Omega-3 and Omega-6 salivary fatty acids as markers of dietary fat quality: A cross-sectional study in Argentina

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ABSTRACT

The use of saliva for analyzing biological compounds has recently been expanded. The aim of this study was to analyze the correlation between specific dietary sources of n-3 and n-6 fatty acids (FA) and their salivary levels to evaluate their role as intake markers.

Seventy-nine healthy volunteers were included. A validated food frequency questionnaire was used for data collection and Interfood v.1.3 software was employed to quantify food intake. Salivary samples were collected following international standards and FA profile was determined by gas liquid-chromatography. Multiple linear regression analyses were performed for dependent variables (salivary FA profile) to detect independent associations with n-3 and n-6 FA food source intake, adjusted by age, gender, body-mass index, total energy intake, regular exercise, alcohol intake and smoking. Salivary concentrations of alpha-linolenic acid (ALA) 18:3 n-3 were significantly associated with nuts intake (β=0.05, 95% CI 0.02-0.07, p=0.04). Salivary concentrations of linoleic acid (LA) 18:2 n-6 and arachidonic acid (AA) 20:4 n-6 were associated with the intake of n-6 vegetable oils and red meat, cold meat and viscera (β=-0.80, 95% CI 0.06-0.09 p=0.03; β=-0.40, 95% CI 0.30-0.50, p=0.02, respectively).

This study supports the hypothesis that salivary concentrations of n-3 and n-6 FA are related to food intake. Monitoring dietary FA though salivary markers is relevant for nutrition epidemiology and for prevention and management of several diseases related to fat intake.

Key words: *fatty acids, diet, saliva, biomarkers.*

Ácidos grasos salivales Omega-3 y Omega-6 como marcadores de la calidad lipídica de la dieta: un estudio de corte transversal en Argentina

RESUMEN

El uso de biomarcadores salivales está en continua expansión. El objetivo de este estudio fue analizar la asociación entre fuentes alimentarias de ácidos grasos (AG) n-3 y n-6 y sus concentraciones salivales como marcador de ingesta.

Participaron 79 voluntarios sanos. Se aplicó un cuestionario validado de frecuencia de consumo alimentario y el software Interfood v.1.3 para su procesamiento. Las muestras salivales se recogieron según estándares internacionales y se determinó el perfil de AG salivales por cromatografía gaseosa. Se desarrolló un modelo de regresión lineal múltiple ajustado por sexo, edad, índice de masa corporal, valor energético total, actividad física, consumo de tabaco y alcohol para analizar la asociación entre el perfil de AG salivales y la ingesta de alimentos fuente de AG n-3 y n-6. Las concentraciones salivales del AG alfa-linolénico (ALA) 18:3 n-3 se asociaron positivamente con la ingesta de nueces (β=0.05, IC 95% 0.02-0.07, p=0.04), mientras que las concentraciones salivales de ácido linoleico (AL) 18:2 n-6 y araquidónico (AA) 20:4 n-6 se asociaron con el consumo de aceites ricos en n-6 (β=0.80, 95% IC 0.06-0.09 p=0.03) y de carnes rojas, fiambres y embutidos y vísceras, (β=0.40, IC 95% 0.30-0.50, p=0.02). De acuerdo a estos resultados, las concentraciones salivales de AG n-3 y n-6 se relacionan a la ingesta de sus alimentos fuente. El monitoreo de la ingesta lipídica a través de biomarcadores salivales constituye un aporte a la epidemiología nutricional y a la prevención y tratamiento de patologías vinculadas a la ingesta de grasas.

Palabras clave: *ácidos grasos, saliva, dieta, biomarcadores.*

INTRODUCTION

The relationship between diet and the risk of developing chronic diseases has been documented in recent decades. Nutritional epidemiology, focusing on dietary intake, involves the development of several tools to estimate calories, proteins, carbohydrates, fats, vitamins, minerals, etc., such as diet records, dietary recalls and food frequency

questionnaires (FFQ). The FFQ is one of the most commonly used methods because it provides insight into the regular dietary intake of a population over time and is relatively cheap, quick and easy to use[1]. Although this progress in dietary recall tools is promising and cost-effective, the methods for assessing dietary intake are still not without intake error, a commonly cited research limitation. The combination of different methods (e.g. administration of different questionnaires and assessment of biomarker levels) could reduce respondent burden and reporting bias[2,3].

