

# Serum Fatty Acids Chemical Characterization after Prolonged Exposure to a Vegan Diet

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**Abstract** The vegan diet excludes animal-derived product consumption and has been linked with cardiovascular benefits. The objective of the present comparative pilot study was to analyze dietary lipid intake, evaluate the conventional clinical lipid profile, extract fatty acids from triglycerides' molecules among Mexican individuals with prolonged exposure to a vegan diet ( $\geq 3$  years), and compare these outcomes with omnivore controls. A case-control and cross-sectional comparative pilot study was performed between 2016 and 2017. Vegans were paired in a 1:1 ratio with omnivores from Merida, Mexico. A 144-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 total cholesterol, stearic, arachidonic, and *trans* fatty acids intake ( $p < 0.001$ , 0.014,  $< 0.001$ , 0.009 respectively), but higher consumption of lauric acid were observed in the vegan group ( $p = 0.007$ ). Vegan-diet consumers also presented greater triglycerides concentrations ( $p = 0.049$ ); however, when analyzing fatty acids, we identified lower levels of stearic, oleic, linoleic, and arachidonic acids ( $p = 0.002$ , 0.030, 0.015, 0.008, 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:** cardiovascular risk factors, feeding patterns, lipids, nutritional status, vegan diet

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## 1. Introduction

The vegan diet entirely excludes animal-derived products consumption. [1] Following this eating pattern could implicate protection against hypertension and ischemic heart disease due to favorable modulations in blood lipids. Lower concentrations of serum lipoproteins, saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs)  $\omega$ -3 and  $\omega$ -6 (particularly arachidonic acid), yet higher monounsaturated fatty acids' (MUFAs) levels had been reported in vegans when compared with meat-eaters. [2-5]

The ideal serum relationship between PUFAs  $\omega$ -6/ $\omega$ -3 remains in a 3:1 ratio. [6] An alteration in this proportion may promote inflammation. [6,7] Arachidonic acid is a precursor of potent pro-inflammatory mediators; thus, a possible reduction in serum concentrations of this  $\omega$ -6 fatty acid among vegans may implicate less biosynthesis

of 2-series thromboxanes, 2-series prostaglandins, and 4-series leukotrienes. [6,8] Furthermore, decreased levels of SFAs could also modulate favorably low-density lipoprotein cholesterol (LDL-C) biosynthesis. [9] These possible differences in the concentrations of lipids between plant-base diet consumers and meat-eaters could implicate diverse risk factors for cardiovascular diseases, and health advantages associated with serum lipids may be assumed in favor of vegans. [3,4,5,10]

However, the Mexican diet typically differs from other regions of the world; consequently, even when a vegan diet is followed, different physiological results may be expected due to local and cultural adaptations. [11] Plant-based diets are not entirely described among the Mexican population and it is pertinent to evaluate health's effects in this unexplored community. Inexistent information regarding serum fatty acids in vegans from Mexico addresses importance to analyze the behavior of serum lipids in this community.

