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Diet, Plasma, Erythrocytes, and Spermatozoa Fatty Acid Composition Changes in Young Vegan Men

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Abstract There has been increasing interest in vegan diets, but how this dietary pattern regulates tissue fatty acids (FA), especially in men, is unclear. Our aim was to evaluate the effect of a vegan diet on plasma, erythrocyte, and spermatozoa FA composition in young men. Two groups consisting of 67 young (18–25 years old) men were studied. One group following an omnivore diet but did not consume fish, shellfish or other marine foods (control, $n = 33$), and another group following a vegan diet (vegan, $n = 34$) for at least 12 months were compared. Dietary intake was assessed *via* a food frequency questionnaire and a 24-h recall. FA composition was measured in plasma, erythrocyte phospholipids, and spermatozoa by gas–liquid chromatography. Compared to controls, the vegan group had higher reported intakes of carbohydrate, dietary fiber, vitamins (C, E, K, and folate), and minerals (copper, potassium) but lower intakes of cholesterol, *trans* FA, vitamins B_6, D, and B_{12}, and minerals (calcium, iron, and zinc). Vegan's reported a lower saturated FA and not arachidonic acid intake, both groups did not intake eicosapentaenoic acid and docosahexaenoic acid (DHA), but vegan's showed a higher alpha linolenic acid ALA intake. Vegans had higher plasma, erythrocyte phospholipid, and spermatozoa ALA, but lower levels of other n-3 polyunsaturated fatty acid (PUFA), especially DHA. Vegans were characterized by higher ALA, but lower levels of other n-3 PUFA, especially DHA in plasma, erythrocytes, and spermatozoids. The biological significance of these findings requires further study.

Keywords Erythrocytes · Healthy men · Plasma · Polyunsaturated fatty acids · Sperm · Vegan diet

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Abbreviations

ALA	alpha-linolenic acid (18:3n-3)
AA	arachidonic acid (20:4n-6)
BMI	body-mass index
BHT	butylated hydroxytoluene
DHA	docosahexaenoic acid (22:6n-3)
EPA	eicosapentaenoic acid (20:5n-3)
FA	fatty acid(s)
FAME	fatty acid methyl esters
FFQ	food frequency questionnaire
LA	linoleic acid (18:2n-6)
MUFA	monounsaturated
n-3 PUFA	n-3 polyunsaturated fatty acids
n-6 PUFA	n-6 polyunsaturated fatty acids
PBS	phosphate-buffered saline
PUFA	polyunsaturated fatty acids
RAE	retinol activity equivalent
SFA	saturated fatty acid(s)
TLC	thin layer chromatography
USDA	United States Department of Agriculture

Introduction

Vegan diets are followed for several reasons including religious, ethical, environmental, health or sociocultural, and

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have become popular, especially among younger adults (Craig et al., 2009; Leitzmann, 2014). Benefits ascribed to a vegan diet include maintenance of body weight, decreased insulin resistance, as well as lower mortality risk from ischemic heart disease and a lower incidence of cancer (Chiu et al., 2015; Dinu et al., 2017; Kwok et al., 2014). In this regard, vegan diets are characterized by higher levels of certain nutrients including dietary fiber, folate, vitamin C, potassium, magnesium, and unsaturated fatty acids (FA) (Craig, 2009). However, several human studies have shown vegan diets also have lower levels of iron, vitamin B_{12}, calcium, and zinc (Craig, 2009; Gibson et al., 2014; Rizzo et al., 2016; Schüpbach et al., 2017; Śliwińska et al., 2018). With regards to FA, vegan diets have lower levels of certain n-3 polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids (Davis and Kris-Etherton, 2003; Kornsteiner et al., 2008; Rosell et al., 2005; Sanders, 2009).

Recent studies have further shed light on the potential benefits of this dietary pattern. One study showed enhanced gut cell metabolism in vegan adults, with changes positively influencing host's health (De Angelis et al., 2020). Although anti-inflammatory properties of the vegan diet have been purposed, recent studies showed no overall differences in inflammatory biomarkers between vegans and omnivore young adults (Lederer et al., 2020; Menzel et al., 2020). The vegan diet, however, has been reported to reduce blood pressure in chronic diseases such as type 2 diabetes (Abbasnezhad et al., 2020), and has gained considerable attention from a planetary health perspective, as recently reviewed (Fresán and Sabaté, 2019).

