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# Plasmatic Fatty Acids Chemical Characterization after the Exposure to a Vegan Diet

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**Abstract:** The vegan diet excludes animal-derived products consumption. The objective of the present study is to analyze dietary lipid intake, nine plasmatic fatty acids concentrations (from C14:0 [lauric acid] to C20:4 [arachidonic acid]), and conventional clinical lipid profile among vegan individuals with omnivore controls. A case-control and cross-sectional study was performed between 2016 and 2017. Vegans were paired in a 1:1 ratio with omnivores from Merida, Mexico. A 150-item Semi-Quantitative Food Frequency Questionnaire was conducted to evaluate eating patterns. Serum fatty acids were determined from total blood with a gas chromatography assay. Lower cholesterol, stearic, arachidonic and trans fatty acids intake, but higher consumption of lauric acid were observed in the vegan group ( $p = <0.001, 0.014, <0.001, 0.005$ , respectively). Decreased plasma concentrations of stearic, arachidonic and linoleic acids were found ( $p = 0.017, <0.001$  and  $0.026$ , respectively). Following a vegan diet for more than three years generate modifications in serum concentrations of saturated and polyunsaturated  $\omega$ -6 fatty acids, which could lower inflammatory markers' biosynthesis. Potential benefits regarding cardiovascular risk may be assumed in favor of vegan individuals.

**Keywords:** Gas chromatography assay; Cardiovascular diseases; Feeding Patterns; Lipids; Nutritional status; Vegan Diet.

## 1. Introduction

The vegan diet is characterized by a full exclusion of animal-derived products consumption [1]. Following this eating pattern may derive into variations in serum lipid profile. Existing evidence reports that vegans have lower concentrations of serum lipoproteins, as well as saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs)  $\omega$ -3 (eicosapentaenoic and docosahexaenoic acid) and  $\omega$ -6 (particularly arachidonic acid) [2-5], but higher monounsaturated fatty acids (MUFAs) compared to their omnivorous counterparts [6].

Ideal serum relationship between PUFAs  $\omega$ -6/ $\omega$ -3 remains in a 3:1 ratio [7]. An alteration in this proportion may promote inflammation and cardiovascular diseases [7,8]. Considering that arachidonic acid is a precursor of potent pro-inflammatory mediators, possible reduction in vegans' serum concentration may implicate less biosynthesis of 2-series thromboxanes, 2-series prostaglandins 4-series leukotrienes [7,9]. Furthermore, less concentrations of SFAs (e.g. stearic acid) could also modulate favorably low-density lipoprotein cholesterol biosynthesis. However, lower  $\omega$ -3 intake due to exclusion of animal-derived products, could promote inflammation with adverse cardiovascular events [2]. Possible differences in the serum fatty acids profile between individuals

**Table 2.** Comparison of lipoproteins and serum triglycerides among subjects exposed to a vegan diet ( $\geq 3$  years) with omnivores controls from a south-eastern Mexican population <sup>1</sup>.

Serum lipids <sup>2</sup>	Vegans (n=12)	Omnivores (n=12)	P-value <sup>3</sup>
TC (mg/dL)	154.0 $\pm$ 36.1	167.4 $\pm$ 45.8	0.483
HDL-C (mg/dL)	51.9 $\pm$ 10.9	55.9 $\pm$ 8.6	0.331
LDL-C (mg/dL)	81.4 $\pm$ 30.6	96.0 $\pm$ 36.8	0.304
TG (mg/dL)	102.6 $\pm$ 46.4	76.7 $\pm$ 36.8	0.145

<sup>1</sup>All data are means  $\pm$  standard deviations.

<sup>2</sup>Conversion factor for TC, HDL-C and LDL-C to SI units is mg/dL/38.67= mmol/L; for TG, conversion factor is mg/dL/88.57 = mmol/L.

<sup>3</sup>Obtained using a *t*-student test for paired variables.

*Abbreviations:* TC= total cholesterol; HDL-C= high density lipoprotein cholesterol; LDL-C= low density lipoprotein cholesterol; TG= triglycerides.

with vegan and omnivore diets could generate different scenarios regarding risk factors for cardiovascular diseases [3,5,6,10].

