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ORIGINAL ARTICLE

Dietary fatty acids and the time elapsed from their intake are related to their composition in rat submandibular gland and salivary flow rates

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Abstract

Objectives The aim of this study was to analyze the influence of dietary fatty acids (FAs) and the time elapsed from their intake on FA tissue profile of rat submandibular gland (SG) and on its salivary flow rate (SFR). Do dietary FAs depending on the intake time modify their profile in SG and consequently the SFR?

Materials and methods Thirty-six adult male Wistar rats were fed on control diet (corn oil, CD, 18:2 n-6 FA) for 7 days and then divided into CD and two groups with replacement of corn oil by olive (OD, 18:1 n-9 FA) or chia (ChD, 18:3 n-3 FA) oils (1 and 30 day intake). Submandibular ducts were canalized to collect saliva for 20 min (μL/min). SG were examined (optical/electron microscopy; ImageJ 1.48 software).

Results SFR values were 6.18 ± 0.34 (CD1), 6.04 ± 0.31 (OD1), and 6.00 ± 0.50 (ChD1) ($p > 0.05$). At 30-day intake, higher SFR values in ChD (7.82 ± 0.7) with respect to CD (4.68 ± 0.44; $p < 0.001$) and OD (6.08 ± 0.2; $p = 0.038$) were found. ChD30 showed a higher serous acinous area percentage than CD30 and OD30, whereas mucous acinous density was greater in CD30 than in OD30 and ChD30 ($p < 0.05$). α-Linolenic (ALA) and eicosapentaenoic and docosahexaenoic acid levels were only detected in SG of ChD30, while arachidonic acid was lower in this group as compared with CD30 and OD30 ($p < 0.05$).

Conclusions SG FA composition and its SFR appear to be modulated by dietary FAs and the time elapsed from their consumption. SFR is highest with n-3 ALA-rich ChD at 30-day intake.

Clinical relevance Diet could contribute to improve secretory dysfunctions.

Keywords Salivary flow rates · Fatty acids · Dietary intake · Submandibular gland

Introduction

Salivary glands are involved in secretion of saliva which is a biological fluid that promotes tooth remineralization, protects against infections, allows taste perception of foods, and initiates digestion, among other functions [1–3]. A profuse secretion is mainly induced during the eating process by physiological stimulation (stimulated saliva). In addition, a lesser amount of unstimulated saliva which reflects basal salivary flow rate (SFR) is also produced. The whole unstimulated saliva—mostly secreted by the submandibular gland—is the most important in the maintenance of the oral and gastrointestinal

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mucosa integrity due to the constant cover and lubrication of the oral hard and soft tissues [4, 5].

Under healthy conditions, the total volume of saliva secreted in humans varies between 500 and 1500 mL/day [6], and the SFR (volume/min) ranges from 0.3 to 7 mL/min [7]. The quantitative and/or qualitative alterations of the salivary secretion cause a clinical imbalance manifested by xerostomia, halitosis, microorganism proliferation, increased caries incidence, taste perception and changes in chewing, difficulties in speech and swallowing, and digestive infections [4, 8].

Salivation may be stimulated or reduced by several physiological and pathological factors; age, circadian rhythm, and dietary intake are among the first. They have an impact on the salivary volume and its electrolyte concentrations [8, 9]. With respect to dietary intake, studies carried out on Japanese subjects have shown that those with a scarce intake of n-3 polyunsaturated fatty acids (FAs); potassium; vitamins D, E, and B6; and folate had a significantly lower SFR than participants consuming vegetables, fish, and shellfish [10].

Regarding pathological conditions, changes in saliva volume and composition were observed in patients taking specific medicines (antidepressants, anxiolytics, etc.), or under cancer cytostatic therapy, or radiation to the head and neck region, and/or suffering from certain autoimmune or systemic diseases such as the Sjögren's syndrome [8, 11]. In addition, Silvestre-Rangil [12] reported xerostomia in patients bearing rheumatoid arthritis. Other oral manifestations associated to a decrease in the salivary secretion are poor oral hygiene, increased accumulation of bacterial plaque, and periodontal disease.

Studies carried out in humans or animals highlighted interactions between diet and saliva volume and/or composition, suggesting salivary gland plasticity according to the dietary intake [13, 14]. However, information referred to the relationship between dietary FA intake and submandibular SFR was not found. The aim of the present study was to analyze the influence of different dietary FAs and the time elapsed from their intake on the FA tissue profile of submandibular gland and on its SFR in an experimental animal model.

