was that the liquid oral environment underwent sharply changeable external influences (carbohydrate attack and fluoride applications) which are very often in oral cavity of children and a metabolomic analysis of fat and protein metabolism in those conditions to be made.

Biochemical metabolomic analysis

The analysis of the saliva's metabolic profile was conducted with the use of highly effective liquid chromatography in combination with high-resolution mass spectroscopy (LC MS/MSn). Analysis was carried out on the hybrid LTQ Orbitrap® Discovery mass spectroscopy system. With the aim of identifying and quantitatively assessing the optimal number of metabolites in saliva we used various types of chromatographic methods (reverse phase, HILIC, ion-exchange) in combination with various mass spectroscopy methods (positive, negative ion detection, electrospray and chemical ionization). For processing and interpretation of the gathered data we used specialized software for non-differential data processing – SIEVE 1.3.

The analyses for the presence of amino acids (AA) in saliva were conducted useing originally developed methods for lab analysis "Analysis and synthesis of biologically active agents" Faculty of Medical chemistry and biochemistry – Medical University Sofia. The methods are based on the use of preliminary specific derivation (marking) of the analyzed substances with acridone containing reagents. The reagents we used are originally developed and synthesized in the laboratory and have, so far, never been documented in scientific literature. The resulting specifically marked derivatives of fatty acids and amino acids, after chromatographic separation, were analyzed with the use of the so called HRAM (high-resolution accurate mass) mass spectroscopy methods. The analytical methods used are distinct in their high sensitivity and classificatory precision. Characteristic of these methods is the fact that they require a minimum sample quantity (under 1 ml) and have a simplified preliminary sample analysis preparation.

Statistical methods

Statistical program SPSS-19 with significance level $P < 0.05$ and Independent T-test was used to compare the average digital variables.

RESULTS

1. Metabolic profile of fatty acids in saliva during oral prophylactic treatments in children.

- *children preconditioned with MiPaste*

The results are presented on the Table 1. The average amount of the isolated fatty acids in saliva were compared in 10 children with a one-week precondition with MiPaste, divided into 2 subgroups: first (code 1 and 2) - with topical application of a fluoride gel before gargle with 10% glucose; second (code 3 and 4) – no application of a topical fluoride gel and with a gargle with 10% glucose.

Table 1. Fatty acids in the saliva, during the experiment (µg / ml)

Fatty acids	Before Code	Mean ± SD (n=5)	After Code	Mean ± SD (n=5)	Independent T-test
Palmitoleic acid	1	4.42 ± 3.03	2	6.34 ± 2.58	T=-1.08 P=0.31
	3	6.86 ± 1.74	4	5.04 ± 1.51	T=1.58 P=0.17
Oleic acid	1	13.26 ± 7.32	2	21.55 ± 11.16	T=-1.39 P=0.20
	3	26.43 ± 10.96	4	19.08 ± 9.18	T=1.03 P=0.34
Linoleic acid	1	31.96±28.21	2	35.43±19.49	T=-0.23 P=0.83
	3	46.78±20.63	4	32.38±16.39	T=1.09 P=0.32
α-,γ-Linolenic acid	1	0.70.65728	2	0.42 ± 0.23	T=0.92 P=0.39
	3	1.07 ± 1.49	4	0.38 ± 0.45	T=0.89 P=0.41
Arachidic acid	1	7.40 ± 2.09	2	4.94 ± 2.98	T=1.51 P=0.17
	3	5.37 ± 1.33	4	4.38 ± 0.96	T=1.20 P=0.28
11 Z, 14 Z-Eicosadienoic acid	1	0.01 ± 0.02	2	0.19 ± 0.28	T=-1.41 P=0.20
	3	0.55 ± 0.83	4	0.32 ± 0.57	T=0.46 P=0.66
11 Z, 14 Z, 17 Z-Eicosatrienoic acid	1	0.22 ± 0.17	2	0.93 ± 1.14	T=-1.39 P=0.20
	3	2.00 ± 1.29	4	0.88 ±1.12	T=1.31 P=0.29
Behenic acid	1	0.28 ± 0.47	2	0.18 ± 0.12	T=0.49 P=0.64
	3	0.12 ± 0.08	4	0.03 ± 0.03	T=2.02 P=0.09
Erucic acid	1	1.48 ± 0.42	2	1.17 ± 0.26	T=1.38 P=0.20
	3	1.07 ± 0.26	4	0.88 ± 0.19	T=1.20 P=0.28
13 Z, 16 Z, 19 Z-Docosatrienoic acid	1	0.00 ± 0.00	2	0.00 ± 0.00	----------
	3	0.26 ± 0.52	4	0.42 ± 0.09	T=0.83 P=0.44
4 Z, 10 Z, 13 Z, 16 Z-Docosatetraenoic acid	1	0.02 ± 0.02	2	0.16 ± 0.23	T=-1.41 P=0.19
	3	0.53 ± 0.59	4	0.47 ± 0.65	T=0.14 P=0.90