Lack of information on this subject in a constantly-growing Mexican population that follows a vegan diet, addresses the importance of analyzing the behavior of serum lipids in this particular population. The objective of our study was to compare concentrations of nine serum fatty acids (from C14:0 [lauric acid] to C20:4 [arachidonic acid]) and conventional clinical lipid profile in Mexican individuals with a minimum of three years of vegan practice with omnivore pairs.

## 2. Results

A case-control and cross-sectional study was performed in twelve Mexican vegans who were paired with omnivores controls in a 1:1 ratio, considering similar anthropometric and sociodemographic characteristics (Table 1). All 24 individuals completed the study (six males and eighteen females). None of them were excluded based on missing data.

**Table 1.** Anthropometric and sociodemographic criteria for 1:1 pairing between vegan and omnivore samples from a south-eastern Mexican population <sup>1</sup>.

Variable	Vegans (n=12)	Omnivores (n=12)	P-value <sup>2</sup>
Weight (kg)	63.2 $\pm$ 14.0	58.2 $\pm$ 10.6	0.161
BMI (kg/m <sup>2</sup> )	22.5 $\pm$ 3.6	22.4 $\pm$ 3.4	0.800
Age (years)	29 $\pm$ 9	29 $\pm$ 10	0.665

<sup>1</sup>All data are means  $\pm$  standard deviations.

<sup>2</sup>Obtained using a Student's *t*-test for paired variables.

*Abbreviations:* BMI = body mass index.

### 2.1. Dietary analysis

Dietary analysis (Table 2) indicated lower cholesterol, as well as stearic, arachidonic and total trans fatty acids intake in the plant-based diet group, due to animal-derived products exclusion. Nevertheless, a regular coconut oil consumption was identified among vegan participants, consequently, lauric acid intake was higher in this group.

**Table 3.** Daily dietary lipid intake comparison among subjects with a vegan diet ( $\geq 3$  years) *vs* omnivore controls from a south-eastern Mexican population <sup>1,2</sup>.

Dietary assess	Vegans (n=12)	Omnivores (n=12)	P-value <sup>3</sup>
Total fat (g)	61.2 $\pm$ 19.0	52.5 $\pm$ 15.1	0.259
Total cholesterol (mg)	8.3 $\pm$ 7.2	204.4 $\pm$ 95.8	<0.001*
Total SFA (g)	15.3 $\pm$ 6.6	13.5 $\pm$ 4.1	0.408
Lauric (g) (C12:0)	3.86 $\pm$ 3.1	0.62 $\pm$ 0.6	0.005*
Myristic (g) (C14:0)	1.54 $\pm$ 1.2	1.04 $\pm$ 0.6	0.149
Palmitic (g) (C16:0)	5.9 $\pm$ 1.9	6.5 $\pm$ 1.7	0.397
Stearic (g) (C18:0)	1.5 $\pm$ 0.7	2.4 $\pm$ 0.7	0.014*
Total MUFA (g)	23.6 $\pm$ 6.5	20.7 $\pm$ 7.1	0.327
Palmitoleic (g) (C16:1)	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2	0.481
Oleic (g) (C18:1)	22.5 $\pm$ 6.7	17.8 $\pm$ 6.6	0.142
Total PUFA (g)	17.4 $\pm$ 10.8	13.0 $\pm$ 6.9	0.245
Total $\omega$ -3 PUFA (g)	1.8 $\pm$ 1.4	1.9 $\pm$ 1.0	0.871
Total $\omega$ -6 PUFA (g)	15.1 $\pm$ 9.7	10.6 $\pm$ 5.8	0.179
Linoleic (g) (C18:2)	14.9 $\pm$ 9.7	9.6 $\pm$ 5.7	0.120
Arachidonic (g) (C20:4)	0.07 $\pm$ 0.05	0.23 $\pm$ 0.09	<0.001*
Total <i>trans</i> fatty acids (g)	0.1 $\pm$ 0.1	0.4 $\pm$ 0.2	0.011*

<sup>1</sup>All data are means  $\pm$  standard deviations.

<sup>2</sup>Conversion factor to System of Units is mg/dL/29.23= mmol/L.

<sup>3</sup>Obtained using a Student's *t*-test for paired variables; *P* < 0.05 consider as significant.