N-6 and n-3 PUFA regulate critical aspects of human physiology and biochemistry (Burns et al., 2018; FAO/WHO, 2010). The synthesis of n-6 and 3 PUFA is a complex process that occurs mostly in the liver and provides a constant supply of these PUFA to different cells and tissues, such as the brain, retina, immune system, and spermatozoa cells (Burns et al., 2018; FAO/WHO, 2010). Linoleic acid (18:2n-6, LA) is the precursor to longer carbon-chain n-6 PUFA, such as arachidonic acid (20:4n-6, AA) (Das, 2006) while alpha-linolenic acid (18:3n-3, ALA) is a precursor to EPA and DHA (Domenichiello et al., 2015), but the efficiency in the conversion process considered to be very low (ALA to EPA less than 5% and ALA to DHA less than 1%) (Brenna et al., 2009). Also, PUFA synthesis is dependent on substrate availability and competition, activity of elongase and desaturase enzymes (e.g. Δ-5 and Δ-6 desaturases), availability of specific nutrients (e.g. zinc), redox cell state (especially hepatic), hormones, and genetics (Burns et al., 2018; Lee et al., 2016; Montanaro et al., 2005; Valenzuela et al., 2018).

It has been reported that vegetarian and vegan men have lower plasma and erythrocyte levels of EPA and DHA, compared to omnivore controls (Elorinne et al., 2016; Fokkema et al., 2000; Rosell et al., 2005; Sanders, 2009). However, to the best of our knowledge, the effect of a vegan diet on FA levels in other body compartments such as spermatozoa has not been reported yet. This is an important area to study as it has been reported there is decreased sperm quality with lower spermatozoa motility in vegan men compared to nonvegetarian men (Orzylowska et al., 2016).The current study aimed to assess the impact of the vegan diet on plasma, erythrocyte, and spermatozoa FA composition compared with men that do not eat fish and shellfish.

Material and Methods

Subjects

For this study, we invited through an open call to participate, young (between 18 and 25 years) healthy vegan and omnivore men. Subjects had to be following an omnivore diet (control, $n = 33$) or a vegan diet (vegan, $n = 34$), for at least the previous 12 months. Exclusion criteria were presence of any acute or chronic medical condition (hypertension, type-2 diabetes, non-alcoholic fatty liver disease, infertility, *etc*.), high alcohol intake, use of any drugs, or the use of nutritional supplements during the previous year. In addition, omnivore men should not have ingested fish, shellfish, or other marine foods (traditional dietary EPA and DHA) for at least the previous 12 months. This study included a group of men that did not consume fish or seafood, to avoid the effect of foods containing EPA or DHA on levels of n-3 PUFA in plasma, erythrocytes, or spermatozoa. Those who voluntarily agreed to participate and fulfilled the inclusion and exclusion criteria were recruited. The study protocol was reviewed and approved by the Ethic Committee of the Faculty of Medicine, University of Chile (Protocol #103-2017). All information regarding the study was given to each participant who voluntarily agreed to participate and signed informed consent was obtained.

Nutritional Assessment

After recruitment, participants were subjected to a clinical and nutritional evaluation. The clinical evaluation was done by a physician to assess overall health status to confirm exclusion criteria. Anthropometric evaluation was done by a trained nutritionist. Weight and height were measured and body-mass index (BMI) was calculated in kg/m^2. BMI classification was used to define underweight

(BMI <18.50 kg/m^2), normal-weight (BMI: 18.50–24.99 kg/m^2), overweight (BMI: ≥25.00 kg/m^2), and obesity (BMI: ≥30.00 kg/m^2) (World Health Organization, 2000).

Diet Analysis

To estimate dietary intakes (energy, macro-, and micronutrients) participants were interviewed by a trained dietitian. Diet evaluation included a structured food frequency questionnaire (FFQ) and a 24-h recall. First, participants were asked to report all consumed foods during the last month. To better estimate the amount of each food/beverage consumed, dietitians used a photographic instrument (*Atlas of Commonly Consumed Foods in Chile)*, as was previously reported (Cerda et al., 2010). This is a validated graphic instrument to help assess foods consumed by the Chilean population. Second, a 24-h dietary recall was applied to evaluate the dietary pattern of the previous day and to potentially include any unreported food in the FFQ. Dietary information was reviewed with the trained dietitian to evaluate accuracy, and to check for potential missing information. In case of missing data, participants were contacted and the data checked immediately. In both groups, the questionnaire included nine food groups (e.g. *cereals; fruits and vegetables; dairy; meats and eggs; legumes; fish and shellfish; high-lipid foods; oils and fats; sugars and processed foods*) which were used for analysis of the FFQ data. In more detail, *cereals* included all cereals and potatoes; *fruits and vegetables* included citrus, apple, peach, banana, berries, grape, watermelon, melon *etc*., natural fruit juice and vegetables lettuce, celery, tomato, cabbage, carrot, onion, cabbage, spinach *etc*.; *dairy* products included milk, cheese, fresh cheese and yogurts; *meats and eggs* included beef, chicken, pork, and turkey meat and all their derived products, as well as eggs; *fish and shellfish* included hake, mackerel, tuna, salmon, and shellfish (fresh and frozen); *legumes* included beans, chickpeas, and lentils; *high-fat foods* included olives, almonds, peanuts, walnuts, avocado, pistachios, and hazelnuts; *oils and fat* included vegetable oils (mainly sunflower, soybean, canola, grape seed, and olive oil) and fats (lard, butter, margarine, mayonnaise and cream); *sugars and processed foods* included sugar, honey, jam, delicacies, soft drinks, artificial juices, chocolates, cookies, sweet, and savory snacks.

