Beef Consumption and Fatty Acids Serum Concentration: Relationship with Salivary Gland Tumors in Córdoba, Argentina

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Abstract. *Aim: The objective of the present study was to analyze beef consumption, conjugated linoleic acid (CLA) and n-3 fatty acid (FA) serum concentration and their relation to salivary gland tumors (SGT). A questionnaire on non-nutritional risk factors and a validated food frequency questionnaire were applied in 20 SGT and 20 control (Co) patients. Materials and Methods: Food data were processed by the Interfood v.1.3 software. Serum CLA was analyzed by chromatography. Results: Non-significant differences were found between SGT and Co regarding lean and fatty BC and serum CLA. Serum n-3 linolenic acid concentration was higher in Co than in SGT (p=0.004). No associations between BC and CLA serum concentration were found, but a strong-positive association between total energy intake and total fat intake and SGT were observed. A significant inverse association between oleic and linoleic FA intake and SGT was recorded. Conclusion: Serum oleic and linolenic FAs showed a significant negative association with SGT.*

Beef is one of the main foods in the Argentinean food pattern. Although it represents an important protein dietary source along with dairy products, it is also responsible for a considerable lipid contribution to the diet. These lipids provide a high total energy intake and most of them could be associated with an increased risk of developing various types of cancers (1), especially breast, colorectal, prostate and ovarian (2, 3).

Although the fat from beef products is considered unhealthy because of its high saturated fatty acid (FA) content (4) and cholesterol, it has been recently found that beef fat contains nutrients such as n-3 polyunsaturated FAs and conjugated linoleic acid (CLA), the latest with potential anticancer activity (5-8).

Alpha-linolenic acid (18:3 n-3) and its derivatives, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are among the n-3 FAs being of nutritional interest. They have shown the ability to suppress the cancer development and to reduce the growth of tumor cell lines, especially those of breast, colon and pancreas in *in vivo* and *in vitro* experiments (3, 9-12).

CLA is a polyunsaturated FA consisting of geometric and positional isomers of linoleic acid (18:2 n-6) which exhibit *cis-trans* double bonds conjugated at different sites in the carbon chain. CLA is an intermediary formed during the biohydrogenation of linoleic or linolenic acid to stearic acid in ruminant animals (cattle, goats and sheep). Therefore, the main CLA food sources are dairy and meat from those animals (8, 13, 14).

The production system and the nutrition offered to the animals may improve the meat FA type, increasing the CLA content and enhancing the ratio of n-6/n-3 FAs. It has been observed that animals fed on pasture, the main farming system in Argentina, receive a higher amount of polyunsaturated FAs and n-3 in particular, as well as a lower proportion of n-6 FAs than those fed on grain. Thus, it is

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Key Words: Beef, conjugated linoleic acid, n-3 fatty acid, serum, salivary gland tumors.

possible to improve the CLA production, mainly the *cis*-9, *trans*-11 CLA, or its precursor, the vaccenic acid, and achieve a lower n-6/n-3 ratio by increasing the dietary polyunsaturated FAs (15-17).

The *cis*-9, *trans*-11 CLA (also called rumenic acid) is the main conjugated isomer found in nature and represents 80-90% of total CLA in milk and beef. In addition, it is the only isomer that has shown a significant anticancer effect, even in small quantities (5, 8, 18, 19).

Salivary gland tumors (SGT) are a non-highly frequent hormone-dependent type of neoplasia with some malignant varieties having poor prognosis. Although no association has been found between the above mentioned nutrients and SGT, a positive relationship could be established between other types of fats, such as cholesterol and saturated fats and neoplasias (20-22). Regarding other types of hormone-dependent neoplasias, antitumor effects or inverse associations between n-3 FAs and CLA and breast tumors have been observed in many studies (23, 24). The relationship between prostate tumors and long chain n-3 FAs still remains unclear (3, 23-27). In *in vivo* and *in vitro* models, the biological mechanisms of n-3 polyunsaturated FAs in mammary carcinogenesis include eicosanoid production inhibition, cyclooxygenase-2 decreased production, altered response of protein kinase C to the stimulation of hormones and growth factors, changes in gene expression through peroxisome proliferator-activated receptor gamma (PPAR-gamma) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) decreased expression and apoptosis induction (3, 10, 28). CLA exerts a carcinogenesis-modulating effect through numerous and complex cellular mechanisms, namely: cell proliferation reduction, lipid oxidation, vitamin A and prostaglandin metabolism. It is also possible that *cis*-9, *trans*-11 CLA interferes in the cellular transformation through signal transduction. CLA may also interfere in the n-6 polyunsaturated FAs metabolism concerning the synthesis of eicosanoids, including prostaglandin E2 (3, 19, 29-33).