*Abbreviations:* SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

## 2.2. Serum lipoproteins assay

Conventional lipid profile did not show differences between groups (Table 3). However, when analyzing individually, hypertriglyceridemia ( $\geq 150$  mg/dL, 1.69 mmol/L) was identified in three of the twelve vegans. This trend is related to an excessive carbohydrates' intake presented in grain-based products (250.8  $\pm$  44.2 *vs* 208.9  $\pm$  55.4, grams in the vegan and omnivore group, respectively). In contrast, two of twelve controls showed high TC concentrations ( $\geq 240$  mg/dL, 6.24 mmol/L), associated to animal fat intake, and no individuals with hypertriglyceridemia were found in this group.

**Table 4.** Serum fatty acids comparison (from C14:0 to C20:4) among subjects with a vegan diet ( $\geq 3$  years) vs omnivore controls from a south-eastern Mexican population <sup>1</sup>.

Fatty acids (mg/dL) <sup>2</sup>	Vegans (n=12)	Omnivores (n=12)	P-value <sup>3</sup>
<i>SFA</i>			
Lauric (C12:0)	3.9 ± 1.10	4.6 ± 3.3	0.618
Myristic (C14:0)	5.8 ± 3.1	6.5 ± 5.3	0.680
Palmitic (C16:0)	60.7 ± 57.9	107.6 ± 58.3	0.061
Stearic (C18:0)	16.8 ± 15.9	38.6 ± 24.0	<b>0.017*</b>
<i>MUFAS</i>			
Palmitoleic (C16:1)	7.1 ± 5.6	6.2 ± 3.4	0.670
Oleic (C18:1)	36.2 ± 32.9	55.4 ± 28.0	0.138
<i>PUFAS (<math>\omega</math>-6)</i>			
Linoleic (C18:2)	40.1 ± 33.5	76.1 ± 39.9	<b>0.026*</b>
Arachidonic (C20:4)	5.5 ± 2.6	11.7 ± 4.9	<b>0.001*</b>
<i>Unsaturated Trans</i>			
Elaidic	4.8 ± 2.5	6.9 ± 2.9	0.080

<sup>1</sup> All data are means ± standard deviations.

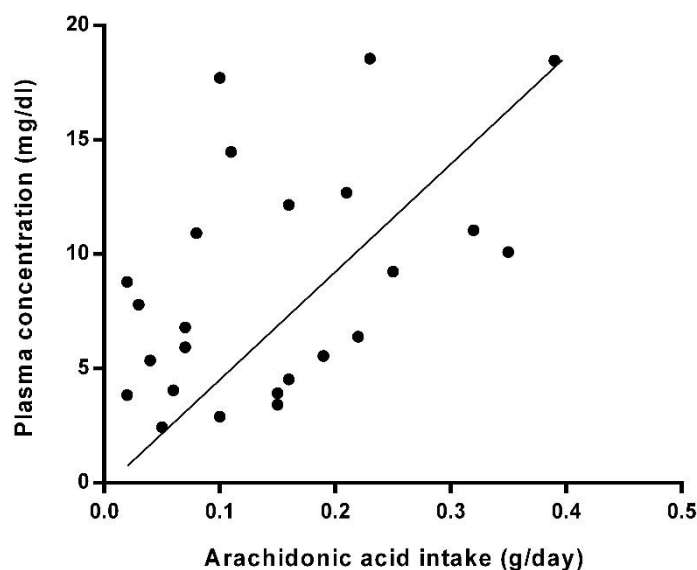
<sup>2</sup> Conversion factor to System of Unit is mg/dL/29.23= mmol/L.

<sup>3</sup> Obtained using a Student's *t*-test for paired variables;  $p < 0.05$  consider as significant.