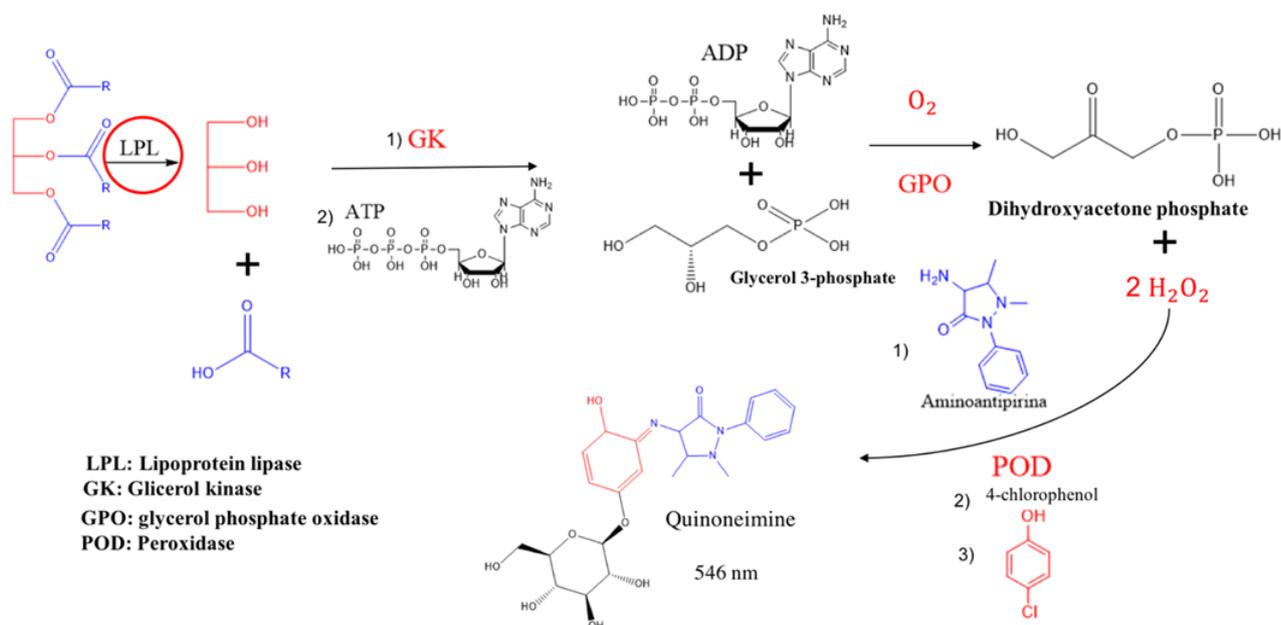


Figure 1. Triglycerides determination by spectrophotometric techniques

It should be noticed, however, that clinical techniques to determine serum triglycerides typically uses a colorimetric method by spectrophotometric technique; this methodology uses a lipase enzyme to cut the triglycerides after separating the glycerol and three mols of fatty acids, and only uses the glycerol to quantify triglycerides molecules as described in Figure 1. Nonetheless, it is relevant to classify the fatty acids that are integrated into triglycerides' structure by spectrophotometric approaches, to generate more information regarding blood lipids. Therefore, we aimed to evaluate not only the conventional clinical lipid profile, but extract fatty acids from triglycerides' molecules among Mexican individuals with prolonged exposure to a vegan diet ( $\geq 3$  years). We also compared the concentrations of fatty acids (from C14:0 [lauric acid] to C20:4 [arachidonic acid]) between plant-based diet consumers vs. omnivore controls and described them as biomarkers of cardiovascular health. We hypothesized that the local vegan diet derives into variations in serum lipoproteins and fatty acids' concentrations when compared with non-vegans, which supposes improvements in cardiovascular biomarkers.

## 2. Materials and Methods

### 2.1. Study Design

A case-control cross-sectional pilot study was conducted in south-eastern Mexican adults (Merida, Mexico) in 2017, following pre-established criteria for an adequate pilot study design and similar previously published studies. [12,13] The present work adhered to the Strengthening the Reporting of Observational Studies in Epidemiology statement for case-control studies and received approval by the Ethics Committee from Universidad Marista de Merida number CE\_UMM002A\_2017, following the regulations regarding research protocols by Secretary of Health of Mexico (NOM-012-SSA3-2012) and Declaration of Helsinki.

### 2.2. Participants

Sixty-eight subjects from a local vegan-vegetarian group were originally identified through printed and electronic advertisement, twenty-five of them met the inclusion criteria, and twelve individuals provided written informed consent to participate in the study. Inclusion criteria contemplated female and male healthy individuals with a vegan diet for an uninterrupted period of  $\geq 3$  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 equal gender and socioeconomic status, similar age ( $\pm 5$  years), and body mass index ( $\pm 1$  kg/m<sup>2</sup>). Controls also signed informed consent. Exclusion criteria contemplated high-performance athletes (we also verified that physical activity did not reach intensities prescribed for therapeutic purposes), those with a previous diagnosis of chronic disease, chronic alcoholism (Alcohol Use Disorder Identification Test score  $>8$ ), 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. Twenty-four participants (vegans and omnivores combined) were included in the study.