The objective of the present study was to analyze total energy intake, beef consumption as well as CLA and n-3 FA serum concentration in relation to the development of salivary gland tumors.

Patients and Methods

Patients. This study included 40 patients selected among those attending the Privado and Córdoba hospitals in the city Córdoba between the years 2006-2009. Twenty adults of both sexes and of mean age 46.55±13.83 were included (10 women and 10 men). They had recent diagnosis (incident) of benign and malignant SGT confirmed by histopathological examination. None of the patients had received any treatment. In addition, 20 controls (Co) each matched by sex and age (±5 years) with respect of the SGT patients (46.8±14.97 mean age) were selected. Exclusion criteria for Co were: tumor diagnosis (at any location), digestive diseases and/or metabolic disorders (ulcers, gout, diabetes) and adherence to special diets.

Participants signed the informed consent approved by the Institutional Committee of Ethics in Health Research (CIEIS) of Cordoba Province, Argentina and this study was in accord with the guidelines of the Argentine National Act 25,326 on protection of personal data (habeas data).

Data and sample collection. The following instruments were applied in interviews in order to collect information about nutritional and non-nutritional aspects: (i) Clinical history: Information about family history of cancer and pathological diagnosis. (ii) Questionnaire on non-nutritional risk factors: Registration of data on toxic habits like tobacco use and alcoholic beverage consumption (type, frequency and time). (iii) Validated semi-quantitative food frequency questionnaire (FFQ): Anthropometric data and information about the type of food consumed. The FFQ included questions related to 257 types of food and drink consumed five years before the time of the interview (34) taking into account the latent period between the consumption of a particular food and its potential consequences on health. The frequency references used were: never, the number of times per month, per week and per day. The portion size was described as small, medium or large. A photographic guide was used in order to better understand these portion size definitions (35). With regard to beef, questions were referred to the consumption of lean (ball back, shoulder, hip, square ham, loin, round stake and buttocks) and fat cuts (roast, cutlet, rose meal, common ground beef and flank steak). Although goat and sheep meat is another source of CLA, questions about their consumption were not included since they are not part of the habitual Argentinean food pattern.

Patients of both groups were interviewed in person by two well-trained nutritionists.

Blood samples were obtained under fasting conditions. Serum was obtained by centrifugation and stored at –20˚C until processing.

Data and sample processing. The food data were processed by Interfood v.1.3 software (36) to produce information about food (g/day) and nutrients (mg/day and μg/day) (available: http://interfoodargentina.com.ar/).

Total lipids were extracted from serum using the Bligh and Dyer method (37). Extracted FAs were methylated using potassium hydroxide (KOH) and FA methyl esters were analyzed by gas chromatography using a Shimadzu 2014 chromatograph equipped with a flame ionization detector. (Supplier: JENCK S.A. Instrumental, Buenos Aires, Argentina). All individual FA results are expressed in weight percent (wt%) of total FA methyl esters. FAs with concentrations of less than 0.5 wt% were considered minor and are not shown unless they are CLA isomers. The temperature of both the injector and the detector was 250˚C and nitrogen was the carrier gas. FA methyl esters were analyzed using a 100 m × 0.25 mm × 0.2 mm film thickness SP-Sil 88 capillary column (Varian, Darmstadt, Germany). FA methyl esters were identified by comparison of their retention times relative to those of commercial standards (AccuStandard, New Haven, USA and Sigma, St. Louis MO, USA). Chromatographic data processing was performed with GC Solutions software (Supplier: JENCK S.A. Instrumental, Buenos Aires, Argentina).

Table I. *Total energy and fat intake*.

Variables	SGT		Co		*P*
	Mean	SD	Mean	SD	
Total energy intake (Kcal/day)	2877.66	925.57	2379.5	1007.34	0.06[1]
Fat intake (g/day)					
Total	120.84	39.71	102.02	40.50	0.15[2]
Saturated	21.32	12.96	18.66	7.70	0.46[2]
Monounsaturated	19.97	10.56	21.81	12.22	0.61[2]
Polyunsaturated	18.61	11.07	14.32	10.99	0.18[1]
EPA	0.06	0.01	2.10	7.10	0.037[1]
DHA	2.50	4.02	4.80	13. 00	0.39[1]

SGT: Salivary gland tumors; Co: controls; SD: standard deviation; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; [1]Wilcoxon test; [2]Student's *t*-test.

Table II. *CLA food source intake and CLA and n-3 fatty acid serum concentration*.