Dietary data were analyzed using the software, Food Processor SQL® (ESHA Research, Salem, OR, USA), to calculate energy and nutrient intake. Diet composition was obtained using a database from the USDA National Nutrient Database for Standard Reference, and from locally generated database including nutrient composition data of common foods and preparations commonly used in Chile. Total dietary fat and FA composition was calculated for each consumed food, expressed as g per 100 g of food. An average of total fat and FA intake was obtained per each food group.

Sampling and Analysis of FA from Erythrocytes, Plasma, and Spermatozoa

Blood and semen samples were obtained at the clinical and nutritional visit after enrollment. Upon a night of fasting for at least 8 h, a venipuncture was done in the nondominant arm and a blood sample (25 mL) was taken. Five BD Vacutainer spray-coated K2-EDTA tubes were filled with 5 mL of blood. Blood samples were immediately centrifuged to obtain erythrocyte and plasma fractions (3500 rpm × 10 min at 20 °C). After centrifugation, plasma and buffy coat were removed with a micropipette; then the red cell fraction was washed with cold phosphate-buffered saline (PBS, 4 °C) until reaching a red cell fraction to PBS ratio of 1:3. The tube's content was mixed with gentle inversion movements and was centrifuged (1500 rpm × 5 min at 4 °C), and the supernatant was removed. The washing process was repeated once more, and, afterward, the washed red cell fraction was stored in a cryotube and then frozen at −80 °C for further analysis.

Semen samples were obtained from each participant who were previously requested to abstain from ejaculating for 4–7 days prior to clinical evaluation. Semen samples were obtained and collected in a pre-weighed sterile cup and incubated for 30 min at 37 °C for liquefaction. Spermatozoa cells were obtained by centrifuging samples at 500 g for 15 min. Seminal plasma was carefully removed and the remaining pellet was washed with 300 μL PBS. The washing procedure was repeated three times. After the last wash cycle, samples were centrifuged at 1000 g for 15 min, and PBS was removed before storing at −80 °C.

Lipid Extraction

Extraction of total lipids in erythrocyte, plasma, and spermatozoa samples was carried out according to Bligh and Dyer (Bligh and Dyer, 1959). Erythrocytes were homogenized in ice-cold chloroform/methanol (2:1 v/v) (containing magnesium chloride 0.5 N and 0.01% (w/v) BHT).

Erythrocytes phospholipids were obtained as described previously (Bascuñán et al., 2014), and the phospholipids were scraped from thin layer chromatography (TLC) plates

Table 1 Age and anthropometrics of the study population

	Control (n = 33)	Vegan (n = 34)	p value
Age, years	22.9 ± 2.3	24.9 ± 3.1	0.083
Weight, kg	69.1 ± 7.2	67.4 ± 8.6	0.074
Height, m	1.75 ± 0.2	1.73 ± 0.1	0.237
BMI, kg/m^2	22.6 ± 1.6	22.4 ± 1.3	0.563

Value are shown as mean ± SD, or as a percentage (%); BMI, body mass index: kg/m^2. The groups were compared with Student's t test for unpaired data ($p < 0.05$).

Table 2 Reported daily food groups intake

Food groups	Control ($n = 33$)	Vegan ($n = 34$)	p value
Cereals (g)	245.4 ± 30.1	371.3 ± 89.8	0.075
Fruits and vegetables (g)	305.3 ± 49.6	519.5 ± 86.7	**0.042**
Dairy (g)	409.5 ± 45.6	No intake	—
Meats and eggs (g)	85.4 ± 17.8	No intake	—
Fish and seafood (g)	No intake	No intake	—
Legumes (g)	5.15 ± 2.6	48.9 ± 8.1	**0.028**
High-fat foods (g)	29.9 ± 8.1	37.5 ± 13.5	0.253
Oils and fats (g)	27.5 ± 7.4	39.8 ± 15.2	0.311
Sugar and processed foods (g)	154.3 ± 35.7	206.9 ± 58.6	0.305

Note: The bold values are significantly different.
Data are expressed as the mean ± SD. p-value for media comparison between groups with Student's t test for unpaired data ($p < 0.05$).