Materials and methods

Animals

Thirty-six healthy adult male *Wistar* rats obtained from the Mercedes and Martín Ferreyra Research Institute and kept at the Institute of Health Sciences Research (Instituto de Investigaciones en Ciencias de la Salud, INICSA), CONICET-UNC, Córdoba, Argentina, were employed in this experimental study. Each rat (300 ± 50 g body weight) was housed in a separate cage under standard temperature conditions (23 ± 2 °C), with 12-h light-dark cycles. Animals had access to food (commercial or laboratory diet) and water ad libitum. Daily food intake (g/day) as well as the initial and final body weight (g) was registered.

The animal handling was done according to the international guidelines for the care and use of laboratory animals. The study protocol was approved by the Institutional Ethics Committee on Health Research (CIEIS) of the School of Dentistry, National University of Córdoba, Argentina (Protocol # 12/2013).

Diets

After weaning, the animals received a standard commercial chow (Asociación Balanceado Cooperación, Gilardoni, Argentina). At the 11th week, all rats were changed to a laboratory-made diet containing corn oil (rich in 18:2 n-6 FA) as fat source. It was based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G) [15], except for the FA source that was based on AIN-76 [16]. The composition of both diets is presented in Table 1. After 7 days, the animals were randomly divided into three dietary groups: (1) control diet (CD) (animals continued on the same diet), (2) olive oil diet (OD), and (3) chia oil diet (ChD), in which the corn oil was replaced by olive oil (rich in 18:1 n-9) or chia oil (rich in 18:3 n-3), respectively. Rats were fed on these diets for 1 to 30 days according to the experimental schedule (CD1, CD30; OD1, OD30; ChD1; and ChD30 groups). All diets were isoenergetic, providing 16.6 kJ/g. FA composition of dietary oils—determined by gas chromatography—is shown in Table 2.

Sample collection

At 1 and 30 days after starting the specific diets, the animals ($n = 18$ *per* experimental time) were weighed and anesthetized through an intraperitoneal injection of chloral hydrate (0.6 mg/kg body weight). A tracheotomy was performed for free pulmonary ventilation. Body temperature was measured through a rectal thermometer and maintained at 37.5 °C. Secretory ducts of both submandibular glands were exposed and cannulated by using fine glass tubes that gave about 45 drops/mL of distilled water [17, 18]. Secretory responses were obtained by injecting isoproterenol and pilocarpine intraperitoneally (5 mg/kg body weight of each one dissolved in isotonic saline solution) [19–21], since both branches of the autonomic nervous system acting synergically are responsible for the nervous control of saliva secretion. Saliva was collected into sterile 1.5-mL tubes for 20 min from the moment in which the first drop appeared at the end of the cannula. Left and right submandibular glands ($n = 72$) were immediately removed. The saliva and left submandibular gland were kept on ice and stored at − 80 °C until processing, whereas the right submandibular gland was fixed for microscopy. Rats were then euthanized by cervical dislocation.

Table 1 Composition of diets

Weaning at 10th week. All animals consumed chow diet → From 11th week, rats consumed experimental diets for 1 or 30 days

	Chow ($n = 36$)		CD		OD		ChD	
			1 day ($n = 6$)	30 days ($n = 6$)	1 day ($n = 6$)	30 days ($n = 6$)	1 day ($n = 6$)	30 days ($n = 6$)
Protein	23%	Protein	20%		20%		20%	
Raw fiber	6%	Fibers	5%		5%		5%	
Total minerals	10%	Minerals	3.5%		3.5%		3.5%	
Calcium	1–1.4%	Vitamins	1%		1%		1%	
Phosphorus	0.5–0.8%							
Chlorine	0.3%	Cystine/methionine/ choline	0.55%		0.55%		0.55%	
Sodium	0.2%							
Potassium	0.7%							
Magnesium	0.2%	Energy (Kjoule/G)	14%		14%		14%	
Sulfur	0.16%	Starch	52.9%		52.9%		52.9%	
		Saccharose	10%		10%		10%	
Fat source	5%	Fat source	7% corn oil		7% olive oil		7% chia oil	
Humidity	12%	Humidity	–		–		–	

CD, corn diet; OD, olive diet; ChD, chia diet

Homogenization

The left submandibular glands were homogenized ten times and then diluted in PBS (0.02 mol L-1, pH 7.0–7.2) by a glass homogenizer (Omni TH, International).

Gas chromatography

Lipids from left submandibular glands were extracted by means of chloroform-methanol 2:1 according to Folch's method [22].