Code 1 and 2 - with fluor gel; Code 3 and 4- with no fluor gel

Eleven of the most common in human organism unsaturated fatty acids were isolated in our study (saturated monocarboxylic acids and saturated aliphatic), which are the main source of energy and important to the composition of complex lipids: triacylglycerols, phospholipids, cholesterol esters and others. Oleic acid, linoleic acid, followed by palmitoleic acid and arachidic acid were isolated in the largest amounts. All other fatty acids are isolated in small amounts up to 3 µg / ml.

On the other hand, prolonged a single time topical fluoride application (NaF) at home, also does not change authentically fatty acid concentrations in saliva. Saturation of the oral environment (saliva and tooth surfaces) with mineral ions (calcium, phosphate and fluoride), high buffer activity and the expected higher level of total protein in the saliva due to the presence of casein in the composition of MiPaste, do not affect the lipid metabolism in saliva.

- children not preconditioned with MiPaste

Table 2. Fatty acids in the saliva, during the experiment (µg / ml)

Fatty acids	Before code	Mean ± SD (n=5)	After code	Mean ± SD (n=5)	Ind T-test
Palmitoleic acid	5	4.26 ± 3.15	6	5.87 ± 4.89	T=-0.62 P=0.55
	7	3.66 ± 2.14	8	2.81 ± 1.87	T=0.67 P=0.52
Oleic acid	5	10.23 ± 6.83	6	15.20 ± 10.23	T=-0.93 P=0.39
	7	10.60 ± 6.09	8	5.86 ± 3.34	T=1.53 P=0.17
Linoleic acid	5	13.60± 7.31	6	20.41±16.14	T=-0.86 P=0.42
	7	17.19±10.93	8	9.49 ± 6.66	T=1.35 P=0.22
α-, γ-Linolenic acid	5	0.13 ± 0.13	6	0.29 ± 0.48	T=-0.69 P=0.51
	7	0.24 ± 0.24	8	0.09 ± 0.09	T=1.36 P=0.21
Arachidic acid	5	4.40 ± 0.69	6	4.04 ± 2.36	T=0.33 P=0.75
	7	4.20 ± 2.51	8	2.80 ± 2.62	T=0.86 P=0.41
11 Z, 14 Z-Eicosadienoic acid	5	0.01 ± 0.02	6	0.06 ± 0.09	T=-1.13 P=0.29
	7	0.01 ± 0.01	8	0.00 ± 0.00	T=1.00 P=0.35
11 Z, 14 Z, 17 Z-Eicosatrienoic acid	5	0.46 ± 0.91	6	0.20 ± 0.20	T=0.62 P=0.55
	7	0.12 ± 0.18	8	0.03 ± 0.07	T=1.11 P=0.32
Behenic acid	5	0.04 ± 0.03	6	0.06 ± 0.08	T=0.46 P=0.66
	7	0.09 ± 0.07	8	0.04 ± 0.05	T=1.23 P=0.26
Erucic acid	5	0.88 ± 0.14	6	0.81 ± 0.47	T=0.33 P=0.75
	7	0.95 ± 0.29	8	0.81 ± 0.25	T=0.82 P=0.44
13 Z, 16 Z, 19 Z-Docosatrienoic acid	5	0.00 ± 0.00	6	0.40 ± 0.32	T=-2.79 P=0.02
	7	0.00 ± 0.00	8	0.00 ± 0.00	----------
4 Z, 10 Z, 13 Z, 16 Z-Docosatetraenoic acid	5	0.11 ± 0.16	6	0.04 ± 0.08	T=0.90 P=0.39
	7	0.09 ± 0.12	8	0.02 ± 0.05	T=1.11 P=0.30