Table 3 Reported daily dietary energy and nutrient intake

	Control ($n = 33$)	Vegan ($n = 34$)	p value
Energy (kcal)	2733 ± 318	2804 ± 362	0.239
Protein (g)	81.0 ± 11.9	74.6 ± 19.4	0.633
Carbohydrate (g)	383.2 ± 90.0	517.2 ± 120.2	**0.039**
Dietary fiber (g)	17.3 ± 10.8	28.4 ± 12.2	**0.002**
Soluble fiber (g)	4.2 ± 1.6	8.11 ± 3.2	**0.001**
Insoluble fiber (g)	12.9 ± 2.9	19.9 ± 6.0	**0.002**
Fat (g)	102.1 ± 25.3	109.3 ± 30.1	0.344
Cholesterol (mg)	299.3 ± 114.3	17.2 ± 8.3	**0.0001**
Trans fatty acid (g)	1.6 ± 1.0	0.5 ± 0.2	**0.031**
Vitamin A (RAE)	1793 ± 902	2955 ± 1211	**0.007**
Thiamin (mg)	1.6 ± 0.5	1.9 ± 0.7	0.269
Riboflavin (mg)	2.1 ± 0.7	1.9 ± 0.6	0.394
Niacin (mg)	17.4 ± 5.7	19.3 ± 7.6	0.955
Vitamin B_6 (mg)	2.7 ± 1.0	1.6 ± 0.5	**0.002**
Vitamin C (mg)	131.1 ± 65.3	229.0 ± 102.1	**0.003**
Vitamin E (mg)	11.6 ± 4.3	17.8 ± 5.9	**0.016**
Vitamin D (IU)	169.4 ± 94.3	100.1 ± 62.3	**0.0003**
Folate (μg)	499.9 ± 151.0	693.8 ± 235.2	**0.0001**
Vitamin B_{12} (μg)	5.9 ± 2.6	1.0 ± 1.3	**0.0001**
Biotin (μg)	31.4 ± 26.9	27.9 ± 17.4	0.059
Vitamin K (μg)	157.9 ± 132.8	401.4 ± 281.4	**0.006**
Pantothenic acid (mg)	5.4 ± 2.6	4.9 ± 2.0	0.852
Calcium (mg)	836 ± 255	95 ± 12.3	**0.021**
Copper (μg)	1.6 ± 0.7	2.1 ± 0.8	**0.001**
Iron (mg)	25.3 ± 4.1	13.1 ± 2.1	**0.031**
Magnesium (mg)	742.4 ± 205.1	533.2 ± 126.3	0.070
Phosphorus (mg)	1055 ± 531.4	1021 ± 446.4	0.452
Potassium (mg)	2951 ± 843.2	4022 ± 1078	**0.034**
Sodium (mg)	3932 ± 1021	3178 ± 919.5	0.533
Zinc (mg)	12.1 ± 3.5	3.2 ± 1.5	**0.001**

Note: The bold values are significantly different.
Data are expressed as the mean ± SD. Folates includes folic acid intake from fortified products (wheat flour); RAE, retinol activity equivalent; 1 RAE = 1 mg retinol. p-value for media comparison between groups with Student's t test for unpaired data ($p < 0.05$).

Table 4 Daily intake of selected fatty acids calculated from dietary survey

FA intake	Control ($n = 33$)	Vegan ($n = 34$)	p value
ΣSFA (g)	31.8 ± 8.2	24.3 ± 4.6	0.064
4:0 (g)	1.65 ± 0.6	0.001 ± 0.004	**0.0001**
6:0 (g)	0.85 ± 0.2	0.001 ± 0.002	**0.0001**
8:0 (g)	0.97 ± 0.2	0.002 ± 0.01	**0.0001**
10:0 (g)	0.76 ± 0.3	0.034 ± 0.1	**0.0001**
12:0 (g)	1.68 ± 0.4	0.52 ± 0.01	**0.032**
14:0 (g)	2.48 ± 1.7	1.13 ± 0.5	**0.044**
16:0 (g)	16.3 ± 4.2	19.43 ± 2.5	0.241
18:0 (g)	5.12 ± 2.1	1.80 ± 0.5	**0.021**
ΣMUFA (g)	35.3 ± 10.2	42.3 ± 16.7	0.384
16:1n-7 (g)	3.73 ± 0.78	4.14 ± 0.58	0.682
18:1n-9 (g)	29.6 ± 11.4	36.3 ± 14.5	0.683
ΣPUFA (g)	36.9 ± 10.7	43.2 ± 12.8	0.079
18:2n-6, LA (g)	30.6 ± 7.2	34.7 ± 6.7	0.054
18:3n-3, ALA (g)	2.01 ± 0.8	7.62 ± 2.5	**0.013**
20:4n-6, AA (mg)	343 ± 76	No intake	—
20:5n-3, EPA (mg)	No intake	No intake	—
22:6n-3, DHA (mg)	No intake	No intake	—
Σn-6 PUFA (g)	32.9 ± 8.2	35.0 ± 6.7	0.057
Σn-3 PUFA (g)	3.97 ± 1.0	8.15 ± 1.5	**0.018**
ΣTrans fatty acid (g)	1.6 ± 1.0	0.5 ± 0.2	**0.031**

Note: The bold values are significantly different.
Values are shown as the mean ± SD; the groups were compared with Student's t test for unpaired data ($p < 0.05$). Saturated fatty acids (SFA) correspond to 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0 and 22:0, and 24:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, 16:1, and 18:1. Polyunsaturated fatty acids (PUFA) correspond to 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3.

and eluted with either diethyl ether or chloroform/methanol (2:1 v/v) (Ruiz-Gutierrez et al., 1992).