Table 2 Fatty acid composition of dietary oils

FA	Common name	Corn	Olive	Chia
14:0	Myristic	0.03	Nd	Nd
16:0	Palmitic	12.21	17.10	6.83
16:1 n-7	Palmitoleic	0.12	1.97	Nd
17:0	Margaric	Nd	0.08	0.12
18:0	Stearic	1.93	1.58	2.62
18:1 n-9	Oleic	31.95	**55.19**	5.05
18:1 n-11	Vaccenic	0.54	4.76	0.96
18:2 n-6	Linoleic	**51.26**	17.21	18.91
20:0	Arachidic	0.50	0.30	0.17
20:1 n-9	Gondoic	0.25	Nd	Nd
20:1 n-11	Gadoleic	Nd	0.25	Nd
18:3 n-3	Alpha-linolenic	0.88	0.75	**65.11**
22:0	Behenic	0.16	0.13	Nd
24:0	Lignoceric	0.15	Nd	Nd
LA/OA/ALA ratio		58/36/1	23/74/1	4/1/13

FA, fatty acid; LA, linoleic acid; OA, oleic acid; ALA, alpha-linolenic acid. Values correspond to percentages of FAMEs. Nd: nondetected. Percentages of the main FAs in each oil are remarked

FAs were then methylated by sodium methoxide [23] and dried into a nitrogen atmosphere. FA methyl esters (FAMEs) were analyzed by gas chromatography by means of a Shimadzu 2014 chromatograph equipped with a flame ionization detector. Temperature of both injector and detector was 250 °C, and nitrogen was the carrier gas. FAMEs were analyzed by using a 100 m × 0.25 mm × 0.2 mm film thickness SP-Sil 88 capillary column (Varian, Darmstadt, Germany). Analysis was focused on the most representative FAs from each dietary group: oleic 18:1 n-9 (OA), linoleic 18:2 n-6 (LA), and alpha-linolenic 18:3 n-3 (ALA) acids as well as arachidonic 20:4 n-6 (AA) and eicosapentaenoic 20:5 n-3 (EPA) acids. The results are expressed as percentages of total FAMEs (%) of the main FAs. FAME values below 0.1 a% were considered minor and are not shown. FAME identification was based on retention times of authenticated FAME commercial standards (AccuStandard, New Haven, USA, and Sigma, St. Louis, MO, USA). Chromatographic data were processed by gas chromatography solution software (Supplier: JENCK S.A. Instrumental, Buenos Aires, Argentina).

Optical microscopy

Samples from right submandibular glands were fixed in 10% buffered formalin solution and embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin (H/E) and periodic acid–Schiff (PAS) according to routine procedures in our laboratory. The evaluation was performed on photomicrographs obtained by a LeicaDM750 light microscope (Leica Microsystems, Wetzlar, Germany) at ×10 and ×40 magnifications. Images were analyzed by means of the Image J 1.48 software (1.48, National Institute of Health, MD, USA).

The density of serous and mucous acini—expressed as image area percentage (a%)—was analyzed in H/E and in PAS/H stained preparations, respectively. Values correspond to the mean obtained *per* treatment. Samples of submandibular glands belonging to the CD30, OD30, and ChD30 groups were just considered since 1-day intake is a very short time for expected structural changes.

Electron microscopy

Salivary gland samples were processed as described by De Paul [24]. They were washed and fixed in a mixture of 4% formaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer for 2 h and then treated with 1% OsO4 for 1 h, before being stained in a block with 1% uranyl acetate in 0.1 M acetate buffer pH 5.2 for 20 min. After dehydration by graded cold acetones, the glands were embedded in Araldite. Thin sections were obtained by using a JEOL ultramicrotome with a diamond knife. They were then stained with uranyl acetate/lead citrate and examined through a Zeiss Leo 906-E electron microscope (Oberkochen, Germany).

Images were analyzed by means of the Image J 1.48 software to determine the average diameter of acinar cell nuclei and secretory vesicles as well as the rough endoplasmic reticulum (RER) occupied area. Electron microscopy analyses were performed in CD30, OD30, and ChD30 groups due to the changes observed by optical microscopy.