Code 5 and 6 – with fluor gel; Code 7 and 8 *– with no fluor gel*

The Table shows that all the isolated fatty acids undergo minimal changes in their average concentrations before and after the glucose attack, irrespective of local application on NaF (P> 0.05). This is proof that the fatty acids present in the saliva as final metabolic products of lipid metabolism are not affected by sudden changes in the biochemistry of saliva as a result of aggressive carbohydrate attack and stimulation of carbohydrate cariogenic metabolism in the dental plaque.

The results are presented on the following Table 2. The average quantities of the isolated fatty acids in saliva in 10 children of the 2 groups with and without topical fluoride application (code 5, 6 and 7, 8) were compared. The result registered in the previous group that the aggressive carbohydrate attack with 10% glucose and local action of fluorine gel does not influence on the fat metabolism in saliva, was confirmed here.

Table 3. Amino acids in the saliva during the experiment (µg / ml)

Amino acids	Before code	Mean ± SD	After code	Mean ± SD	Ind T-test
Asp	1	41.79 ± 29.63	2	67.61 ± 22.11	T=-1.56 P=0.16
	3	26.31 ± 16.58	4	22.79 ± 16.54	T=0.30 P=0.77
Glu	1	90.81 ± 57.39	2	123.67 ± 43.88	T=-1.02 P=0.34
	3	7.66 ± 6.76	4	9.85 ± 7.54	T=-0.43 P=0.68
Asn	1	8.00 ± 2.47	2	5.04 ± 1.15	T=2.43 P=0.04
	3	5.62 ± 1.59	4	4.76 ± 1.34	T=0.83 P=0.44
Pro	1	330.40 ± 123.94	2	324.80±129.79	T=0.07 P=0.95
	3	261.29 ± 32.42	4	204.56±120.54	T=0.91 P=0.40
Gln	1	352.16 ± 191.36	2	818.46±326.69	T=-2.75 P=0.03
	3	378.76 ± 206.23	4	309.04±132.69	T=0.57 P=0.59
His	1	17.92 ± 14.16	2	35.20 ± 22.14	T=-1.47 P=0.18
	3	25.56 ± 5.43	4	17.11 ± 12.35	T=1.25 P=0.26
Ser	1	37.80 ± 23.25	2	72.77±14.92	T=-2.83 P=0.02
	3	30.81 ± 13.84	4	19.64 ± 2.48	T=1.59 P=0.16
Gly	1	211.48±77.64	2	221.60 ± 71.01	T=-0.22 P=0.84
	3	175.48 ± 20.57	4	140.76 ± 64.89	T=1.02 P=0.35
Thr	1	19.33 ± 11.63	2	27.74 ± 6.47	T=-1.41 P=0.20
	3	9.42 ± 5.44	4	9.10 ± 2.46	T=0.11 P=0.92
Arg	1	13.68 ± 14.56	2	40.45 ± 13.67	T=-2.99 P=0.02
	3	41.49 ± 32.42	4	39.85 ± 12.06	T=0.10 P=0.93
Met	1	10.08 ± 4.99	2	10.32 ± 3.84	T=0.09 P=0.93
	3	4.51 ± 1.19	4	3.57 ± 0.51	T=1.45 P=0.20
Tyr	1	46.82 ± 17.27	2	52.84 ± 17.47	T=-0.55 P=0.60
	3	32.72 ± 20.41	4	23.26 ± 3.86	T=0.91 P=0.40
Val	1	63.40 ± 29.89	2	61.87 ± 28.84	T=0.08 P=0.94
	3	22.44 ± 23.57	4	12.12 ± 6.31	T=0.85 P=0.43
Leu	1	47.38 ± 27.28	2	58.42 ± 21.38	T=-0.71 P=0.50
	3	20.86 ± 11.92	4	13.75 ± 0.78	T=1.19 P=0.28
Phe	1	38.43 ± 19.23	2	57.61 ± 17.05	T=-1.67 P=0.13
	3	33.92 ± 10.80	4	21.63 ± 4.93	T=2.07 P=0.08
Trp	1	10.06 ± 5.17	2	11.95 ± 3.91	T=-0.65 P=0.53
	3	6.04 ± 3.85	4	3.66 ± 2.29	T=1.06 P=0.33
Orn	1	35.88 ± 17.17	2	37.48 ± 8.19	T=-0.19 P=0.85
	3	32.71 ± 10.28	4	23.90 ± 9.10	T=1.28 P=0.25
Lys	1	99.74 ± 62.49	2	118.35 ± 59.55	T=-0.48 P=0.64
	3	72.34 ± 6.41	4	51.41 ± 17.88	T=2.04 P=0.07
Put	1	161.59 ± 60.40	2	101.42 ± 24.41	T=2.07 P=0.07
	3	85.28 ± 43.77	4	51.01 ± 19.72	T=1.43 P=0.20
Spm	1	17.16 ± 13.66	2	10.43 ± 2.58	T=1.08 P=0.31
	3	7.33 ± 2.87	4	6.48 ± 2.26	T=0.47 P=0.66
Spd	1	11.24 ± 5.89	2	10.88 ± 6.24	T=0.09 P=0.93
	3	2.74 ± 1.92	4	6.06 ± 6.52	T=-0.98 P=0.36