### 2.3. Dietary Analysis

A previously validated 144-item Semi-Quantitative Food Frequency Questionnaire [14] was conducted to assess monthly eating patterns in both dietary groups and to ensure full exclusion, consciously and unconsciously, of animal-derived products in the vegan sample. Daily nutrients intake was calculated by blinded nutritionists through The Food Processor Software<sup>®</sup> (ESHA research) Version 10.15.41. Healthcare professional aid regarding diet planning or supplementation habits and other health-related behaviors were also assessed in both groups.

## 2.4. Anthropometric Assessment

For paring performance, body mass index (BMI) was determined. A previously calibrated Tanita BC-418 Segmental Body Composition Analyzer<sup>®</sup> scale was used for weight measurement, following the protocol proposed by Khalil and colleagues. [15] For height evaluation a SECA stadiometer (model 700) was used following the methods established by the International Society for the Advancement of Kinanthropometry. [16]

## 2.5. Serum Lipid Assessment

### 2.5.1. Lipoproteins and Triglycerides

Five milliliters were collected from total blood in SST tubes (without anticoagulant) under eight-hours fasting conditions. Total cholesterol (CT), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were immediately determined by blinded-professionals through analytic chemistry. Cut-off points used were those stipulated by the Adult Treatment Panel III. [17] 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<sup>®</sup> 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<sup>®</sup> 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 the final point with a 546 nm filter and Randox<sup>®</sup> reagents. Friedewald formula was used to estimate LDL-C concentrations (in milligrams/deciliter), as presented in equation (1). [18]

$$\text{LDL} - \text{C} = [(\text{TC}) - (\text{HDL} - \text{C})] - \text{TG} / 5 \quad (1)$$

### 2.5.2. Fatty Acids Assay

Fatty acids' extraction was performed from 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 (μL) 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). The 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 Gas chromatography-mass spectrometer (GC-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.

Results were obtained as chromatogram of 37 fatty acid methyl ester (FAME) according to the gas chromatography column the main separate it is based on boiling point, for that, we observed between 10 to 35 min the saturated FAME, and from 40 to 60 min the C18 to C24 FAMES.

### 2.5.3. Gas Chromatography-Mass Spectrometer (GC-MS) Calibration and Validation Parameter

A certified reference material (TraceCERT, Supelco USA) of 37 FAME (C6 to C24, saturated, monounsaturated, and polyunsaturated) was used to calibrate de Gas chromatography coupled to mass spectrometry, in range concentration of 0.5 mg/L to 50 mg/L. In that same range of calibration, we used with a deuterated standard reference material of Myristid-d27 acid, Palmiditic-d31 acid, and Stearic-d35 acid all from Sigma-Adirch (USA). The deuterated FAMES were used as an internal standard to fortified the samples. The validation parameters evaluate according to de International Council for Harmonization-Quality (ICH Q2A) for linearity, limit of detection (LOD), precision, and accuracy.

## 2.6. Statistical Analysis

A Shapiro-Wilk normality test was performed. Variables with a normal and non-normal distribution were compared between paired dietary groups through a two-tailed Student's *t*-test and a Wilcoxon signed-rank test, respectively. The association between dietary intake and serum concentrations of arachidonic acid was assessed with a Pearson's correlation coefficient. According to distribution, data is presented in means ± SD and medians (25th and 75th percentiles). A  $p \leq 0.05$  value was considered as significant. Statgraphics Centurion<sup>®</sup> software version XVII was used.