Statistical analysis

The Student's *t* test was applied to compare SFR (μL/min) between dietary groups according to different experimental times. The nonparametric Kruskal-Wallis test was employed to compare submandibular gland FA profile and microscopic data between groups. The Spearman correlation coefficient test was employed to quantify the association between the SFR and the submandibular gland FAs of each dietary group. According to its strength, the statistical association may be very strong (0.8 to 1.0 or − 0.8 to − 1.0), strong (0.6 to 0.8 or − 0.6 to 0.8), moderate (0.3 to 0.5 or − 0.3–0.5), weak (0.1 to 0.3 or − 0.1 to − 0.3), and very weak or none (0.1 to − 0.1) [25]. A $p \leq 0.05$ significance level was considered. The *Infostat* statistical program [26] was employed.

Results

Salivary flow rate

The stimulated submandibular SFR of the three dietary groups according to time is presented in Table 3. No statistically significant differences between CD1, OD1, and ChD1 groups ($p > 0.05$) were observed. On the other hand, higher values in ChD30 with respect to CD30 ($p < 0.001$) and OD30 ($p = 0.038$) were found.

Table 3 Submandibular salivary flow rate according to dietary group and time

Time	Dietary group		
	CD	OD	ChD
1 day	6.18 ± 0.34^{A}	6.04 ± 0.31^{A}	6.00 ± 0.50^{A}
30 days	4.68 ± 0.44^{A}	6.08 ± 0.21^{B}	7.82 ± 0.71^{C}

CD, corn diet; OD, olive diet; ChD, chia diet. Values are expressed as μL/min and correspond to media ± SE. Student *t* test. Different superscript letter indicates a significant difference ($^{A/B/C}$ $p \leq 0.05$) between dietary groups

Within-group analyses showed that the SFR had a significant decrease of 24% at 30-day intake ($p = 0.01$) with respect of CD1, whereas OD30 maintained similar values than 1-day intake ($p > 0.05$). In contrast, the SFR showed an increase of 30.4% in ChD30 group as compared with ChD1 ($p = 0.02$).

SFR and submandibular gland FA profile according to dietary intake and time

Table 4 shows the submandibular gland FA profile. At 1 day-intake, ALA was just detected in ChD. EPA (nondetected in CD1), and DHA were higher, and AA was lower in ChD1 than in CD1 and OD1. DGLA was higher in OD than in ChD1 and CD1. At 30 day-intake, ALA, EPA, and DHA levels were just detected in SG of ChD30, while AA was lower in this group as compared with CD30 and OD30. OA was higher in OD30 than in the other dietary groups. In addition, some correlations between them were observed: *CD group*: weak negative correlation between SFR and AA ($r = -0.24$, $p > 0.05$). *OD group*: moderate significant negative correlation between SFR and LA ($r = -0.59$, $p = 0.05$) and AA ($r = -0.61$, $p = 0.05$). *ChD group*: moderate significant negative correlation and weak positive nonsignificant association between SFR and AA ($r = -0.53$, $p = 0.05$) and EPA ($r = 0.25$, $p > 0.05$), respectively.

Quantification of submandibular gland serous and mucous acini

Figure 1 shows serous and mucous acinous density according to different experimental groups. The ChD30 group showed a higher area percentage of serous acini than CD30 and OD30 ($p < 0.05$), whereas the density of mucous acini was greater in CD30 than in OD30 and ChD30 ($p < 0.05$).

Table 4 Submandibular gland FA profile according to dietary groups and time from intake

FA	Dietary groups								
	CD			OD			ChD		
	1 day	30 days	*p**	1 day	30 days	*p**	1 day	30 days	*p**
PA	29.03 ± 0.71	24.39 ± 2.31	*0.066*	28.87 ± 0.18	27.56 ± 0.55	*0.185*	29.12 ± 0.47	26.5 ± 1.56	*0.095*
POA	2.77 ± 0.21	2.38 ± 0.41	*0.275*	2.52 ± 0.41	2.62 ± 0.17	*0.689*	2.38 ± 0.11	2.62 ± 0.49	*0.585*
SA	11.04 ± 0.58	9.22 ± 0.58	*0.118*	11.95 ± 0.33^{A}	9.03 ± 0.28^{B}	***0.007***	10.88 ± 0.47	10.66 ± 0.33	*0.219*
OA	11.35 ± 1.09	10.95 ± 1.36	*0.766*	10.11 ± 0.59^{A}	15.77 ± 0.81^{B}	***0.049***	12.97 ± 0.71	10.42 ± 0.73	*0.066*
LA	12.94 ± 1.24	11.82 ± 1.74	*0.368*	10.31 ± 0.36	11.9 ± 0.71	*0.057*	12.4 ± 0.81^{A}	15.5 ± 1.05^{B}	***0.009***
ALA	Nd	Nd	–	Nd	Nd	–	0.81 ± 0.15^{A}	3 ± 0.59^{B}	***0.007***
DGLA	3.84 ± 0.31	2.92 ± 0.21	*0.096*	5.06 ± 0.31	2.25 ± 0.17	*0.065*	4.13 ± 0.19^{A}	1.98 ± 0.09^{B}	***0.002***
AA	23.82 ± 1.01^{A}	15.11 ± 1.71^{B}	***0.007***	22.52 ± 1.09^{A}	15.01 ± 1.31^{B}	***0.001***	16.42 ± 1.11^{A}	9.76 ± 0.12^{B}	***0.003***
EPA	Nd	Nd	–	0.12 ± 0.02	Nd	–	0.81 ± 0.09^{A}	4.08 ± 0.56^{B}	***0.001***
DPA	0.5 ± 0.17	Nd	–	0.39 ± 0.04	Nd	–	0.48 ± 0.06^{A}	1.28 ± 0.11^{B}	***0.016***
DHA	0.62 ± 0.04	0.57 ± 0.13	*0.905*	0.63 ± 0.04	0.89 ± 0.12	*0.057*	0.84 ± 0.11	0.77 ± 0.11	*0.683*
n-6/n-3 PUFA	36.25	51.67	–	33.23	32.83	–	11.20	2.98	–