Fatty Acid Methyl Ester and Gas Chromatography Analysis

Fatty acid methyl esters (FAME) were obtained from *erythrocyte* phospholipids, plasma, and spermatozoa upon treatment with methanolic boron trifluoride (12% methanolic solution) and sodium hydroxide (0.5 N methanolic solution) (Morrison and Smith, 1964). FAME samples were cooled and extracted with 0.5 mL of hexane. After, FAME were separated and quantified by gas chromatography in Hewlett-Packard equipment (model 7890A, CA, USA) using a capillary column (Agilent HP-88, 100 m × 0.250 mm; I.D. 0.25 μm), and a flame ionization detector (FID). Details on the gas chromatography FA analysis of FA were previously described by Bascuñán et al. (2014). The amount of each FA was given as mole percentage of the total FA content.

Statistical Analyses

We estimated that a sample size of 30 participants per group gave a power of 96% to achieve a significant difference between vegan and semi-omnivores adults, with an estimated effect size of 0.98 and an α-error of 5% (two-tailed t test), based on the difference in long-chain n-3 PUFA in erythrocytes phosphatidylserine [mean difference (±SD) 1.69 ± 1.72 mol% of total FA], as reported by Kornsteiner et al. (2008). Data are presented as means ± SD. A descriptive analysis was conducted and variables' distribution was assessed using the Shapiro–Wilk test. Between-group differences regarding diet, erythrocytes, plasma, and spermatozoa FA composition were assessed using the Student's t test for independent samples. The statistical software used was SPSS v.24.0 (Chicago, IL, USA). The level of significance was set at an α level 5% ($p < 0.05$).

Results

Anthropometric Characteristics

Table 1 shows the background characteristics of the subjects. No statistically significant differences were detected regarding age and body composition (weight, height and BMI) between the two groups. In both groups, all of the subjects had a normal BMI.

Table 5 Plasma fatty acid composition

FA (mol% of FA)	Control ($n = 33$)	Vegan ($n = 34$)	p value
ΣSFA	29.9 ± 2.3	27.9 ± 2.3	0.742
12:0	0.09 ± 0.02	0.11 ± 0.04	0.864
14:0	0.95 ± 0.3	0.87 ± 0.5	0.754
16:0	21.1 ± 1.9	20.1 ± 1.7	0.636
18:0	7.08 ± 0.8	6.32 ± 1.2	0.071
20:0	0.06 ± 0.05	0.07 ± 0.1	0.858
22:0	0.19 ± 0.07	0.20 ± 0.1	0.861
24:0	0.17 ± 0.08	0.20 ± 0.1	0.346
ΣMUFA	25.0 ± 4.3	28.1 ± 5.3	0.077
14:1	0.01 ± 0.01	0.01 ± 0.01	0.983
16:1n-7	1.73 ± 0.7	1.64 ± 0.8	0.642
18:1n-9	22.7 ± 3.4	25.9 ± 4.9	0.313
20:1	0.25 ± 0.1	0.29 ± 0.2	0.072
ΣPUFA	45.1 ± 4.9	43.9 ± 5.7	0.574
18:2n-6, LA	33.7 ± 4.5	34.5 ± 5.1	0.393
18:3n-6	0.36 ± 0.1	0.40 ± 0.2	0.372
18:3n-3, ALA	0.69 ± 0.1	0.95 ± 0.29	**0.039**
20:2n-6	0.18 ± 0.07	0.23 ± 0.05	0.078
20:3n-6	1.29 ± 0.3	1.38 ± 0.42	0.252
20:4n-6, AA	6.27 ± 1.6	4.82 ± 1.52	**0.003**
20:5n-3, EPA	0.50 ± 0.2	0.28 ± 0.13	**0.001**
22:4n-6	0.13 ± 0.07	0.12 ± 0.07	0.409
22:5n-3	0.35 ± 0.09	0.27 ± 0.10	**0.015**
22:6n-3, DHA	1.56 ± 0.40	0.64 ± 0.26	**0.0001**
Σn-6 PUFA	41.9 ± 4.2	41.5 ± 4.5	0.387
Σn-3 PUFA	3.11 ± 0.5	2.15 ± 0.3	**0.041**

Note: The bold values are significantly different.
Data are expressed as g fatty acid per 100 g of Fatty acid methyl esters (FAME) and represent the mean ± SD; the groups were compared with Student's t test for unpaired data ($p < 0.05$). The identification of saturated and unsaturated fatty acids and their relationships are shown in Table 4.