## 3. Results

### 3.1. General Characteristics of Subjects

A case-control and cross-sectional comparative pilot study was performed in twelve Mexican vegans who were randomly paired with omnivores controls in a 1:1 ratio. Subjects conditions did not differ significantly among groups (BMI= 63.2 ± 14.0 vs. 58.2 ± 10.6,  $p= 0.445$ ; age= 29 ± 9 vs. 29 ± 10,  $p= 0.777$ ; vegans and omnivores, respectively), nor socioeconomic or gender. All individuals completed the study (six males and

eighteen females). None of them were excluded based on missing data.

### 3.2. Nutritional Data

Dietary analysis is presented in Table 1. Plant-based diet group exhibited lower cholesterol, stearic, arachidonic, and total *trans* fatty acids intake, due to animal-derived products dietary exclusion. Contrarily, lauric SFA consumption was greater among vegans, associated with regular dietary inclusion of coconut oil ( $6.9 \pm 6.0$  grams). It should be noticed, however, that vegan-diet consumers only showed daily consumption of soy oil ( $3.0 \pm 5.5$  grams), olive oil ( $9.3 \pm 8.9$  grams), almonds or nuts ( $18.5 \pm 10.4$  grams), and avocado ( $29.3 \pm 13.5$  grams) as unique vegetable oils' sources. Regular consumption of peanuts, cashews, pistachios, walnuts, and hazelnuts was not identified.

### 3.3. Serum Lipoproteins Assay

Conventional lipid profile analysis (Table 2) revealed greater triglycerides' concentration among vegans, and

hypertriglyceridemia ( $\geq 150$  mg/dL, 1.69 mmol/L) was prevalent in three of them. This finding is related to excessive grain-based products and carbohydrates intake observed in vegans ( $250.8 \pm 44.2$  vs.  $208.9 \pm 55.4$  grams) as a replacement for animal-derived product consumption. No controls with hypertriglyceridemia were identified.

### 3.4. Fatty Acids chemical characterization

Fatty acids from TG molecules were extracted and quantified. Results are presented in Table 3. In agreement with dietary consumption, a significantly lower concentration of stearic acid was observed among vegans. Regarding PUFAs, a reduced concentration of oleic, linoleic, and arachidonic was also found in vegan-diet consumers. A positive association between arachidonic acid plasmatic concentrations and dietary intake was found ( $r = 0.477$ ,  $p = 0.018$ ). Furthermore, the concentrations of elaidic acid (the *trans* isomer of oleic acid) was decreased among vegans, this finding corresponds with the dietary intake of total *trans* fatty acids observed in plant-based diet consumers.

**Table 1. Comparison of serum lipoproteins and triglycerides among subjects following a vegan diet with omnivores controls from a Mexican population**

Serum lipids (mg/dL) <sup>a</sup>	Vegans (n= 12)	Omnivores (n= 12)	p-value <sup>b</sup>
TC	154.0 $\pm$ 36.2	167.4 $\pm$ 45.8	0.273
HDL-C	51.9 $\pm$ 10.9	55.9 $\pm$ 8.6	0.337
LDL-C	81.4 $\pm$ 30.6	96.0 $\pm$ 36.8	0.091
TG	102.6 $\pm$ 46.4	76.7 $\pm$ 36.8	0.049*

Data presented in means  $\pm$  SD

<sup>a</sup>International System of Units conversions: 1 mg/dL of TC, HDL-C and LDL-C= 0.26 mmol/L; 1mg/dL of TG= is 0.01 mmol/L.

<sup>b</sup>Obtained using a Student's *t*-test for paired variables, considering 95% confidence interval.