*Abbreviations:* SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

### 2.3. Plasmatic fatty acids chemical analysis

Regarding serum fatty acid assay (Table 4), we found that the most abundant in both populations comprises the palmitic acid. We identified a trend to develop a higher concentration of this long-chained SFAs in the omnivore group, which is contained in most animal-derived products usually consumed by this sample group (e.g. meat and milk, but also present in vegetables oils such as olive oil), although it can also be synthesized endogenously [11,12]. In addition, a significantly lower serum concentration of stearic acid was observed in the vegan sample. Nevertheless, lauric and miristic (SFAs) did not show differences between groups. For unsaturated fatty acids, MUFAs did not vary among the vegan and omnivore individuals. However, a significant difference on both PUFAs ( $\omega$ -6) was identified, where the vegans showed a lower concentration of linoleic acid and arachidonic acid. This outcome can be associated to the absence of animal-derived products consumption, specially meat and fish [13,14]. A positive association between arachidonic acid plasmatic concentrations and dietary intake was found for the combined groups (Figure 1;  $n=24$ ,  $r= 0.477$ ,  $p=0.0184$ ). Furthermore, elaidic acid (trans isomer of oleic acid) was measured, but no significant difference between groups was identified in total blood, despite significant difference towards a lower intake of total trans fatty acid that was observed in the vegan group.



**Figure 1.** Association between dietary intake and plasmatic concentrations for omnivores and vegans.

### 3. Discussion

A comparative analysis was conducted between individuals who follow a vegan and omnivore diet, to evaluate possible variations in serum lipoproteins, TG, and specific fatty acids as potential indicators of cardiovascular risk.

Despite a lower intake of cholesterol in the plant-based diet sample, no difference between groups was observed regarding conventional lipid profile. However, two individuals from the omnivore sample presented TC values  $\geq 240$  mg/dL ( $\geq 6.24$  mmol/L), which represent a higher cardiovascular risk as stipulated by the Adult Treatment Panel III [14]. No hypercholesterolemia was reported in any participant with vegan-type diet. In this sense, Wang and colleagues [15] carried out a systematic review with meta-analysis which concluded that vegetarian diets effectively reduce TC and LDL-C concentrations, being a useful non-pharmaceutical intervention to control hypercholesterolemia. Although vegan diets have been associated with blood lipids concentration's reduction, which suggests lower cardiovascular risk [16], there is discrepant evidence founding that this eating pattern does not always generate favorable changes in serum lipid levels [17]. In this sense, our statistical analysis indicated that exposure to a vegan diet did not modify levels of lipid markers regularly used in clinical practice. Serum lipoproteins can also be influenced by confounding factors, different than diet, that generally distinguish vegans' lifestyle: regular practice of physical activity, management of stress through meditation techniques, support groups that could improve the attachment to a healthier lifestyle and a reduction of alcohol and tobacco consumption are some examples [18-20]. However, these lifestyle factors are not necessarily widespread, since we did not identify those particular health-related behaviors in all vegans.

Variations in vegans' food selection among different populations could also lead to diverse physiological results [26], due to different availability of food within regions, as well as lack of professional advice when following a vegan diet. We identified an imbalance in dietary macronutrients, as a consequence of a large grain-based products consumption for meat substitution, which imply larger carbohydrates intake with potential metabolic adverse effects (serum TG levels addressed). In agreement with our results, other studies have reported that vegetarians have higher TG concentrations, as well as a lower level of HDL-C, increased systolic pressure and higher prevalence of antihypertensive drug consumption compared to an omnivore population [21,22]. The

authors also attributed these results to changes in diet macronutrients distribution, which supposes an excessive intake of carbohydrates as substitution method for animal-derived products [21]. Regarding Mexicans' food composition, a direct relationship has been reported between undesirable eating habits, environmental, and sociocultural factors, with dyslipidemia (high TG and low HDL-C) and Metabolic Syndrome [23]. Contrary to conventional plant-based dietary recommendations (frequent and vast intake of vegetable oils present in flaxseed, canola oil, walnuts, chia [*Salvia hispanica*] or hemp seeds, and key supplements) [24,35], our findings only showed a feeble intake of walnuts, olive oil, and avocado as vegetable oils source in the vegan group. No  $\omega$ -3/  $\omega$ -6 PUFA supplement consumers were identified, and professional nutritional guidance was reported by only two participants. In countries consuming other types of vegetable oils, vegans may likely show different serum lipoproteins [26]. Consequently, sociocultural and food elements around the vegan diet in Mexican population could limit the benefits reported in other latitudes regarding serum lipoproteins.