CD, corn diet; OD, olive diet; ChD, chia diet; FA, fatty acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Values of n-6/n-3 PUFA ratio are referred to 1 (e.g., 36.25/1). Values are expressed as percentages of total FAMEs and correspond to mean ± SE. Nd: nondetected. * Kruskal-Wallis test. Different superscript letter indicates a significant difference ($^{A/B}$ $p \leq 0.05$)

Analysis of submandibular gland acinar cells

Figure 2 shows submandibular glands of CD30, OD30, and ChD30 groups through electron microscopy. Secretory vesicles were smaller in ChD30 than in CD30 and OD30 ($p < 0.05$) (Table 5). No statistically significant differences in acinar cell nucleus diameter, RER, and vesicular a% were observed between the dietary groups.

Discussion

The present study was designed to analyze the influence of different dietary FAs and the time elapsed from their intake, on the FA composition of the submandibular gland and on its SFR in an experimental animal model. The results highlight that 30-day intake of chia diet (rich in ALA) increases the SFR; olive diet (rich in OA) maintains their values, whereas at that time, the corn diet (rich in LA) consumption decreases it. Iwasaki [10] reported that the intake of n-3 polyunsaturated FAs, potassium, folates, and vitamins present in vegetables, fish, and shellfish induces a SFR increase in humans. Other experimental studies have shown that the diet-induced changes in the membrane lipid composition of rat submandibular salivary glands may alter $Na^{+}K^{+}$-ATPase and adenylate cyclase activities [27, 28]. According to Rodríguez-Cruz [29], highly unsaturated FAs like EPA, DHA, and AA are mainly esterified into phospholipids and contribute to the maintenance of plasma membrane fluidity. Ahmad [30] reported that exogenous FAs are incorporated into membrane or cellular phospholipids as a result of the phospholipases and acyltransferases action which modifies some of their functions, such as cellular transport, receptor characteristics, and activities of some membrane-associated enzymes.

We found that the CD30 group showed a saliva flow reduction as compared with CD1, while the OD group maintained their SFR values after 30 days of intake. Calderón [31] reported modifications in the FA composition of urothelial membrane in animals fed on OA or LA enriched diets which had differential effects on its rigidity and, especially, on the vesicle secretion. They observed that an OA-enriched diet increased the endocytic vesicle release, whereas a high LA diet modifies neither the mechanism nor the number of vesicles released with respect to the control group [32]. In addition, Delporte [28] confirmed that the dietary FA supplementation generates biochemical modifications in the submandibular gland membrane lipids and consequently in the fluidity of its phospholipid bilayer.

Murakami [33, 34] employed perfused rat submandibular glands and demonstrated that most of the glandular water is transported through the paracellular pathway, whereas a small fraction does it by a transcellular route, working in conjunction and not by compensation. Hashimoto and Murakami [35] reported that agonist-induced salivary secretion such as carbachol and isoproterenol implies great paracellular permeability, in addition to aquaporin-5-mediated transcellular transport.

