5-2017

Improvement of cardiometabolic markers after fish oil intervention in young Mexican adults and the role of PPARα L162V and PPARγ2 P12A

Aristea Binia

Carolina Vargas-Martínez

Mónica Ancira-Moreno

Laura M. Gosoniu

Ivan Montoliu

See next page for additional authors

Recommended Citation

Binia, A., Vargas-Martínez, C., Ancira-Moreno, M., Gosoniu, L. M., Montoliu, I., Gámez-Valdez, E., Soria-Contreras, D. C., Angeles-Quezada, A., Gonzalez-Alberto, R., Fernández, S., Martínez-Conde, D., Hernández-Morán, B., Ramírez-Solano, M., Pérez-Ortega, C., Rodríguez-Carmona, Y., Castan, I., Rubio-Aliaga, I., Vadillo-Ortega, F., Márquez-Velasco, R., Bojalil, R., … Tejero, M. E. (2017). Improvement of cardiometabolic markers after fish oil intervention in young Mexican adults and the role of PPARα L162V and PPARγ2 P12A. The Journal of nutritional biochemistry, 43, 98–106. https://doi.org/10.1016/j.jnutbio.2017.02.002

Authors

Aristea Binia, Carolina Vargas-Martínez, Mónica Ancira-Moreno, Laura M. Gosoniu, Ivan Montoliu, Elí Gámez-Valdez, Diana C. Soria-Contreras, Adriana Angeles-Quezada, Rocío Gonzalez-Alberto, and Juan Carlos Lopez Alvarenga

Improvement of cardiometabolic markers after fish oil intervention in young Mexican adults and the role of *PPARα* L162V and *PPARγ2* P12A[☆,☆☆]

Aristea Binia[a], Carolina Vargas-Martínez[b], Mónica Ancira-Moreno[c], Laura M. Gosoniu[a], Ivan Montoliu[d],
Elí Gámez-Valdez[b], Diana C. Soria-Contreras[c], Adriana Angeles-Quezada[b], Rocío Gonzalez-Alberto[e],
Silvia Fernández[b], Diego Martínez-Conde[e], Brianda Hernández-Morán[b], Marisol Ramírez-Solano[b],
Carlos Pérez-Ortega[b], Yanelli Rodríguez-Carmona[b], Isabelle Castan[f], Isabel Rubio-Aliaga[a, 1],
Felipe Vadillo-Ortega[c], Ricardo Márquez-Velasco[g], Rafael Bojalil[g], Juan Carlos López-Alvarenga[h],
Philippe Valet[f], Martin Kussmann[d, i], Irma Silva-Zolezzi[a], M Elizabeth Tejero[b,*]

[a]*Nestlé Research Center, Switzerland*
[b]*Instituto Nacional de Medicina Genómica (INMEGEN), Mexico City, Mexico*
[c]*Unidad de Vinculación de la Facultad de Medicina, Universidad Nacional Autónoma de Mexico en el INMEGEN, Mexico City, Mexico*
[d]*Nestlé Institute of Health Sciences, Lausanne, Switzerland*
[e]*Departamento de Salud, Universidad Iberoamericana, Mexico City, Mexico*
[f]*Institut des Maladies Metaboliques et Cardiovasculaires, Institut national de la santé et de la recherche médicale (INSERM), Toulouse, France*
[g]*Departamento de Inmunología, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico*
[h]*Hospital General de México Eduardo Liceaga, Mexico City, Mexico*
[i]*Systems Biology in Nutrition and Health, The Liggins Institute, The University of Auckland, New Zealand*

Abstract

Polyunsaturated fatty acids (PUFA) contained in fish oil (FO) are ligands for peroxisome proliferator-activated receptors (PPAR) that may induce changes in cardiometabolic markers. Variation in PPAR genes may influence the beneficial responses linked to FO supplementation in young adults. The study aimed to analyze the effect of FO supplementation on glucose metabolism, circulating lipids and inflammation according to *PPARα* L162V and *PPARγ2* P12A genotypes in young Mexican adults. 191 young, non-smoking subjects between 18 and 40 years were included in a one-arm study. Participants were supplemented with 2.7 g/day of EPA + DHA, during six weeks. Dietary analysis, body composition measurements and indicators for glucose metabolism, circulating lipids, and markers for inflammation were analyzed before and after intervention. An overall decrease in triglycerides (TG) and an increase in HS-ω3 index were observed in all subjects [−4.1 mg/dL, (SD:±51.7), P=.02 and 2.6%, (SD:±1.2), P<.001 respectively]. Mean fasting insulin and glycated hemoglobin (HbA1c%) were significantly decreased in all subjects [−0.547mIU/L, (SD:±10.29), P=.034 and −0.07%, (SD:±0.3), P<.001 respectively], whereas there was no change in body composition, fasting glucose, adiponectin and inflammatory markers. Subjects carrying the minor alleles of *PPARα* L162V and *PPARγ2* P12A had higher responses in reduction of TG and fasting insulin respectively. Interestingly, doses below 2.7 g/day (1.8 g/day) were sufficient to induce a significant reduction in fasting insulin and HbA1c% from baseline (P=.019 and P<.001). The observed responses in triglycerides and fasting insulin in the Mexican population give further evidence of the importance of FO supplementation in young people as an early step towards the prevention of cardiometabolic disease.
© 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: PPARα L162V; *PPARγ2* P12A; Fish Oil; PUFA; Epistasis