The main outcome of our study is that the exposure to a vegan diet for more than three years generates modifications in the serum fatty acid profile, which imply a reduction in absolute serum fatty acid concentrations, with significance in stearic, linoleic and arachidonic acid when compared to an omnivore group. These differences coincide partially with what has been reported in previous studies, where a significant reduction in absolute serum fatty acids levels have been shown in vegetarians when compared with an omnivore group (linoleic, oleic, palmitic palmitoleic, stearic and arachidonic) [27].

Literature reports that vegans have lower plasma concentrations of SFAs than their omnivores counterparts, which could represent a protective factor against cardiovascular events [7]. These findings correspond with our results, where a significantly lower intake and serum concentration of stearic acid was found, as well as a trend towards a lower proportion of palmitic acid. SFAs' are linked to LDL-C biosynthesis and inflammatory processes which contributes on the development of cardiometabolic disease [4,28]. Specifically, palmitic acid affects fibrinolytic activity in plasma [29]. Nevertheless, frequent consumption of tropical oils such as coconut, rich in SFAs (e.g. lauric acid), could reduce the optimal  $\omega$ -6/ $\omega$ -3 ratio and disservice the conversion of  $\alpha$ -linolenic acid into PUFAs in vegans [30]. However, a recent systematic review with meta-analysis carried out by Chowdhury and colleagues [31] has questioned the recommendations regarding the reduction of SFAs consumption to decrease cardiovascular risk. This is a topic of current scientific debate.

Despite some literature report that vegans have higher plasma concentration of unsaturated fatty acids (MUFAs among them) than those that follow an omnivore diet [6], other studies, in agreement with our results, did not show differences in MUFAs plasmatic concentration between vegans and meat eaters [32]. Thus, we did not corroborate the expected benefits related to a favorable lipid profile after MUFAs consumption in this Mexican sample, since dietary intake among groups did not present variations.

We found a lower plasmatic concentration of arachidonic and linoleic PUFAs ( $\omega$ -6) in vegans. Individuals following a plant-based diet have lower serum concentrations of arachidonic acid compared with omnivore individuals due to greater proportions contained in animal-derived products [13,14]. Opposing to our findings, other authors have reported through serum determination of fatty acids by gas chromatography, an increased concentration of linoleic acid in vegans ( $p < 0.001$ ) [26]. However, arachidonic acid can be synthesized through an enzymatic process of elongation and desaturation from linoleic acid. Therefore, despite having a higher linoleic intake, vegans rely on this biochemical pathway to synthesize arachidonic; thus, plasma concentrations of both PUFAs in this particular sample is expected to be lower than omnivores [13,33,34].

In those who follow a vegan diet, the reduction in  $\omega$ -6 fatty acids concentrations can limit 2-series prostaglandins' biosynthesis (PGE<sub>2</sub>, PGI<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2</sub> $\alpha$ ), as well as 2-series thromboxanes

(TXA<sub>2</sub>, TXB<sub>2</sub>), 4-series leukotrienes (LTA<sub>4</sub>, LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>) and proinflammatory or vasoconstriction factors (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ), which contribute to the development and progression of cardiovascular diseases [7,35]. By having statistically diminished serum concentrations of  $\omega$ -6 fatty acids, especially arachidonic, vegan participants from this study may have fewer inflammatory factors, which could imply a lower risk of developing cardiovascular complications.

We identified a lower trans-fatty acid intake; however plasmatic concentrations of elaidic acid did not variate significantly among groups. Sarter and colleagues [14] partially coincide with our results: authors found a significant reduction of plasma trans-fatty acids (18:1 and 18:2 isomers) in vegans. They attributed this result to a higher intake of products containing partially-hydrogenated vegetable oils in the omnivore population. A meta-analysis and systematic review conducted by Huang and colleagues [36] associated large intakes of trans-fatty acids with a greatest impact on the development of cardiovascular diseases, the authors found a 16% lower mortality rate from circulatory diseases in vegetarians than in omnivores.

Evidence shows some results that contrast with what was found in our study. However, eating habits in Mexico and Latin America are culturally and socially uncommon to other countries, consequently, even when a plant-based diet is followed, different physiological results may be expected because of local and cultural adaptations. Individuals who follow a vegan diet have a lower risk of cardiovascular diseases due to regular consumption of a variety of vegetables, fruits, grains, legumes, and nuts [3]. However, these ideal patterns were not repeated strictly in this Mexican population.