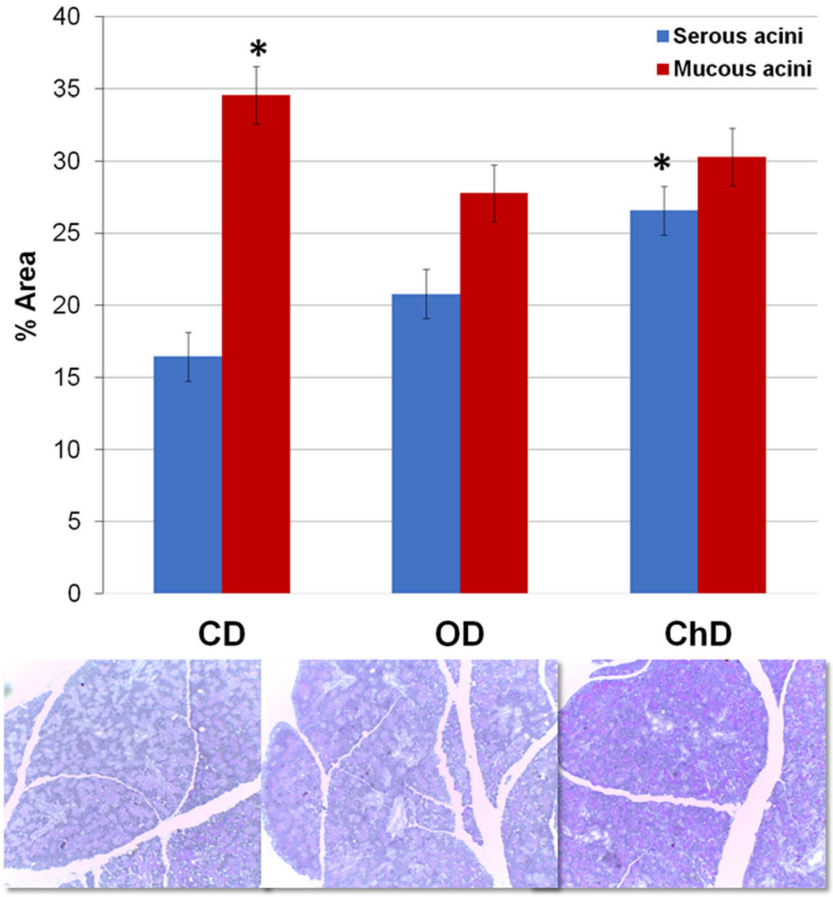

Fig. 1 Serous and mucous acinous density at 30-day intake according to dietary groups. PAS stained histological slides, original magnification ×40. CD, corn diet; OD, olive diet; ChD, chia diet. Values are expressed as area percentage (a%) (optical microscopy) and correspond to mean ± SE. Kruskal-Wallis test. *Indicate a significant difference ($p \leq 0.05$)