Table 6 Erythrocyte phospholipids fatty acid composition

FA (g/mol% of FA)	Control ($n = 33$)	Vegan ($n = 34$)	p value
ΣSFA	42.1 ± 4.6	39.5 ± 1.8	0.653
14:0	0.73 ± 0.2	0.71 ± 0.2	0.764
16:0	25.9 ± 3.0	25.4 ± 1.1	0.816
18:0	13.2 ± 1.2	11.0 ± 1.2	0.091
20:0	0.14 ± 0.1	0.17 ± 0.1	0.087
22:0	0.50 ± 0.2	0.53 ± 0.1	0.510
24:0	1.71 ± 0.7	1.68 ± 0.4	0.792
ΣMUFA	20.8 ± 2.9	24.1 ± 3.4	0.422
14:1	0.03 ± 0.1	0.03 ± 0.06	0.392
16:1n-7	0.60 ± 0.3	0.74 ± 0.4	0.561
18:1n-9	18.4 ± 2.4	20.7 ± 3.0	0.069
20:1	0.90 ± 0.2	1.2 ± 0.41	0.078
ΣPUFA	37.1 ± 6.7	36.4 ± 2.7	0.089
18:2n-6, LA	14.8 ± 2.2	16.5 ± 1.9	0.065
18:3n-6	0.03 ± 0.04	0.04 ± 0.06	0.052
18:3n-3, ALA	0.12 ± 0.01	0.37 ± 0.1	**0.006**
20:2n-6	0.29 ± 0.05	0.33 ± 0.1	0.081
20:3n-6	1.50 ± 0.4	1.70 ± 0.4	0.066
20:4n-6, AA	11.9 ± 2.8	10.6 ± 1.8	0.092
20:5n-3, EPA	0.32 ± 0.2	0.28 ± 0.3	0.059
22:4n-6	2.50 ± 0.7	2.71 ± 0.7	0.286
22:5n-3	1.40 ± 0.5	1.38 ± 0.3	0.207
22:6n-3, DHA	3.44 ± 1.2	1.41 ± 0.6	**0.0001**
Σn-6 PUFA	30.1 ± 3.4	31.5 ± 3.0	0.356
Σn-3 PUFA	5.20 ± 0.6	3.70 ± 0.2	**0.038**

Note: The bold values are significantly different.
Data are expressed as g fatty acid per 100 g of Fatty acid methyl esters (FAME) and represent the mean ± SD; the groups were compared with Student's t test for unpaired data ($p < 0.05$). The identification of saturated and unsaturated fatty acids and their relationships are shown in Table 4.

Daily Intake According to Food Groups

Table 2 shows the food group intake in both groups. No significant differences were observed in the intake of cereals, high-fat foods, oils and fats, and sugar and processed foods were observed between the two group. The vegan group reported a significantly higher intake of fruits and vegetables, and legumes as compared to the omnivore controls. The vegan group did not consume dairy, and meats and eggs, and both groups did not report intake of fish or seafood.

Energy and Nutrient Intake

The reported energy and nutrient intakes are shown in Table 3. No significant differences were observed in the intake of energy, protein, fat, thiamin, riboflavin, niacin, biotin, pantothenic acid, magnesium, and sodium between the two groups. The vegan group reported a significantly lower intake of cholesterol, *trans* FA, vitamins B_6, D, B_{12}, and the following minerals: calcium, iron, phosphorus, and zinc, compared to the control group. However, the vegan group had a higher intake of carbohydrates, dietary fiber (both soluble and insoluble), vitamins A, C, E, folate and K, copper, and potassium.

Dietary FA Intake

Table 4 summarizes the reported daily intake of the most relevant FA. No significant differences were observed in total saturated fatty acids (SFA), monounsaturated (MUFA), or PUFA intake between the groups. However, several differences appeared when examining specific SFA and PUFA. The vegan group reported a significantly lower intake of the following SFA: 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, and 18:0. Regarding PUFA, the vegan group reported a significantly higher intake of ALA and total n-3 PUFA, AA intake was not detected. In both groups, the intake of EPA