Code 1 and 2 – with fluor gel; Code 3 and 4 – with no fluor gel

No significant differences in all isolated fatty acids between the 2 groups (with and without fluoride application) before and after the attack carbohydrate (P> 0,05). The results here are similar to those in children preconditioned with MiPaste. It has been supposed that the isolated fatty acids had mainly from the cellular origin (membrane lipids of microorganisms and macro-organism's cells and enzymes), which determined their stability to external influences. This was confirmed by other authors (Zhang A *et al*. 2012).

2. Metabolic profile of amino acids in saliva during oral prophylactic treatments in children.

- children preconditioned with MiPaste

The results are presented on the following Table 3. The average amounts of isolated amino acids in saliva of the 10 children (preparation with MiPaste): (1) with topical application of fluorine gel (with code 1 and 2) and (2) without fluorine gel (code 3 and 4) were compared.

The Table shows that in the saliva of the studied children almost all existing 20 amino acids and 4 biogenic amines isolated were. The concentrations of glycine (Gln), glutamine (Glu) and proline (Pro) were highest.

Amino acids isolated in saliva of the studied children did not change their concentrations during the experiment, for children preconditioned with MiPaste (P> 0,05). Prolonged daily fluorine and remineralizing applications as well as single time fluorine and remineralizing applications immediately before carbohydrate attack with glucose do not change the profile of amino acids in saliva and do not affect their homeostasis.

- children not preconditioned with MiPaste

In children not preconditioned with MiPaste at home exactly the same tendencies and no significant differences between groups before and after the carbohydrate attack (P> 0,05) were observed.

DISCUSSION

Nowadays, metabolomic analysis is perceived as one of the best ways to assessing the human phenotype and forecasting its manifestation. Investigation of the metabolites profile in biological fluids, and especially in the saliva is a promising strategy for the detection of various diseases - cardiovascular, renal, autoimmune, viral and bacterial infections and especially cancers.

Saliva diagnostics based on metabolomics technology is a new direction that offers promising clinical strategy characterized the relations between salivary metabolites and certain diseases.