Blood is the most commonly used biological fluid for biomarker determination and is sensitive to dietary intake. Urine is a good indicator of hydrosoluble compound intake, since their concentration in urine depends on the nutrient saturation degree in tissues[4,5]. The use of saliva for analyzing biological compounds to evaluate nutritional status has recently been expanded[6-8]. Collecting saliva is non-invasive, more comfortable than venipuncture and requires no special equipment, and it is easily stored prior to analysis[9-11].

Fatty acid (FA) intake is reflected in a wide variety of biological tissue samples, such as serum and subcutaneous adipose tissue[12,13]. A previous study showed a significant increase of α-linolenic acid 18:3 n-3 (ALA) in saliva from vegetarians or semi-vegetarians compared to people who consumed a mixed diet rich in animal products, who had significantly higher levels of salivary arachidonic acid 20:4 n-6 (AA)[14]. This finding could be related to the significant intake of food rich in n-3 essential fatty acids (EFA), such as soy, dried fruits, sunflower and corn oils.

Competition between metabolic pathways may lead to changes in FA composition independent of dietary content. It is reasonable to expect that the best markers of dietary intake would be FA that cannot be endogenously synthetized such as *trans*, linoleic acid 18:2 n-6 (LA) and ALA. Other FAs whose synthesis depends on EFA intake include eicosapentaenoic acid 20:5 n-3 (EPA) and docosahexaenoic acid 22:6 n-3 (DHA), also present in fish and seafood[15]. EFA are important compounds which play a role in the complex biological process of inflammation. EFA and their metabolites are known to have pro- and anti-inflammatory actions and to regulate gene expression, enzyme activity, immune response and gluconeogenesis[16].

We hypothesized that FA salivary levels may be affected by dietary sources of FA. The main aim of this study was to analyze the correlation between specific dietary sources of n-3 and n-6 FA and salivary levels of EFA and derivatives, to evaluate their role as intake markers.

MATERIALS AND METHODS

Study participants

A cross-sectional study was carried out involving non-probability sampling of 79 male and female adults aged eighteen to eighty years who visited two hospitals in Córdoba (Privado and Córdoba), Argentina for a routine check-up. Subjects on special diets and those with digestive and/or metabolic dysfunction (such as celiac disease, lactose intolerance or diabetes) were excluded. All participants provided informed consent to participate in this study. The study complies with the Helsinki Declaration and was carried out according to the guidelines for the protection of the volunteers' rights. It was approved by the Institutional Ethics Committees (Córdoba, Argentina).

Dietary assessment

Participants were interviewed by two well-trained professional nutritionists. A validated qualitative and quantitative FFQ was used to collect data. It included questions related to 257 types of food and drinks consumed, with emphasis on food sources of fat[17]. This instrument includes questions on intake of whole and low-fat dairy products, cheese, eggs, beef, poultry, pork, fish, canned fish, seafood, viscera, sausages, vegetables, tomato derivatives, herbs, fruits, nuts, legumes, cereals, bakery products, animal fat, oils, seasonings, sugar, confectionery, pastry, beverages (alcoholic and non-alcoholic), snacks, ice cream and soy products. The frequency references used were the number of times each item was consumed per month, per week or per day and never. Portion size was described as small, medium or large using a photographic guide to help subjects understand these definitions[18].

Food data were processed using a software package called *Interfood v.1.3*, to produce information in grams/day of each food item consumed[19]. This software is an open-source program that has three basic components: dietary intake FFQ; a database of common foods and their composition considering 131 substances (macro- and micronutrients and

phytochemicals); and a relational database that links the FFQ data with the food database.

Collection of saliva samples

Saliva was collected from 8:00 to 10:00 a.m. after a minimum of six to eight hours fasting. With the subject leaning forward, unstimulated but spontaneously flowing whole saliva was collected in sterile plastic test tubes (5 mL or more). Prior to saliva collection, the study subjects did not consume any drinks and were at rest with no previous physical activity, tooth brushing and/or oral rinse[9]. Samples were frozen at -20ºC until they were processed by centrifuging at 80xg at 5ºC for 30 minutes. The supernatant was analyzed.