Variables	SGT		Co		*P*
	Mean	SD	Mean	SD	
Total CLA food source intake (g/day)*	385.17	228.41	340.61	199.37	0.51[1]
Intake of (g/day)					
Total beef	125.39	74.75	129.53	151.80	0.39[1]
Lean cuts[3]	52.73	62.00	71.60	150.14	0.81[2]
Fatty cuts[4]	72.66	60.44	57.93	51.47	0.40[2]
Serum concentration (mg/ml)					
CLA	0.08	0.03	0.09	0.03	0.21[1]
Linolenic acid	0.07	0.03	0.22	0.85	0.004[2]
EPA	0.23	0.44	0.04	0.04	0.90[1]
DHA	0.16	0.26	0.20	0.22	0.19[1]

SGT: Salivary gland tumors; Co: controls; SD: standard deviation; CLA: conjugated linoleic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; *Includes milk. cheese and fatty foods (butter. cream and animal fat); [1]Student's *t*-test; [2]Wilcoxon test; [3]Ball back, Shoulder, Hip Square ham, Loin, Round stake and buttocks; [4]Roast, Cutlet, Rose meal, Common ground beef and flank steak.

Statistical analysis. Chi-square, Student's *t* and the Wilcoxon tests were applied to compare the variables between SGT and Co. The Pearson correlation coefficient was used to analyze the correlation between beef consumption and serum CLA and n-3 FAs. A logistic regression model was employed to estimate the odds ratio (OR) with a 95% confidence interval (CI). The OR adjusted by sex, tobacco and alcohol intake as well as the family history of cancer was also calculated. CLA intake in Argentina varies from 0.3 to 1.5 g/person/day (8). It has been described that the CLA serum concentration required to play its therapeutic and protective role on cancer corresponds to 0.8-3 g/day (8). Since the serum CLA and n-3 FA exact amount producing their protective effects is still unknown, the median value was used as a cutoff point for the risk estimation (OR), considering the range below the median as the risk category for both variables. Thus, the risk of developing these tumors was estimated in relation to serum CLA and n-3 FA as well as lean and fat beef consumption. Variables like total energy intake, body mass index, total fat, saturated, monounsaturated and polyunsaturated FA intake (n-3 and n-6) were analyzed the same way.

Results

The mean body mass index reached 25.89±4.55 kg/m^2 and 24.94±4.06 kg/m^2 in SGT and Co, respectively. 60% of the SGT and 25% of Co were smokers ($p<0.05$). Nevertheless, non-significant differences ($p>0.05$) between both groups regarding alcohol intake and family history of cancer were found.

Patients were categorized according to the mean total energy intake and total, saturated, monounsaturated and polyunsaturated fat intake (Table I). EPA consumption was significantly higher in Co than in SGT ($p=0.037$). Although total energy intake was higher in SGT than in Co ($p=0.06$), no statistically significant differences were found for this and the other variables.

Non-significant differences were found between SGT and Co regarding CLA food sources like lean and fatty beef intake and the serum CLA. Serum n-3 linolenic acid concentration was significantly higher in Co than in TGS ($p=0.004$) (Table II).

Total beef consumption was 12% of total energy intake in SGT, 8% and 4% corresponding to fatty and lean cuts, respectively. Total beef consumption in Co was 14% of total energy intake, fatty and lean cuts representing 7.5% and 6.5%, respectively. Thus, the lean cut consumption which provides the greater CLA proportion was higher in Co.

The Pearson correlation test showed no correlation between total beef consumption and serum CLA concentrations. On the other hand, the Spearman correlation test showed a non-significant positive correlation between total beef consumption and total serum EPA, DHA and linolenic acid concentration in the SGT group. A significant positive correlation between beef consumption and serum EPA ($r=0.337$, $p<0.005$) and DHA ($r=0.337$, $p=0.045$) concentrations as well as a non-significant positive correlation with the serum linolenic acid concentration was found in Co.

Table III presents the association between the different variables and SGT. No associations between beef intake and CLA serum concentration and SGT were observed. However, a strong significant positive association between total energy

Table III. *Relationship between variables and SGT.*

Variables	SGT		
	aOR	CI	*p*-Value
Age	0.959	0.179-5.143	0.961
BMI	2.982	0.646-13.765	0.162
TEI	23.833	2.453-231.514	0.006
Fat intake			
Total	8.310	1.343-51.424	0.023
Saturated	1.672	0.351-7.962	0.518
Monounsaturated	1.188	0.282-5.012	0.815
Oleic acid	0.157	0.031-0.802	0.026
Polyunsaturated	0.758	0.169-3.410	0.718
Linoleic acid	0.104	0.017-0.623	0.013
Linolenic acid	0.539	0.126-2.300	0.404
EPA	1.085	0.188-6.282	0.927
DHA	0.961	0.230-4.023	0.957
Beef intake			
Total beef	1.199	0.271-5.300	0.811
Lean cuts[1]	0.587	0.138-2.494	0.470
Fatty cuts[2]	2.295	0.501-10.516	0.285
Serum concentration			
CLA	3.004	0.592-15.251	0.184
Oleic acid	0.170	0.034-0.863	0.032
Linolenic acid	0.142	0.022-0.930	0.042
EPA	1.360	0.321-5.768	0.676
DHA	2.515	0.574-11.024	0.221