In saliva of the examined by us children almost all existing 20 amino acids and 4 biogenic amines isolated were. Glycine (Gln), glutamine (Glu) and proline (Pro) showed the highest concentrations.

Table 4. Amino acids in the saliva during the experiment (µg / ml)

Amino acids	Before code	Mean ± SD	After code	Mean ± SD	Ind T-test
Asp	5	15.78 ± 15.68	6	23.44 ± 11.48	T=-0.88 P=0.44
	7	15.03 ± 8.74	8	16.89± 15.04	T=-0.24 P=0.82
Glu	5	8.48 ± 10.56	6	17.07 ± 19.66	T=-0.86 P=0.41
	7	3.26 ± 6.80	8	7.43 ± 11.52	T=-0.70 P=0.51
Asn	5	5.65 ± 3.64	6	3.76 ± 1.62	T=1.06 P=0.32
	7	6.43 ± 2.33	8	5.46 ± 0.82	T=0.88 P=0.41
Pro	5	102.48 ± 67.93	6	157.12 ± 73.91	T=1.22 P=0.26
	7	175.22 ± 71.55	8	140.90 ± 23.05	T=1.02 P=0.38
Gln	5	295.95 ± 192.34	6	320.55±124.72	T=0.24 P=0.82
	7	469.07 ± 494.18	8	443.67±336.57	T=0.10 P=0.93
His	5	19.50 ± 22.06	6	24.46 ± 19.22	T=-0.38 P=0.72
	7	43.61 ± 21.69	8	23.48 ± 15.34	T=1.70 P=0.13
Ser	5	16.81 ± 11.41	6	24.99 ± 12.21	T=-1.09 P=0.31
	7	33.41 ± 26.05	8	22.64 ± 10.49	T=0.86 P=0.42
Gly	5	129.18 ± 69.3	6	120.11 ± 40.68	T=0.25 P=0.81
	7	199.56 ± 115.53	8	94.59 ± 26.76	T=1.98 P=0.08
Thr	5	5.44 ± 1.65	6	7.53 ± 3.80	T=-1.13 P=0.29
	7	12.84 ± 11.28	8	6.89 ± 2.32	T=1.16 P=0.28
Arg	5	14.52 ± 13.83	6	19.65 ± 12.88	T=-0.61 P=0.56
	7	33.16 ± 27.41	8	22.56 ± 14.49	T=0.77 P=0.47
Met	5	3.35 ± 0.37	6	3.87 ± 1.35	T=-0.83 P=0.43
	7	4.20 ± 1.86	8	3.08 ± 0.34	T=1.32 P=0.22
Tyr	5	20.47 ± 13.00	6	22.67 ± 11.96	T=-0.28 P=0.79
	7	34.20 ± 16.00	8	18.56 ± 7.83	T=1.96 P=0.09
Val	5	10.98 ± 5.52	6	22.12 ± 18.24	T=-1.31 P=0.23
	7	15.02 ± 11.02	8	10.21 ± 10.72	T=0.70 P=0.50
Leu	5	9.17 ± 2.54	6	16.81 ± 9.99	T=-1.66 P=0.14
	7	17.79 ± 14.25	8	11.66 ± 9.09	T=0.81 P=0.44
Phe	5	21.08 ± 12.13	6	26.40 ±12.28	T=-0.69 P=0.51
	7	29.49 ± 13.94	8	18.34 ± 9.71	T=1.47 P=0.18
Trp	5	2.37 ± 2.30	6	3.38 ± 2.50	T=-0.67 P=0.52
	7	2.89 ± 2.30	8	1.64 ± 0.84	T=1.14 P=0.29
Orn	5	30.72 ± 23.72	6	38.22 ± 13.08	T=-0.62 P=0.55
	7	32.60 ± 12.67	8	28.82 ± 11.28	T=0.50 P=0.63
Lys	5	45.74 ± 24.98	6	62.44 ± 25.87	T=-1.14 P=0.33
	7	51.68 ± 42.58	8	31.84 ± 21.98	T=0.93 P=0.38
Put	5	76.23 ± 53.02	6	64.87 ± 42.97	T=0.37 P=0.72
	7	62.86 ± 38.95	8	58.85 ± 32.13	T=0.18 P=0.86
Spm	5	8.42 ± 9.58	6	5.39 ± 3.44	T=0.66 P=0.52
	7	5.37 ± 2.24	8	4.71 ± 1.88	T=0.50 P=0.63
Spd	5	1.87 ± 1.53	6	3.94 ± 5.30	T=-0.84 P=0.43
	7	4.36 ± 4.82	8	6.15 ± 9.29	T=-0.38 P=0.71