Fatty acids analysis

Total salivary lipids were extracted with chloroform-methanol 2:1 according to Folch's method and FA were methylated by sodium methoxide[20,21]. Salivary FA was separated, identified and quantified using a capillary column (BPX 70.30 m long, 0.25 mm ID, 0.25 um film, Phenomenex, Torrance, USA) in a gas chromatograph (Clarus 500 Perkin Elmer, Waltham, USA). Oven temperature began at 170ºC, rising 1.2ºC/min up to 240ºC, with a total chromatographic run time of 30 minutes[22]. The free FA were identified using commercial standards. Values, expressed as a percentage of detected FA, corresponded to the mean of two chromatographic runs on each sample.

Assessment of other variables

Certified personnel following standardized procedures took all additional measurements. Body weight and height were measured using a professional mechanical scale equipped with calibrated stadiometer. Participants were in underwear and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Other variables such as physical activity and alcohol and tobacco use were also evaluated.

Statistical analysis

Baseline demographic characteristics were calculated as means and standard deviations for continuous variables or as numbers and percentages for categorical variables. Gender differences in variables of lifestyle status, food intake and salivary FA concentrations were compared by Wilcoxon's test for continuous variables and Fisher's test for categorical variables. Multiple linear regression analyses were performed for dependent variables (salivary FA profile) to detect independent associations with n-3 and n-6 FA food source intake in grams/day (fish and seafood, nuts, vegetables oils rich in n-6, and red meat, cold meat and viscera), adjusted by age, gender, BMI, total energy intake, regular exercise, alcohol intake and smoking. All tests were two-sided and significance was considered at $p<0.05$. All analyses were performed with Stata® statistical software package (v.11.0).

RESULTS

Table 1 shows the basic characteristics of the study subjects. Mean age was 57.7 years for men and 49.2 years for women. 84.7% of men and 72.2% of women were current drinkers and 57.9% of men and 41.1% of women were current smokers. Average body mass index (BMI) was similar in men and women (27.7 and 25.5 kg/m^2, respectively). Regular physical activity was low in both men and women (47.3% and 45.1%, respectively). Calorie intake was 2678 ± 105 kcal/day for men and 2468 ± 387 kcal/day for women.

Dietary fat source profile is shown in Figure 1. Overall, the foods most often consumed were dairy products, red meat, confectionary and stuffed pasta. Regarding specific dietary n-6 FA food source

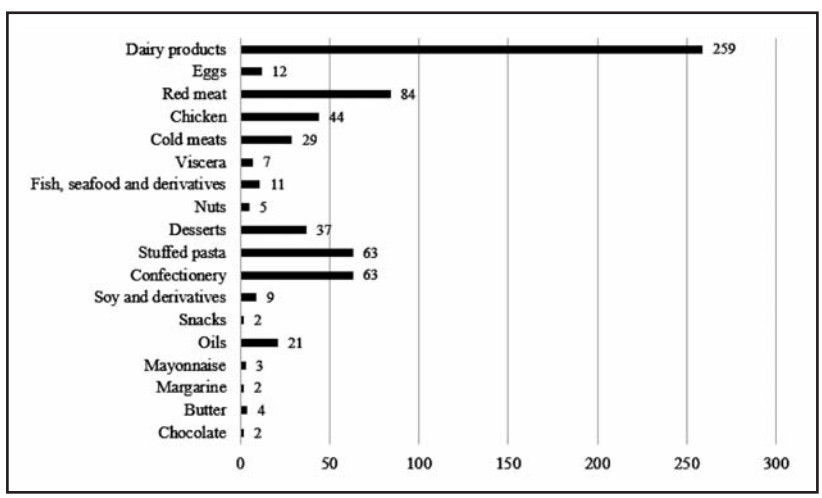

Fig. 1: Fat dietary source intake, grams per day.

intake, sunflower, soybean and corn oils were the oils most frequently consumed (oil average 21 ± 3 g/day) followed by red meat (84 ± 12 g/day). Total intake of red meat, viscera and cold meats was 120 ± 21 g/day). Regarding n-3 FA food source, fish, seafood and derivatives intake was 11 ± 5 g/day. Hake, mackerel, dorado and tuna were the most common fish species in the diet. Nuts intake (5± 2 g/day) included mainly walnuts and peanuts. Non-significant statistical differences were found for food intake, alcohol and tobacco use and physical activity by gender (p>0.05).