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

**Table 2. Daily dietary lipid intake comparison between vegan-diet consumers and omnivore controls from a Mexican population**

Dietary assessment	Vegans (n= 12)	Omnivores (n= 12)	p-value <sup>a</sup>
Total fat (g)	61.2 $\pm$ 19.0	52.5 $\pm$ 15.1	0.259
Total cholesterol (mg)	7.1 (2.9 - 11.6)	180.9 (139.4 - 221.7)	<0.001
Total SFA (g)	15.3 $\pm$ 6.6	13.5 $\pm$ 4.1	0.408
Lauric (C12:0) (g)	3.6 (0.9 - 6.6)	0.3 (0.2 - 0.9)	0.007
Myristic (C14:0) (g)	1.5 $\pm$ 1.2	1.0 $\pm$ 0.6	0.151
Palmitic (C16:0) (g)	5.5 $\pm$ 1.4	6.5 $\pm$ 1.7	0.128
Stearic (C18:0) (g)	1.5 $\pm$ 0.7	2.4 $\pm$ 0.7	0.014
Total MUFA (g)	22.0 (17.9 - 31.2)	21.8 (16.1 - 26.9)	0.519
Palmitoleic (C16:1) (g)	0.7 (0.5 - 1.0)	0.8 (0.6 - 1.0)	0.482
Oleic (C18:1) (g)	20.2 (16.1 - 30.5)	18.9 (12.5 - 22.9)	0.233
Total PUFA (g)	14.5 $\pm$ 3.5	13.0 $\pm$ 6.9	0.472
Total $\omega$ -3 PUFA (g)	1.9 $\pm$ 1.0	1.4 $\pm$ 0.5	0.219
Total $\omega$ -6 PUFA (g)	12.5 $\pm$ 3.1	10.6 $\pm$ 5.8	0.296
Linoleic (C18:2) (g)	12.3 $\pm$ 3.2	9.6 $\pm$ 5.7	0.158
Arachidonic (C20:4) (g)	0.07 $\pm$ 0.05	0.22 $\pm$ 0.09	<0.001
Total <i>trans</i> fatty acids (g)	0.1 (0.01- 0.2)	0.3 (0.2 - 0.4)	0.009

According to distribution, data is presented as means  $\pm$  standard deviations or median (25<sup>th</sup> percentile - 75<sup>th</sup> percentile).

<sup>a</sup>Obtained using a two- tailed Student's *t*-test for paired variables or a Wilcoxon signed-rank test, considering 95% confidence interval.

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

**Table 3. Fatty acids comparison between subjects with a vegan diet vs. omnivore controls from a Mexican population**

Fatty acids	Vegans (n=12)		Omnivores (n=12)		p-value <sup>a</sup>
	(mg/dL) <sup>b</sup>	%	(mg/dL) <sup>b</sup>	%	
<b>SFA</b>					
Lauric (C12:0)	4.0 ± 1.1	2.2	3.6 ± 0.6	1.4	0.269
Myristic (C14:0)	5.1 ± 1.9	3.2	5.1 ± 1.3	2.1	0.987
Palmitic (C16:0)	60.7 ± 57.9	33.5	107.6 ± 58.3	34.3	0.061
Stearic (C18:0)	13.0 ± 8.8	9.3	33.6 ± 16.4	12.3	0.002
<b>MUFAS</b>					
Palmitoleic (C16:1)	5.7 ± 3.1	3.9	5.4 ± 1.6	2.0	0.757
Oleic (C18:1)	27.9 ± 15.7	20.0	55.5 ± 28.1	17.7	0.030
<b>PUFAS (<math>\omega</math>-6)</b>					
Linoleic (C18:2)	32.0 ± 18.2	22.1	76.1 ± 39.9	24.2	0.015
Arachidonic (C20:4)	5.5 ± 2.6	3.0	11.7 ± 4.9	3.7	0.008
<b>Unsaturated Trans</b>					
Elaidic	4.8 ± 2.5	2.7	6.9 ± 2.9	2.2	0.030
<b>Summary</b>	<b>181.3 ± 20.6</b>		<b>314.0 ± 37.5</b>		<b>0.034</b>