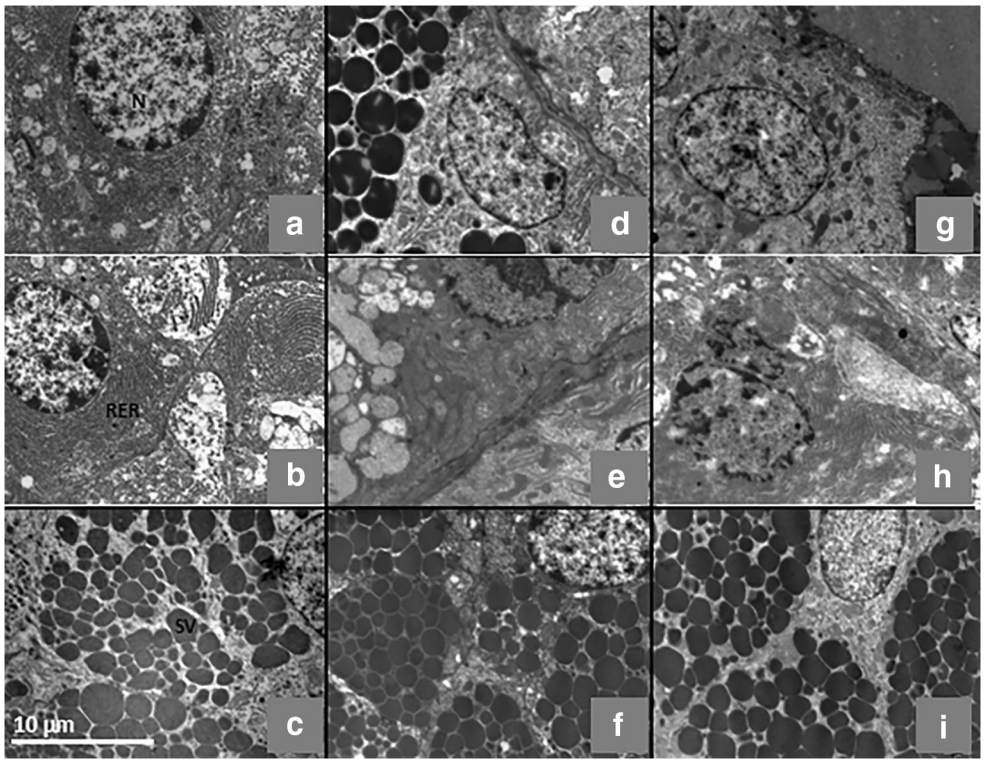

Fig. 2 Rat submandibular glands according to dietary group at 30 day-intake. Electron microscopy. Original magnification ×6000. CD30 (**a**, **b**, **c**); OD30 (**d**, **e**, **f**); ChD30 (**g**, **h**, **i**). N, nuclei; RER, rough endoplasmic reticulum; SV, secretory vesicles

Table 5 Secretory vesicle size at 30-day intake according to dietary groups

Dietary group		
CD	OD	ChD
0.69 ± 0.12^{A}	0.91 ± 0.15^{A}	0.25 ± 0.19^{B}

CD, corn diet; OD, olive diet; ChD, chia diet. Values are expressed as micrometers (μm) (electron microscopy) and correspond to mean ± SE. Kruskal-Wallis test. Different superscript letter indicates a significant difference ($^{A/B}p \leq 0.05$)

Kawedia [36] showed that the absence of aquaporin-5 in the mouse salivary glands resulted in a decrease in water transport through the plasma membrane and intercellular junctions.

With respect to the relationship between SFR and submandibular gland FA profile, the present study showed that SFR was lower and AA levels were higher in CD30 than in OD30 and ChD30 groups. In this respect, Prestifilippo [37] reported that anandamide, main endocannabinoid derived from the AA metabolism, decreases the methacholine and norepinephrine-stimulated salivary secretion of the rat submandibular gland through the activation of the CB1 and CB2 cannabinoid receptors located in the peripheral acinar cells, ductal system, and nerve endings which are coupled to the Gi/o proteins of the gland membrane. Activation of the endocannabinoid system produces an inhibitory effect of adenylyl cyclase activity which, as a consequence, decreases the cAMP production. Bruce [38] reported that the adenylyl cyclase/cAMP complex is the intracellular signaling pathway that results in an increase of cytosolic Ca^{++} levels. Fernandez-Solari [39] observed that a low Ca^{++} concentration could inhibit the neurotransmitter release from the submandibular gland presynaptic terminals resulting in a decrease of saliva secretion and, according to Kopach [40], in a modification of its composition.

Calcium represents the main second messenger involved in salivary secretion. Its concentration is fundamental for the fusion of secretory vesicles with the plasma membrane as well as for the exocytosis process. Therefore, the inhibition of the cytosolic adenylyl cyclase/cAMP/Ca^{++} pathway produced by AA-derived anandamide would have the capacity to inhibit the neurotransmitter secretion—norepinephrine or acetylcholine—by the sympathetic or parasympathetic nerve endings that innervate the glandular parenchyma resulting in a decrease in the nerve stimulus and in a lower salivary secretion. In addition, anandamide may also be directly coupled to the CB1 and CB2 receptors of glandular cells and inhibit the exocytosis process, decreasing saliva production by transcellular route [39, 41].

In addition, Oddi [42] reported that these bioactive products can be stored in intracellular lipid vesicles or released as needed. In contrast to classical neurotransmitters, endocannabinoids may function as retrograde synaptic messengers. They are released from postsynaptic neurons and go backwards across synapses, activating cannabinoid receptors on presynaptic axons and suppressing neurotransmitter release. Therefore, the decrease in SFR observed in our study could be related to the high dietary LA-derived AA levels as well as to the absence of n-3 polyunsaturated FAs.