Table 1: Characteristics of study subjects.

	Men (n=37)	Women (n=42)	*p-value*
Age (years)	57.7 ± 12.4	49.2 ± 15.8	0.16
BMI	27.7 ± 3.2	25.5 ± 1.2	0.36
Energy intake (Kcal/day)	2678 ± 105	2468 ± 387	0.68
Current smoking (%)	57.9	41.1	0.27
Current alcohol drinking (%)	84.7	72.2	0.14
Regular physical activity (%)	47.3	45.1	0.88

p-values for sex differences are based on t tests for continuous variables and chi-square tests for categorical variables.
Values are mean ± standard deviation for continuous variables and number (percentage) for categorical variables.

Eighteen salivary FAs were analyzed by gas chromatography. Table 2 shows salivary FA composition. Non-significant statistical differences were found between men and women (p>0.05).

The results of multivariate analyses are shown in Table 3. Salivary concentrations of ALA 18:3 n-3 were significantly associated with the intake of nuts (β=0.05, 95% CI 0.02-0.07, p=0.04). On the other hand, salivary concentrations of LA 18:2 n-6 and AA 20:4 n-6 were associated with vegetable oils and with red meat, cold meat and viscera intakes, respectively (β=0.80, 95% CI 0.06-0.09 p=0.03; β=0.40, 95% CI 0.30-0.50, p=0.02, respectively).

DISCUSSION

This study showed a significant correlation between salivary ALA 18:3 n-3, LA 18:2 n-6 and AA 20:4 n-6, and dietary sources in a healthy adult

Table 2: Salivary fatty acid profile*.

FAs	Men	Women	Total	*p-value*	FAs	Men	Women	Total	*p-value*
4:0	5.8	3.1	4.4	0.69	**18:3 n-3**	3.9	4.4	4.1	0.20
	± 0.9	± 1.1	± 1.3			± 1.2	± 0.4	± 0.4	
6:0	4.3	2.8	3.5	0.20	**20:0**	1.8	0.5	1.1	0.37
	± 1.2	± 0.9	± 1.3			± 0.8	± 0.1	± 0.5	
12:0	3.1	4.2	3.6	0.42	**20:3 n-3**	1.5	2.3	1.8	0.43
	± 1.8	± 0.7	± 0.9			± 0.3	± 0.7	± 0.4	
14:0	2.3	4.1	3.6	0.22	**20:4 n-6**	5.2	6.3	5.6	0.41
	± 0.6	± 1.1	± 0.7			± 0.9	± 2.1	± 1.1	
16:0	22.2	23.6	22.5	0.40	**20:5 n-3**	1.3	0.9	1.0	0.46
	± 4.2	± 5.6	± 3.8			± 0.4	± 0.3	± 0.3	
16:1 n-7	2.2	3.1	2.7	0.36	**22:0**	1.7	0.9	1.4	0.19
	± 0.5	± 1.1	± 0.6			± 0.4	± 0.2	± 0.5	
18:0	13.5	15.4	14.7	0.45	**22:5 n-3**	2.6	1.8	2.2	0.80
	± 3.9	± 4.2	± 2.2			± 1.0	± 0.4	± 0.6	
18:1 n-9	11.2	8.3	9.2	0.44	**22:6 n-3**	2.2	1.8	2.1	0.22
	± 2.7	± 1.2	± 2.1			± 0.4	± 0.6	± 0.7	
18:2 n-6	13.4	15.5	14.1	0.66	**24:1 n-9**	1.8	1.1	1.5	0.52
	± 4.4	± 3.8	± 3.1			± 0.6	± 0.3	± 0.6	

*Values are expressed as **mean** percentage (**± standard deviation**) of total detected fatty acids.

population. Although the presence of FA in adipose tissue, serum or plasma is accepted as a reliable biomarker of their intake in food, there is still scanty literature referring to the correlation between dietary and salivary lipids[23,24].