Table 7 Spermatozoa fatty acid profile

FA (g/mol% of FA)	Control ($n = 33$)	Vegan ($n = 34$)	p value
ΣSFA	44.3 ± 7.9	42.5 ± 8.4	0.655
12:0	0.10 ± 0.2	0.13 ± 0.1	0.374
14:0	2.10 ± 0.5	2.02 ± 0.7	0.485
16:0	26.5 ± 3.0	25.2 ± 2.8	0.181
18:0	11.3 ± 1.5	10.9 ± 1.3	0.548
20:0	0.91 ± 0.6	0.90 ± 0.9	0.915
22:0	1.00 ± 0.7	0.98 ± 0.7	0.923
24:0	0.95 ± 0.6	0.99 ± 0.6	0.801
ΣMUFA	37.0 ± 4.6	40.8 ± 4.1	0.167
14:1	0.04 ± 0.01	0.05 ± 0.02	0.740
16:1n-7	2.92 ± 1.3	3.14 ± 1.2	0.092
18:1n-9	31.2 ± 8.2	34.1 ± 8.4	0.295
20:1	1.38 ± 0.38	1.31 ± 0.3	0.467
22:1	0.47 ± 0.25	0.52 ± 0.2	0.855
24:1	0.41 ± 0.42	0.56 ± 0.3	0.130
ΣPUFA	18.6 ± 4.4	16.5 ± 5.3	0.200
18:2n-6, LA	8.14 ± 2.6	9.04 ± 4.1	0.865
18:3n-3, ALA	0.25 ± 0.1	0.92 ± 0.2	**0.034**
20:2n-6	0.12 ± 0.2	0.25 ± 0.2	**0.021**
20:3n-6	1.82 ± 0.9	1.47 ± 1.0	0.237
20:4n-6, AA	2.03 ± 1.0	1.62 ± 1.1	0.197
20:5n-3, EPA	0.13 ± 0.2	0.15 ± 0.3	0.743
22:4n-6	0.04 ± 0.01	0.04 ± 0.1	0.311
22:5n-3	0.05 ± 0.02	0.10 ± 0.05	0.074
22:6n-3, DHA	4.63 ± 0.7	1.43 ± 0.3	**0.004**
Σn-6 PUFA	13.1 ± 2.7	13.5 ± 2.1	0.485
Σn-3 PUFA	5.25 ± 0.8	2.61 ± 0.5	**0.035**

Note: The bold values are significantly different.
Data are expressed as g fatty acid per 100 g Fatty acid methyl esters (FAME) and represent the mean ± SD; the groups were compared with Student's t test for unpaired data ($p < 0.05$). The identification of saturated and unsaturated fatty acids and their relationships are shown in Table 4.

and DHA was not detected. The vegan group had a low intake of trans FA compared with control group.

Plasma FA Composition

No significant differences were observed for plasma SFA, MUFA, PUFA, and n-6 PUFA levels between the two groups (Table 5). However, the vegan group had significantly lower levels of AA, EPA, 22:5n-3 (n-3 DPA), DHA, and n-3 PUFA compared to controls. The vegan group had significantly higher levels of ALA (Table 5).

Erythrocyte Phospholipid FA Composition

Table 6 summarizes the FA composition of erythrocyte phospholipids. No significant between-group differences were observed for erythrocyte phospholipid SFA, MUFA, PUFA, and n-6 PUFA. N-3 PUFA and DHA levels were lower in the vegan group compared to controls. However, the ALA level was significantly higher in vegans compared to controls.

Spermatozoa FA Composition

Table 7 shows the FA composition of spermatozoa in both groups. No significant differences were observed for SFA, MUFA, PUFA, and n-6 PUFA levels between the two groups. The vegan group had significantly higher levels of ALA, 20:2n-6 compared to the control group. In vegans, however, DHA and n-3 PUFA levels were significantly lower compared to the control group (Table 7).

Discussion

This study finds that healthy young vegan men from Chile report higher intakes of ALA and total n-3 PUFA. In addition, the vegan group had lower DHA levels in plasma, erythrocyte phospholipids, and spermatozoa. As expected, we also found differences in the reported intake of several nutrients (carbohydrates, vitamins, minerals, and FA) compared to the omnivore controls. We found similar dietary intakes of total SFA, MUFA, and PUFA but lower intakes of specific SFA and PUFA in the vegan group. Overall our findings are similar to several previous reports (Kristensen et al., 2015; Rosell et al., 2005; Sanders, 2009). Furthermore, the vegan group reported relatively lower intakes of the majority of the SFA (4:0 to 14:0 and 18: 0), AA intake was not detected (Table 4).

A vegan pattern of increased ALA intake accompanied by a lower EPA and DHA intake has been reported by others (Kornsteiner et al., 2008; Rosell et al., 2005; Sanders, 2009; Welch et al., 2010). In our study, we did not detect intake of EPA and DHA in vegans and omnivore men that do not eat fish and shellfish (Table 4). This apparent discrepancy is most likely due to the fact that we recruited omnivores that refrained from eating fish over the past year. It is also noteworthy that the omnivore group also reported a relatively low intake of EPA and DHA as compared to other reports (Sanders, 2009; Welch et al., 2010). Nevertheless, FA levels in plasma, erythrocytes, and spermatozoa indicated that vegan men had higher levels of ALA and lower levels of other n-3 PUFA, especially EPA and DHA (Tables 5–7). This differential pattern could be related to (1) the low rate of conversion of ALA to DHA in humans (less than 1%) (Brenna et al., 2009), or (2) the preferential incorporation of preformed EPA and DHA into these tissues. Regarding the conversion of ALA into n-3 PUFA studies have shown that a higher intake of ALA increases the levels of EPA in erythrocytes in humans (Barceló-Coblijn et al., 2008;

Goyens et al., 2006; Greupner et al., 2018). In our study, vegans consumed 190% more ALA than omnivores and no significant differences were observed in the intake of LA between groups.