SGT: Salivary gland tumors; aOR: OR adjusted by sex. tobacco use. alcohol intake and family history of cancer; CI: confidence interval, BMI: body mass index; TEI: total energy intake; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; CLA: conjugated linoleic acid; [1]Ball back, shoulder, hip, square ham, loin, round stake and buttocks; [2]Roast, cutlet, rose meal, common ground beef and flank steak.

intake and total fat intake and SGT was found. Regarding FAs, a significant inverse association between oleic (p=0.026) and linoleic (p=0.013) acid intake and the development of SGT was observed. Serum oleic (p=0.032) and linolenic acid (p=0.042) showed a significant negative association with the SGT risk.

Discussion

Diet is one of the main factors related to the development of various types of cancer. Fat consumption, depending on the quantity and composition is related to the promotion or prevention of some tumors (3, 21, 38). Most studies have referred the relationship between lipids and cancer focused on monounsaturated, polyunsaturated (n-3 and n-6) and saturated FAs, with their results still being controversial. No information could be found regarding the relationship between beef or CLA consumption and SGT. Nevertheless it was shown that CLA inhibits the development of breast, skin, colon and stomach tumors (28, 39, 40).

The present study showed significant correlations between EPA and DHA intake and their serum concentrations in the Co group. Féart *et al*. found similar results associated with the consumption of certain foods and serum FA concentrations (41). In addition, non-significant correlations between beef consumption and serum CLA, linolenic acid, EPA and DHA in the SGT group have been reported.

According to our results, no association was found between body-mass index and SGT. Nevertheless, there are many reports indicating that overweight and obesity have certain influence on the development of certain types of cancer such as breast and prostate (1, 39, 42).

A significant strong-positive association between total energy intake and SGT was observed. This result is coincident with that of Donaldson (2004) suggesting that a high energy intake would be the major risk factor for developing various types of cancers (1). On the other hand, a moderate positive association was found between total fat intake and SGT. In this respect, experimental studies showed that cell proliferation in salivary glands and other locations is influenced by dietary lipids (3, 38, 43, 44). The consumption of saturated fats showed a moderately positive association with SGT. Similarly, numerous studies have demonstrated that a high intake of these fats is positively-associated with different types of cancer like breast, colon and rectum, prostate, lung and endometrium (28, 43, 44, 45).

A significant inverse association between dietary monounsaturated oleic acid and SGT was observed. In relation to this FA, Granados *et al*. (2006) refers to conflicting results concerning the possible effect of oleic acid on the different stages of cancer development. Some studies cited on that review show a suppressor role of this FA in tumorigenesis while other authors have not found such effect (3).

A non-significant positive association was observed between polyunsaturated FA intake and SGT development, with the exception of linoleic acid which showed a significant inverse association. No human studies could be found in this respect. However, experimental research indicated that just n-3 FAs produced a protective effect on murine dimethylbenzanthracene-induced salivary tumors (21, 46).

According to most studies, EPA and DHA are inversely-associated with endometrium, ovary and breast tumors with the latest being hormone-dependent neoplasias like SGT (3, 25, 47). On the other hand, both positive and inverse associations between those FAs and prostate tumors have been reported (26, 27, 48, 49).

Non-significant associations between the consumption of lean and fatty beef and SGT development were found. Nevertheless, higher oleic and linolenic acid serum concentrations showed to be inversely-associated with SGT. These results could not be found with respect of salivary or other type of tumors.

Beef from Argentina has high n-3 FA and CLA concentrations due to cattle feeding (16, 17). This study showed no associations between beef consumption and CLA serum concentration and SGT. Although no evidence could be found regarding this association, it is known that CLA would have a protective effect on mammary (50) and prostate tumors (13), both hormone-dependent neoplasias like SGT.

The present results showed that serum oleic and linolenic, as well as dietary oleic and linoleic FAs related to foods like Argentinean beef, due to its composition, could be associated with SGT. Similar results were not be found regarding beef intake. Further research is required in order to relate the circulating or dietary levels of n-3 FAs as well as CLA and the development of SGT and other neoplasias.

Acknowledgements

The Authors wish to acknowledge the assistance of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Universidad Nacional de Córdoba, both of which support facilities used in this investigation. They also want to thank the following funding institutions: Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (Res. 214/10), Ministerio de Ciencia y Tecnología, Provincia de Córdoba, Argentina (Res. 121/08) and CONICET.

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Received April 29, 2014
Revised June 24, 2014
Accepted June 26, 2014