Salivary LA 18:2 n-6 and ALA 18:3 n-3 would be expected to have the strongest association with dietary intake because they cannot be synthetized endogenously. In our study, nut intake, a source of n-3 FA, was associated with 18:3 n-3 salivary levels. Nuts are natural foods rich in unsaturated FA; most nuts contain substantial amounts of monounsaturated FA, while walnuts are especially rich in both n-6 and n-3 FA[25]. Although there is no evidence about nuts intake and salivary lipids, frequent consumption of nuts is related with lower concentrations of inflammation markers and is also recommended by the WHO Guideline on Sugar Intake for Adults and Children for dental health professionals[26-28]. The American Heart Association promotes the consumption of four servings (30-40 gram per portion) per week of nuts, seeds or legumes[29]. The study population shows inadequate intake of nuts according recommendations, but even though it was low, it was reflected in the salivary n-3 profile. On the other hand, we found no association between fish intake and salivary levels of long chain n-3 FAs due to the very low fish intake recorded, which was considerably lower than current guidelines (100 grams, twice a week). In this respect, other authors have shown a correlation between fish intake and plasma n-3 FA[30,31].

Our results showed significant association between oil intake –mainly sunflower oil, followed by soybean and corn oils– and salivary LA 18:2 n-6. Previous studies have reported high intake of sunflower oil in Argentina[32]. The aforementioned oils are the main sources of 18:2 n-6 FA[33,34]. The effects of 18:2 n-6 on health and disease are still controversial. An imbalance in the n-3:n-6 ratio, produced by an increased n-6 consumption or a reduced n-3 intake, may promote the endogenous synthesis of inflammatory molecules[35].

Significant association was observed between red meat intake, cold meat and viscera and AA 20:4 n-6 salivary concentrations. The presence of this FA in human saliva as related to diet and inflammatory conditions has been previously demonstrated [10,36]. Westernized diets characterized by high intake of red meat and processed foods have been related to inflammation biomarkers such as leukotrienes-2 and thromboxanes-4, which are associated with cardiovascular diseases and certain types of cancer[37]. EFA metabolites, derived by lipoxygenase and cyclooxygenase enzymatic pathways, have been quantified in human mixed saliva as well as in saliva fractions. The level of free AA and the quantitative hydroxyeicosatetraenoic acid (HETE) act as markers for the inflammatory processes occurring in the oral mucosa and in saliva in response to the development of squamous cell carcinoma[38]. In addition, pro-inflammatory lipid mediator precursors have been detected as potential markers for aggressive periodontitis[39]. In this sense, monitoring dietary FAs such as LA and ALA and their metabolites is a relevant strategy in the prevention and management of several fat intake-related diseases, not for only oral diseases, but also cardiovascular disorders, diabetes and tumors such as prostate and mammary, among others.

Secretion of saliva plays an important role in numerous significant biological processes. Although some salivary components are well characterized, data for characterizing salivary lipids and their functions is scarce and controversial. However, the

Table 3: Linear regression analysis (ß coefficient) by food intake.

Food intake	ß	95% Conf. Interval	*p-value*
Fish and seafood			
20:5 n-3	0.006	0.002-0.001	0.65
22:5 n-3	-0.0003	-0.0001-0.001	0.41
22:6 n-3	0.001	0.0004-0.002	0.59
Nuts			
18:2 n-6	0.02	0.008-0.07	0.36
18:3 n-3	0.05	0.02-0.07	**0.04**
Vegetable oils high in n-6 FA			
18:2 n-6	0.80	0.06-0.09	**0.03**
20:4 n-6	0.02	0.01-0.05	0.19
Red meat, cold meat and viscera			
20:4 n-6	0.40	0.30-0.50	**0.02**

ß: coefficient in multivariable model adjusted for age, sex, smoking status, body mass index, energy intake, alcohol consumption and physical activity.

qualitative and quantitative content of salivary lipids may change in pathological states (e.g. cystic fibrosis and diabetes), and according our results, due to nutritional exposure[40]. Thus, salivary lipids could be used for validating dietary measurement or as markers of food consumption.

Some limitations of the present study must be considered. It is cross-sectional design and therefore we cannot infer causality from these results. In addition, these findings cannot yet be generalized due to the sample size and the fact that a particular population was analyzed.