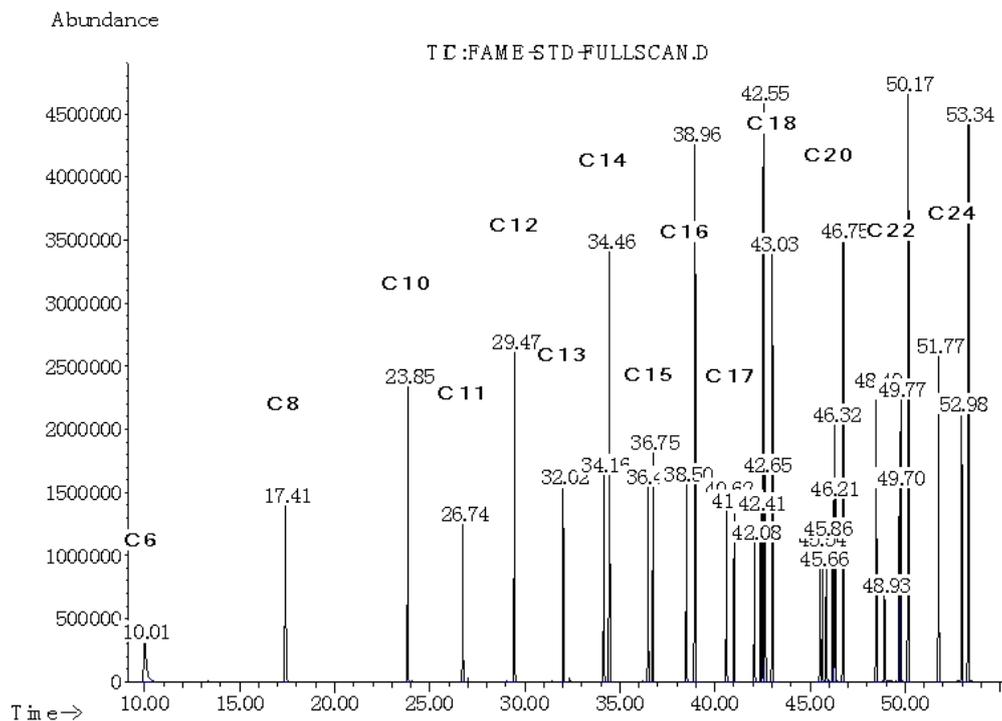
\* The equipment's limit of detection only allowed the observation of fatty acids from C14:0 to C20:4.

Data presented in means ± SD.

<sup>a</sup>Obtained using a Student's *t*-test for paired variables, considering 95% confidence interval.

<sup>b</sup>International System of Units conversions: 1mg/dL of free fatty acids = 0.04 mmol/L.

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



**Figure 2.** Chromatogram by CG-MS at 50 mg/L of 37 FAMES

Figure 2 presents the chromatogram of 37 FAMES, in which a good resolution between each signal and high selectivity are observed, including relevant fatty acids such as  $\alpha$ -linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid, among other  $\omega$ -6 fatty acids. Our methodology and results met with the parameters established with the ICH Q2A as follows: linearity range between 0.5 mg/L to 50 mg/L, all signals showed a proportional increase to the concentration with a correlation coefficient more than 0.99, with a precision less than 1.5%

and recovery percent more than 93.5% on fortified blood samples with FAMES and deuterated FAMES.

## 4. Discussion

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

risk. Despite a lower cholesterol intake among the plant-based diet sample, no difference between groups was observed in TC serum concentrations. Even without statistical significance, two non-vegan individuals 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. [17] No hypercholesterolemia was reported among vegan-diet consumers. In this sense, Wang and colleagues [19] carried out a systematic review and 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 when compared with meat-eaters, [19] there is discrepant evidence founding that this eating pattern does not always generate favorable changes in serum lipids. [20] In this sense, our statistical analysis indicated that a vegan diet did not significantly modify serum lipoproteins when compared with controls. It should be notice, that blood lipids 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. [21,22,23] However, these beneficial lifestyle factors are not necessarily widespread, since we did not identify those health-related behaviors among the vegan sample. Variations in vegans' food selection in different populations could also lead to diverse physiological results, [11] due to the different availability of food within regions, as well as cultural adaptations of the vegan diet.