Our results showed that the SFR in OD30 group maintained similar values to the baseline, whereas the OA percentage was higher in this group as compared with CD30 and in ChD30. In addition, a high level of AA was observed in the submandibular gland of OD30, while ALA, EPA, and DHA were not detected. Thus, it could be inferred that OA would have a little influence on SFR and on the endocannabinoid system. Matias [43] found that OA and PA have no effects on the endocannabinoids production by mature adipocytes

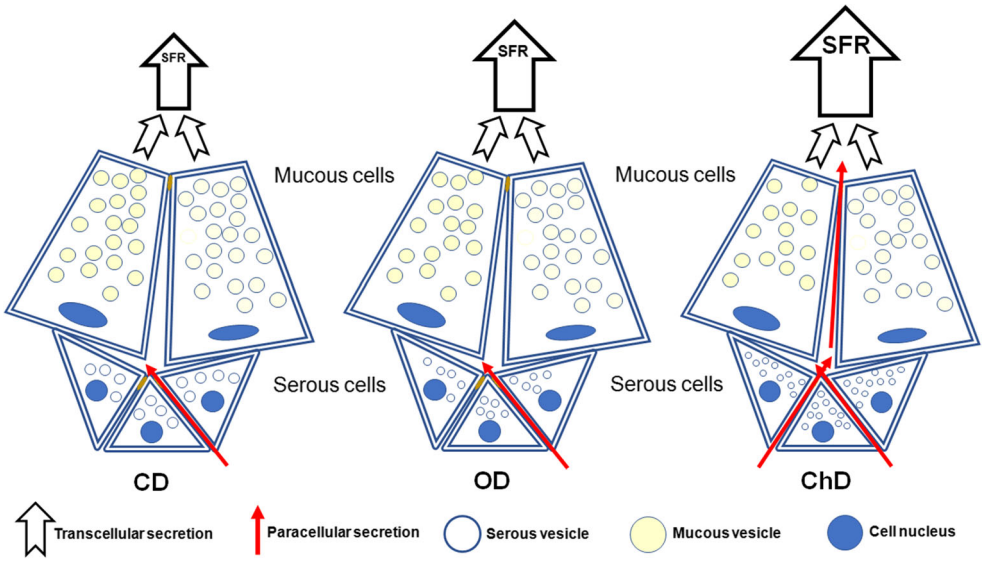

Fig. 3 Hypothetical explanation of the effects of dietary FA on submandibular salivary glands at 30-day intake

after 72 h of incubation. However, they observed an increase in n-palmitoylethanolamine (subtype of endocannabinoid) level without changes in phospholipid and triacylglycerol structure of adipocytes.

Finally, we observed that SFR was higher, and AA was lower in ChD30 as compared with CD30 and OD30 groups. ALA, EPA, and DHA were just detected in ChD30. These results are similar to those of Alvheim [44] who reported that diets or supplements rich in EPA and DHA reduce the AA level incorporated in phospholipids and therefore normalize the endocannabinoid production. Our results in rats would indicate that the SFR increase in chia group could be related to the low AA levels in submandibular gland resulting in a low endocannabinoid production and a low activation of its receptors.

With respect to the density of submandibular gland serous and mucous acini, the present study showed a higher a% of serous acini in ChD30 than in CD30 and OD30 groups. On the other hand, the mucous acinous density was higher in CD30 group than in OD30 and ChD30. These results coincide with El-Nozahy and Ismail [45] who concluded that diets produce structural and functional modifications on rat submandibular gland. They observed a decrease in acinous and duct size in animals receiving corn and soybean diets for 2 months. Therefore, it can be inferred that, on average, there is a greater amount of secretory vesicles in cells of ChD30 than in those of the other groups, considering that the total area occupied by vesicles was similar in all groups (Fig. 3).

This is the first report about the effects of dietary FAs and the time elapsed from their intake on the FA composition of the submandibular gland and on its SFR in an experimental model. Our results suggest that submandibular saliva volume is modulated not only by dietary FAs but also by the time elapsed from their consumption, being highest with chia oil diet—rich in ALA—at 30-day intake. Nevertheless, further studies involving longer time of chia oil intake and also the analysis of action mechanisms in submandibular glands are required both in animals and in humans. Knowledge about the beneficial effects of diet on salivary flow rate would contribute to improve the life quality of people suffering from salivary gland secretory dysfunctions, such as hyposalivation.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Jorge Dario Escandriolo Nackauzi, Raquel Gallará, Gastón Repossi, Claudio Bernal, and Adriana Actis. The original manuscript was written by Jorge Dario Escandriolo Nackauzi, and all authors worked on it in reviewing and editing, and they read and approved the final version.

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Compliance with ethical standards

Conflict of interest Jorge Dario Escandriolo Nackauzi has received a research grant from "Secretaría de Ciencia y Tecnología", Universidad Nacional de Córdoba. Gastón Repossi declares that he has no conflict of interest. Claudio Bernal declares that he has no conflict of interest. Adriana Actis declares that he has no conflict of interest. Raquel Gallará declares that he has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

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