In conclusion, the present study supports the hypothesis that salivary concentrations of n-3 and n-6 FA are related to the intake of certain foods. Further studies are needed to confirm these results and to determine the causal direction of this relationship.

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REFERENCES

1. Ortiz-Andrellucchi A, Sánchez-Villegas A, Doreste-Alonso J, de Vries J, de Groot L, Serra-Majem L. Dietary assessment methods for micronutrient intake in elderly people: a systematic review. Br J Nutr 2009; (102 Suppl 1):S118-149.
2. Naska A, Lagiou A, Lagiou P. Dietary assessment methods in epidemiological research: current state of the art and future prospects. F1000 Res 2017; 6:926. doi: 10.12688/f1000research.10703.1. eCollection 2017.
3. Jenab M, Slimani N, Bictash M, Ferrari P, Bingham SA. Biomarkers in nutritional epidemiology: applications, needs and new horizons. Hum Genet 2009; 125: 507-525.
4. Corella D, Ordovás JM. Biomarkers: background, classification and guidelines for applications in nutritional epidemiology. Nutr Hosp 2015; 31(Suppl 3):177-188.
5. Blanck HM, Bowman BA, Cooper GR, Myers GR, Miller DT. Laboratory issues: use of nutritional biomarkers. J Nutr 2003; 133:888S-894S.
6. Karjalainen S, Sewón L, Söderling E, Larsson B, Johansson I, Simell O, Lapinleimu H, Seppänen R.Salivary cholesterol on healthy adults in relation to serum cholesterol concentration and oral health. J Dent Res 1997; 76:1637-1643.
7. Morzel M, Truntzer C, Neyraud E, Brignot H, Ducoroy P, Lucchi G, Canlet C, Gaillard S, et al. Associations between food consumption patterns and saliva composition: Specificities of eating difficulties children. Physiol Behav 2017; 173:116-123.
8. Defagó MD, Valentich MA, Actis AB. Lipid characterization of human saliva. J Calif Dent Assoc. 2011; 39: 874-880.
9. Hofman LF. Human saliva as a diagnostic specimen. J Nutr 2001;131:1621S-1625S.
10. Kaufman E, Lamster IB. The diagnostic applications of saliva. A review. Crit Rev Oral Biol Med 2002; 13:197-212.
11. Lawrence HP. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. J Can Dent Assoc 2002; 68:170-174.
12. Astorg P, Bertrais S, Laporte F, Arnault N, Estaquio C, Galan P, Favier A, Hercberg S.Plasma n-6 and n-3 polyunsaturated fatty acids as biomarkers of their dietary intakes: a cross-sectional study within a cohort of middle-aged French men and women. Eur J Clin Nutr 2008; 62:1155-1161.
13. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 2008; 47:348-380.
14. Actis AB, Perovic NR, Defagó D, Beccacece C, Eynard AR. Fatty acid profile of human saliva: a possible indicator of dietary fat intake. Arch Oral Biol 2005; 50:1-6.
15. Burdge GC. Metabolism of alpha-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids 2006; 75:161-168.
16. Das UN. Essential fatty acids and their metabolites as modulators of stem cell biology with reference to inflammation, cancer, and metastasis. Cancer Metastasis Rev 2011; 30: 311-324.
17. Perovic NR, Defagó MD, Aguinaldo A, Joekes S, Actis AB. Validación y reproducibilidad de un cuestionario de frecuencia de consumo alimentario para valorar la ingesta de lípidos y fitoquímicos Fac Cien Med Univ Nac Cordoba 2015; 72:69-77.
18. Vázquez MB, Witriw AM. Modelos visuales de alimentos y tablas de relación peso/volumen. 1st ed. Buenos Aires: Argentina, 1997.
19. Defagó MD, Perovic NR, Aguinaldo CA, Actis AB. Desarrollo de un programa informático para estudios nutricionales. Rev Panam Salud Publica 2009; 25:362-366.
20. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226:497-508.
21. Cantellops D, Reid AP, Eitenmiller RR, Long AR. Determination of lipids in infant formula powder by direct extraction methylation of lipids and fatty acid methyl esters (FAME) analysis by gas chromatography. J AOAC Int 1999; 82:1128-1139.