We identified an imbalanced distribution of macronutrients among the plant-based diet consumers, associated with larger grain-based product consumption as a substitution for meat, which implies greater carbohydrates daily intake with potential metabolic adverse effects, such as hypertriglyceridemia. In agreement with our results, other studies have reported that vegetarians have higher TG concentrations, as well as lower levels of HDL-C, increased systolic pressure and higher prevalence of antihypertensive drug consumption compared to an omnivore population. [11,24] The authors also attributed these results to changes in dietary macronutrient distribution, which supposes an excessive intake of carbohydrates as a substitution method for animal-derived products. [11] A direct relationship has been reported between environmental and sociocultural factors around the Mexican diet with dyslipidemia (high TG and low HDL- C). [25] These factors may also be affecting local vegan-diet consumers. Nevertheless, our statistical result for TG may be interpreted with caution.

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), [26,27,28] our findings only showed a feeble intake of nuts, olive oil, and avocado as sources of vegetable oils in the vegan group. No  $\omega$ -3/ $\omega$ -6 PUFA supplement consumers were identified, and professional nutritional guidance was reported by only two vegan participants. In countries consuming different types and proportions of

vegetable oils, vegans may likely show different serum fatty acids concentrations. [29] Consequently, sociocultural factors and food availability around the Mexican vegan diet could limit the benefits reported in other latitudes regarding serum lipids.

The main outcome of our study is that the prolonged exposure to the local plant-based diet generates modifications in the triglycerides' fatty acid profile, which imply a reduction in absolute fatty acid concentrations, with an emphasis in stearic, oleic, linoleic, arachidonic, and elaidic acids when compared to omnivore controls ( $p= 0.002, 0.030, 0.015, 0.008, 0.030$ , respectively). These findings coincide partially with what has been previously reported. A substantial reduction in absolute serum fatty acids levels -especially in stearic, oleic, linoleic, arachidonic, palmitic, and palmitoleic- have been shown among vegetarians when compared with omnivores. [30]

Literature reports that vegans have lower plasma concentrations of SFAs, which could represent a protective factor against cardiovascular events. [6] These findings correspond with our results, where a lower intake and serum concentration of stearic acid were found. SFAs are linked to LDL-C biosynthesis and inflammatory processes which contribute to the development of cardiometabolic. [9] However, a recent systematic review and meta-analysis performed 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.

Some evidence report that vegans have higher serum MUFAs' levels than non-vegan individuals. [5] Contrarily, we identified less oleic acid concentrations in vegans, and MUFAs dietary intake did not present variations between groups. In agreement with our results, similar studies had also identified lower serum oleic acid concentrations in vegetarian-diet consumers. [30] Based on these findings, we could attribute the differences in food selection between regions of the world, since we did not identify a vast quantity of MUFAs' food sources in the vegan group, such as peanuts, cashews, almonds, pistachios, macadamia, or hazelnuts. [32] Thus, benefits regarding the cholesterol-lowering effect may not be expected in this vegan community. [33]

Regarding PUFAs, we found decreased arachidonic and linoleic acids' concentrations in vegans' TG molecules. Individuals following a plant-based diet typically have lower serum concentrations of arachidonic acid due to animal-derived products consumption avoidance, which vastly contain in this  $\omega$ -6 fatty acid. [34,35] 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 ( $p < 0.001$ ). [29] 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 acid; thus, serum concentrations of both PUFAs in this sample are expected to be lower than omnivore controls. [34,36,37].

The reduction in the concentration of  $\omega$ -6 fatty acids can limit the biosynthesis of 2-series prostaglandins (PGE2, PGI2, PGD2, PGF2 $\alpha$ ), 2-series thromboxanes (TXA2, TXB2), 4-series leukotrienes (LTA4, LTB4, LTC4,

LTD4, LTE4) and other proinflammatory or vasoconstriction factors (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ), which contribute to the development and progression of cardiovascular diseases. [6,38] By having statistically diminished the concentration of arachidonic acid, vegan participants may have fewer inflammatory factors, which could imply a lower risk of developing cardiovascular complications.