Code 5 and 6 – with fluor gel; Code 7 and 8 – with no fluor gel

On the other hand, there are conflicting results on the relations between the metabolic products and oral diseases - caries, periodontal diseases or mucosal diseases (Zhang *et al*., 2002; Miller *et al*., 2006; Monton, 2007; Nordlund *et al*., 2009). Metabolomic analysis of liquid oral environment was not conducted in our country. Literature review of that problem showed that modern biochemical techniques made it possible to examine the final products of protein, carbohydrate and fat metabolism, which would enable conclusions about the processes in the oral cavity associated with oral or systemic pathology to be drawn. In our study, among the products of fat metabolism oleic acid, linoleic acid, followed by palmitoleic acid and arachidic acid in the bigest quantitiy isolated were. The origin of the fatty acids in saliva has been discussed in many publications. There are three possible directions: from serum in the salivary glands (by active or passive transport); food - it was shown that the fatty acids are retained in the mouth one hour after consumption; cell membranes and enzyme systems of oral microorganisms and macroorganism's cells (Schipper *et al*., 2007).

Scientific research determined Proline (Pro) and glycine (Gln), as the most frequent decarboxylated amino acids derived from proteins in the saliva. These two amino acids together represent 70-85% of the amino acid sequences in the structure of collagen, which glycine (Gln), proline (Pro) and hydroxyproline (Hyp) predominate in. Collagen is the major structural unit of the connective tissue which makes up the bigger part of the oral structures. On the other hand, proline (Pro) is a precursor of the glycoproteins in saliva (19 Schipper *et al*., 2007). It is involved in the most common repeating sequence of proline-rich proteins (PRP) that represent 25-30% of the total protein in the saliva and have high affinity to hydroxyapatite (Edwards *et al*., 2006). Glycine (Gln) is not an essential amino acid and it is synthesized from the amino acid serine (Ser) in the human organism. Glycine (Gln) is a precursor of porphyrins at microorganisms prevalent in the oral environment. It is an inhibitor of neurotransmitters in the central nervous system in the organism.

The conclusion that can be made from our study is that the lipid metabolism in saliva is a relatively constant and various prophylactic products applications and manipulations with different duration on the oral environment do not influence significantly on it. This supports the view that such metabolites may be used as markers to some common diseases and various physiological and pathological processes in the body and they are insignificantly affected by factors such as cariogenic nutrition, oral prophylactic procedures etc. (Dunn *et al*., 2011; Perinpanayagam *et al*., 1995).

The main conclusion in this study is related to demonstrate the stability of fat and protein metabolites in the oral environment during the prophylactic treatment with fluoride and remineralizing products as well as glucose attack. This fact is proof for predominance of endogenous source of metabolites derived from salivary glands, inflammatory tissue reactions (periodontal, mucosal) and microbial metabolism.

Conclusions

- The fatty and amino acids in saliva, as metabolic end products of metabolism are not affected by sudden changes in biochemistry of saliva (aggressive carbohydrate attacks or stimulation of carbohydrate cariogenic metabolism in the plaque).
- Topical fluoride application (NaF) also does not change the concentration of the fatty and amino acids.
- Lipid metabolism in saliva is a constant homeostatic process on which the various prophylactic products and manipulations with different duration impacted on the oral environment do not influence significantly.
- Similar metabolites can be used as markers to some common diseases and various physiological and pathological processes in the body and they are insignificantly influenced by external factors.

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