This study provides substantial, correlative and statistical evidence, adjusted to the Mexican population, therefore comparable with a Hispanic population, regarding the relationship between vegan diet and serum fatty acids modifications. Our results coincide partially with what has been reported in the limited international literature, specifically about fatty acids plasmatic concentrations. This reinforces the hypothesis that a vegan diet implies lower concentrations of arachidonic acid. However, we recognize sample size as a limitation, due to an ideological resistance to participate, as well as the transversal and observational nature of our design. Nevertheless, statistical analyzes remain valid to this observational study, interpretations are relevant to this particular population, and valuable for clinical practice; thus, both limitations are not major concerns.

#### 4. Materials and Methods

A case-control cross-sectional study was conducted in a healthy south-eastern Mexican adult sample (Merida, Mexico) between 2016 and 2017. The present work adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for case-control studies and received approval by the Ethics Committee from "Universidad Marista de Merida", number CE\_UMM002A\_2017 in accordance with regulations about research protocols by Secretary of Health of Mexico (NOM-012-SSA3-2012) and Declaration of Helsinki.

Considering that sociodemographic characteristics among Merida's vegan, as well as in the rest of the country are unknown, a convenience-sample selection was performed. Furthermore, sixty-eight subjects from a local vegan-vegetarian group were originally identified, twenty-five of them met the inclusion criteria. However, ideological beliefs allowed only twelve to participate in the study. Samples included female and male individuals with a history of vegan eating habits for an uninterrupted period  $\geq 3$  years. They were randomly paired with controls in a 1:1 ratio. Each control was assigned arbitrarily from a triple-shortlist of candidates with an omnivore diet, considering same gender and socioeconomic status, age ( $\pm 5$  years), and body mass index ( $\pm 1$  kg/m<sup>2</sup>).

A 150-item Semi-Quantitative Food Frequency Questionnaire [37] was conducted to assess eating patterns in both groups, and to ensure full exclusion (consciously and unconsciously) of animal-derived products in the vegan sample. For a representative diet survey, monthly food frequency consumption was obtained and then escalated to daily intake. Nutrients were calculated by blinded-nutritionist through The Food Processor Software® (ESHA research) Version 10.15.41.

We also verified that physical activity did not reach intensities prescribed for therapeutic purposes. Exclusion criteria contemplated high-performance athletes, those with a previous diagnosis of chronic disease, chronic alcoholism, pregnant and lactating women. Users of medications (particularly absorption-inhibition or modifiers of lipid metabolism drugs) or variations in sleep patterns that could influence variables of interest were excluded. Volunteers who followed a partial vegetarian diet were not considered either. All participants gave their approval on informed consent.

A short-questionnaire was applied to identify healthcare professional aid regarding diet planning or supplementation habits and other health-related behaviors in both groups. For paring performance, body mass index (BMI) was determined, which includes variables of weight and height. For weight measurement, a Tanita BC-418 Segmental Body Composition Analyzer® scale was used following the protocol proposed by Khalil and colleagues [38]. For height evaluation, methods established by the International Society for the Advancement of Kinanthropometry (ISAK) were followed [39], using a SECA stadiometer (model 700). In both groups, four conventional lipid biomarkers were determined by trained professionals: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), as well as the serum concentration of nine fatty acids (from C14:0 to C20:4) as potential indicators of cardiovascular risk. Blinded-professionals performed lipid quantification through analytic chemistry.