22. Tomita Y, Miyake N, Yamanaka S. Lipids in human parotid saliva with regard to caries experience. J Oleo Sci 2008; 57,115-121.
23. Arab L, Akbar J. Biomarkers and the measurement of fatty acids. Public Health Nutr 2002; 5:865-871.
24. Øverby NC, Serra-Majem L, Andersen LF. Dietary assessment methods on n-3 fatty acid intake: a systematic review. Br J Nutr 2009; (102 Suppl 1):S56-63.
25. Ros E, Mataix J. Fatty acid composition of nuts—implications for cardiovascular health. Br J Nutr 2006; (96 Suppl 2):S29-S35.
26. Rajaram S, Connell KM, Sabaté J. Effect of almond-enriched high-monounsaturated fat diet on selected markers of inflammation: a randomised, controlled, crossover study. Br J Nutr 2010; 103:907-912.
27. Ros E. Nuts and novel biomarkers of cardiovascular disease. Am J Clin Nutr 2009; 89:1649S-1656S.
28. Moynihan P, Makino Y, Petersen PE, Ogawa H. Implications of WHO Guideline on Sugars for dental health professionals. Community Dent Oral Epidemiol 2018; 46:1-7.
29. Van Horn L, Carson JA, Appel LJ, Burke LE, Economos C, Karmally W, Lancaster K, Lichtenstein AHet al. Recommended dietary pattern to achieve adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: a scientific statement from the American Heart Association. Circulation. 2016; 134:e505-e529.
30. Wilk JB, Tsai MY, Hanson NQ, Gaziano JM, Djoussé L. Plasma and dietary omega-3 fatty acids, fish intake, and heart failure risk in the Physicians' Health Study. Am J Clin Nutr 2012; 96:882-888.
31. Lucas M, Proust F, Blanchet C, Ferland A, Déry S, Abdous B, Dewailly E. Is marine mammal fat or fish intake most strongly associated with omega-3 blood levels among the Nunavik Inuit? Prostaglandins Leukot Essent Fatty Acids 2010; 83:143-150.
32. Navarro A, Muñoz SE, Lantieri MJ, Fabro EA, Eynard AR.Composition of saturated and unsaturated fatty acids of foods frequently consumed in Argentina. Arch Latinoam Nutr 1997; 47:276-281.
33. Matthaus B, Ozcan MM. Fatty acid and tocopherol contents of several soybean oils. Nat Prod Res 2014; 28:589-592.
34. Alezones J, Avila M, Chassaigne A, Barrientos V. Fatty acids profile characterization of white maize hybrids grown in Venezuela. Arch Latinoam Nutr 2010; 60:397-404.
35. Marangoni F, Novo G, Perna G, Perrone Filardi P, Pirelli S, Ceroti M, Querci A, et al. Omega-6 and omega-3 polyunsaturated fatty acid levels are reduced in whole blood of Italian patients with a recent myocardial infarction: the AGE-IM study. Atherosclerosis 2014; 232:334-338.
36. Elabdeen HR, Mustafa M, Szklenar M, Rühl R, Ali R, Bolstad AI.Ratio of pro-resolving and pro-inflammatory lipid mediator precursors as potential markers for aggressive periodontitis. PLoSOne 2013; 8:e70838.
37. Kim E, Coelho D, Blachier F. Review of the association between meat consumption and risk of colorectal cancer. Nutr Res 2013; 33:983-994.
38. Prasad S, Tyagi AK, Aggarwal BB. Detection of inflammatory biomarkers in saliva and urine: Potential in diagnosis, prevention, and treatment for chronic diseases. Exp Biol Med (Maywood) 2016; 241:783-799.
39. Elabdeen HR, Mustafa M, Szklenar M, Rühl R, Ali R, Bolstad AI.Ratio of pro-resolving and pro-inflammatory lipid mediator precursors as potential markers for aggressive periodontitis. PLoS One 2013; 8:e70838.
40. Matczuk J, Żendzian-Piotrowska M, Maciejczyk M, Kurek K. Salivary lipids: A review. Adv Clin Exp Med 2017; 26:1021-1029.