In the context of *trans*-fatty acids, we identified both lower intake and decreased serum concentrations of elaidic acid in vegan participants. Sarter and colleagues [35] coincide with our results, by reporting a reduction of plasma *trans*-fatty acids (18:1 and 18:2 isomers) in vegan-diet consumers. The authors attributed this outcome to a lower intake of products containing partially hydrogenated oils in vegans. A systematic review and meta-analysis conducted by Huang and colleagues [39] associated large intakes of *trans*-fatty acids with a greatest risk for developing cardiovascular diseases, the authors found a 16% lower mortality rate from circulatory diseases in vegetarians when compared with omnivores. Thus, benefits associated with positive modulations of *trans*-fatty serum concentrations may be extrapolated in favor of our vegan sample.

Evidence shows some results that contrast with what was found in our study. However, eating habits in Mexico are culturally and socially inequivalent to other countries, consequently, even when a plant-based diet is followed, different physiological results may be expected due to local adaptations. Individuals who follow a vegan diet typically present a 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 pertinent evidence to the Mexican population regarding the relationship between the vegan diet and serum fatty acids modifications. Our preliminary results coincide partially with what has been reported in limited international literature. This reinforces the hypothesis that a vegan diet may imply lower concentrations of stearic, oleic, linoleic, arachidonic, and elaidic acids. However, we recognize sample size with unequal numbers of male and female participants as a limitation. Nevertheless, it is considered appropriate that a minimum of 12 subjects per group be included for pilot studies -such as the present research- based on the feasibility and precision around the estimates to be used to design future studies. [13,40] Although a full-scan was performed and 37 FAMES were measured, some fatty acids were not expressed in grams, because they were found in quantities below the equipment's LOD.

We used a dietary tool that specifically assesses "fats and oils" in one of the nine food categories. It was validated in a Latin-American country in the Spanish language, which was easily understood by our participants and allowed us to accurately collect representative data relevant for our main outcome. Furthermore, our dietary findings and novel technique for the determination of fatty acids obtained directly from TG molecules in this unexplored population are relevant to international literature and valuable for clinical practitioners, which promotes further larger research regarding this matter. Future investigations may also consider a prospective randomized trial design, to diminish the expected bias of the transversal and

observational nature of our study. The cardiovascular biomarkers modifications observed after the prolonged exposure to a plant-based diet befell in normal-weight healthy young adults; nonetheless, the inclusion of participants outside normal parameters and the usage of a technique that allows a full spectrum fatty acid quantification may also be taken into consideration for future projects. The preliminary results of the present study enable that task, by presenting innovative findings that can be translated into clinical practice and proposing additional research as a complementary biochemical screening in vegan individuals.

## 5. Conclusions

This study incorporates a quantitative extraction of fatty acids from TG molecules, providing a chemical characterization which contributes to precisely depict modifications of serum lipids induced by the exposure to a vegan diet.

Vegan-diet consumers presented lower stearic and arachidonic serum concentrations, which could favorably modulate the biosynthesis of LDL-C, prostaglandins, thromboxanes, and leukotrienes with proinflammatory effects related to cardiovascular diseases. Therefore, potential benefits regarding cardiovascular risk could be in favor of those Mexican individuals who follow a vegan diet. Nonetheless, we observed that the local vegan diet may differ from other regions due to cultural adaptations. Consequently, reduced concentrations of linoleic and oleic acid were found. Hypertriglyceridemia produced by an excessive carbohydrates consumption may decrease the expected benefits in cardiovascular biomarkers.

The analysis by gas chromatography coupled with mass spectrometer generated precise information regarding serum lipids and can be applied to characterize the FAME in blood samples with excellent and safe results that can be employed in future research.

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## Conflict of Interests

The authors have no competing interests.

## List of Abbreviations

MTBE= methyl tert-butyl ether

FAME= fatty acid methyl ester

ICH Q2A= International Council for Harmonization-Quality.

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