To determine lipid biomarkers, a blood sample was obtained in SST tubes (without anticoagulant) under fasting conditions for eight hours. Subsequently, a serum sample was collected and processed immediately. Cut-points used were those stipulated by the Adult Treatment Panel III [40]. For serum TC measurement, a Trinder enzymatic method was used, which evaluates the activity of Cholesterol-Esterase-Oxidase-Peroxidase enzyme, using enzymatic endpoint method with a Randox® cholesterol reagent (CHOL) (cat ch201). The analysis was performed spectrophotometrically at 546 nanometers (nm) with a Vital Scientific analyzer (Selectra XL). HDL-C was determined by a direct method of immunoinhibition, using a Vital Scientific Selectra XL equipment, calibrated at two points with a 578 nm filter and Randox® reagents. For TG quantification, an enzymatic hydrolysis lipase-reaction was carried out. Total activity was determined in a Vital Scientific Selectra XL spectrophotometer, calibrated to final point with a 546 nm filter and Randox® reagents. Friedewald formula was used to estimate LDL-C concentrations, as following:  $LDL-C = [(TC)-(HDL-C) - (TG/5)]$  expressed in milligrams/deciliter (mg/dL) [41].

Fatty acids extraction was performed in 15 milliliters (mL) conical tubes, in which 1 mL of total blood was added with 1 mL of saturated sodium chloride solution, then sonicated in an ultrasound bath (Brason 1800, USA) for 2 min at 40 kHz. Following, a 300 microliters ( $\mu$ L) of high-performance liquid chromatography (HPLC) grade methanol solution (Fermont, Mexico) was added and centrifuged at 4,500 rpm at 4° C. For lipid phase extraction, a liquid-liquid microextraction was carried out, adding 2 mL solution of methyl tert-butyl ether: n-hexane (MTBE: Hx, 1:1, v/v). Organic phase was concentrated to dryness with a rotary evaporator.

Derivatization of lipid phase was carried out by adding 5 mL of trifluoride boron (14% in methanol, Sigma Aldrich, USA) and refluxing for 15 min at 220° C. The solution was transferred to a 25 mL conical tube, 10 mL of HPLC grade water was added (J.T. Baker, USA) and stirred. Finally, 1 mL of MTBE: HX solution (1:1, v/v) was added and centrifuged at 4,500 revolutions per minute for



10 min at 20° C. The quantitative fatty acid assay was performed in a 6890N gas chromatograph (Agilent, San Jose, CA, USA) coupled with an MS 5973 Network using a DB-5MS fused silica capillary column (50m × 0.25mm × 0.25 mm, J&W GC Column; Agilent Technologies, USA). A 1 µL solution of MTBE: HX with fatty acids was injected into pulsed splitless injection (70 psi for 2 min to vent) to elevate the sensitivity of the equipment. Helium (99.999%) was used as carrier gas at 230 KPa. The CG-MS oven temperature was programmed initially at 60° C for 5 min and increased to 315° C at 5° C min<sup>-1</sup>. Fatty acids' identification was performed with NIST Research Data Base.

A Shapiro-Wilk normality test was performed for continuous variables. A paired Student's t-test was conducted for means comparison between groups. Finally, association between dietary intake and biochemical profile was assessed with a Pearson's correlation coefficient. A  $p \leq 0.05$  value was considered as significant. IBM SPSS Statistics software version 21.0 was used.

## 5. Conclusions

We observed that following a vegan diet (≥3 years), did not generate evident modifications in the lipid profile commonly used in clinical practice (TC, HDL-C, LDL-C, and TG) in a south-eastern Mexican population compared with apparently healthy individuals with omnivore diet. Nevertheless, this study incorporates a quantitative identification of fatty acids, providing a chemical characterization of each one of them, contributing on a precise depiction of serum lipids modifications induced by the exposure to a vegan diet.

Vegan population presented lower SFAs (stearic acid) serum concentration, which could mean a lower biosynthesis of LDL-C, but higher intake of other SFAs (lauric acid) due to frequent coconut oil consumption. Vegans showed reduced intake and plasmatic levels of arachidonic acid associated with the biosynthesis of prostaglandins, thromboxanes, and leukotrienes with proinflammatory effects related to cardiovascular diseases, serum concentrations of linoleic acid were also reduced in this group. Therefore, potential benefit in terms of cardiovascular risk among both types of diets in Mexican individuals could be in favor of those who follow a vegan diet. However, other serum lipids in vegans need to be considered for a comprehensively interpretation. In this sense, hypertriglyceridemia was found in three vegan participants due to excessive carbohydrates consumption. PUFAs ω-3 should also be considered in future research. Cultural-influenced eating behaviors and the lack of professional advice when following a plant-based diet in the Mexican population studied decreased the benefits reported in other studies.

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