Furthermore, other nutrients can regulate the activity of the Δ-6 desaturase including magnesium, zinc, and calcium (Brenner, 1981). For example, zinc deficiency in rats decreases the activity of the Δ-5 and Δ-6 desaturase enzymes, reducing tissue levels of PUFA, particularly EPA and DHA (Ayala and Brenner, 1983; Eder and Kirchgessner, 1995). Obesity and nonalcoholic fatty liver disease can also decrease the synthesis of longer chain PUFA (Araya et al., 2010), as well as the activity of the Δ-5 and Δ-6 desaturases and tissue (adipose tissue) and circulating (erythrocytes) levels of PUFA (Elizondo et al., 2007). In our study, all the subjects had a normal BMI, and all participants did not have any chronic noncommunicable diseases such as type-2 diabetes or nonalcoholic fatty liver disease. Thus, it is unlikely these factors influenced our results. Future research is required to determine the mechanism by which DHA is reduced in the spermatozoa of vegan men compared to non-fish eating omnivores.

Regarding the level of EPA plus DHA in erythrocytes (as calculated by the omega-3 index), both groups showed relatively low values: 3.8% for the control group and 1.7% for vegan group. This is of interest as several studies have reported that an omega-3 index less than 4% is associated with an increased risk for developing cardiovascular disease (Harris et al., 2017; Tribulova et al., 2017). However, the vegan group had a significantly lower intake of trans FA, which are associated with an increased risk of cardiovascular disease (Wanders et al., 2017), suggesting that this group consumes fewer processed foods that contain trans FA (Martin et al., 2007; Zhu et al., 2019).

One interesting aspect of our study was the evaluation of FA composition in spermatozoa. In humans, DHA is present in high levels in sperm (Arterburn et al., 2006). The vegan group showed a significant reduction (236%) in the DHA level when compared to the omnivore controls. FA, and especially PUFA, regulates the activity of the spermatozoa cell membrane, cellular signaling pathways, and gene expression (Esmaeili et al., 2015). It has been reported that levels of n-3 PUFA, especially DHA, in spermatozoids are relatively lower in infertile compared to fertile men (Martínez-Soto et al., 2016; Safarinejad et al., 2010). Furthermore, clinical studies have shown that DHA supplementation (from fish oil) protects against damage induced by oxidative stress in the sperm (Safarinejad, 2011). Also, the DHA content in the spermatozoa in both groups is low, being possible to establish that a very low intake of DHA would be affecting the content of DHA in the spermatozoa (Craig et al., 2019), especially in vegans (Sanders, 2009). Furthermore, low levels of DHA in the sperm would correlate with quantity and quality of these cells (Craig et al., 2019). Additionally, a recent meta-analysis demonstrated that supplementing infertile men with n-3 PUFA (specifically DHA) resulted in a significant improvement in sperm motility and concentration of DHA in seminal plasma (Hosseini et al., 2019). In the spermatozoa, there are FA unsaturated with 24 or more carbon atoms (Coniglio, 1994). However, we detected a very low value for this FA (data not shown); therefore, it is necessary to further evaluate the impact of diet and FA in the spermatozoa. Future studies are needed to assess the biological relevance, if any, of lower DHA levels in the sperm of vegans. Importantly, these studies could be carried out with DHA obtained from microalgae oil (DHA from vegetal origin), which can be consumed by vegans without producing an ethical conflict in vegan subjects.

Our study also has several important limitations. We worked with a sample of healthy young men; therefore, it is not clear of our results can be extrapolated to women, other age groups, subjects with obesity or other diseases. Dietary intake was assessed using dietary surveys (FFQ), a method that has limitations considering it relies on memory recall (Archer et al., 2018); however, we used a previously validated instrument focusing on food sources of fat and FA intake (Barrera et al., 2018; Bascuñán et al., 2014; Valenzuela et al., 2015), and also used a second instrument (24-h recall) to corroborate the regular dietary structure the previous days' dietary pattern. Also, the fact that dietary surveys rely on food composition databases is another limitation in our study (Primorac et al., 2000). Several factors have been reported to potentially affect food composition data, including food sampling processes as well as analytical, environmental, and biological sources of variability. All those factors will ultimately influence food component levels in food databases leading to misclassification. Regarding dietary surveys, the choice of food items and specific varieties used will affect estimated nutrient intake (Pennington, 2008; Primorac et al., 2000). Here, we used the USDA National Nutrient Database for Standard Reference but also included information on local food composition and from the manufacturer for specific national products. A strength of our study is that the FFQ was complimented by tissue FA measures. Finally, in this study we examined omnivore men who did not consume foods of marine origin (fish, shellfish, *etc*.) as a control group. Thus, it would be of interest to carry out another, including a group of omnivore men who consume foods of marine origin.

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Author Contributions R.V., R.C, K.A.B. and C.B. designed the study and analyzed and interpreted the data. M.F.G., R.A., V.G., and

C.B. performed clinical and nutritional evaluations. C.B. and R.V. conducted the analysis. R.V., R.C. and R.P.B. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest The authors declare that they have no conflict of interest.

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