1. Introduction

Ω3 fatty acids (O3FA) are essential nutrients taking part in multiple metabolic processes, which explains their pleiotropic effects [1,2]. In the context of human health, multiple studies have shown effects of these compounds on human lipid metabolism, insulin sensitivity and inflammatory response, among others [3–5]. The decrease of plasma triglycerides (TG) concentrations in subjects with and without hypertriglyceridemia after the intake of O3FA is a consistent finding across different studies [6–9]. The reported effects of O3FA on other circulating lipoproteins, such as HDL and LDL, is less clear. Similarly, the effects of the intake of these FA on insulin sensitivity and inflammation are controversial [10,-12], and comparison across studies is difficult due to the variety of study designs and characteristics of participants. Recent systematic reviews and meta-analyses that assessed the effect of O3FA intake on cardiovascular disease prevention [11–12] and their anti-inflammatory effects have revealed controversial findings [13–15]. Numerous factors may contribute to the observed variability of the response to the intake of O3FA, such as gender, body mass index (BMI), age, diet, metabolic condition, and genetic factors [9,16–18]. As well, the role of genetic variation in the response to fish oil (FO) supplementation has been investigated across different studies (8, 9).

The peroxisome proliferator activated receptors (PPAR) are a family of transcription factors involved in the regulation of energy metabolism [19,20]. These transcription factors are activated by long chain fatty acids, including O3FA [21,22]. Genetic variation in the coding region with functional effects has been reported for both genes. A polymorphism (rs1800206) in the *PPARα* gene results in the substitution of leucine to valine at codon 162. This polymorphism is located in the DNA binding domain site, rendering a protein differential ligand-mediated activation. Some investigations have evaluated the effect of the interaction between this genotype and the consumption of O3FA supplementation on phenotypes such as lipid metabolism and gene expression [23–26]. In a study conducted by Rudkowska et al. macrophages from carriers of the Val162 variant showed lower expression of *PPARα*, *ApoA1* and *LPL* than the Leu162 variant after exposure to O3FA [26]. Other studies have found that the effect of this variant depends on the availability of O3FA (9). Variation in the *PPARγ2 gene*, particularly the Pro12Ala (rs1801282) has been associated with glucose metabolism and type 2 diabetes (T2D) in numerous studies [27,28]. The combined effect of these two genotypes has been investigated in subjects with metabolic syndrome [29] and in the response to weight change in obese women [30].

Although the potential role of O3FA in the primary prevention of chronic diseases has been extensively investigated [31–46], the effects are controversial, and a limited number of intervention studies have been conducted in generally healthy young adults with the purpose of assessing an interaction between genetic variation, and supplementation with these fatty acids on lipid and glucose metabolism, and inflammation [45,46]. Gene expression in peripheral blood mononuclear cells (PBMC) has been used to shed light on these on these effects of dietary fatty acids on lipid and glucose metabolism, as well as inflammation, in human studies [47–48].

The Mexican population has one of the highest rates in the world of overweight and obesity (71% of adults over 20 years of age) and, as a result, a very high rate of metabolic syndrome-related phenotypes [49,50]. In addition, a very low consumption of O3FA has been documented in the most recent national nutrition survey in Mexico, supporting it as a highly relevant and possibly receptive population to test the potential benefits of O3FA [51]. Thus, we hypothesized that supplementation with O3FA in FO will improve phenotypes related to lipid and glucose metabolism, markers of inflammation in a generally healthy, young adult population. These effects would be influenced by *PPARα* and *PPARγ2* functional variants that have been shown to have an effect on the response to O3FA intake [19,20,22,27,29,30,46]. To the best of our knowledge, no previous study has assessed the combined effects of these two genotypes on the response to supplementation with FO on the mentioned phenotypes, in generally healthy adults. The aim of the study was to investigate the effect of two functional genetic variants in *PPARα* and *PPARγ2* on the response to O3FA supplementation on parameters of circulating lipids, glucose metabolism and selected proteins related to inflammatory response in young Mexican adults.

2. Materials and methods

2.1. Participants and study design

Eligible participants were between 18 and 40 years of age, with BMI between 18.5 and<30, without any medication, vitamin or lipid supplements before or during the study. They had sedentary to moderate physical activity, according to the IPAQ questionnaire [52]. Exclusion criteria were as follows: active smoking, any concomitant consumption of dietary supplements and medications that could affect the study outcomes, excessive alcohol consumption, illness two weeks prior to the study start, or any active systemic infection, or medical condition that would require treatment during the study, medical condition related to coagulation and participation to another clinical trial during the last 4 weeks prior to the beginning of the study.

The present was a 6-week, one-arm study, conducted at two centers (Universidad Iberoamericana and Universidad Nacional Autonoma de Mexico, UNAM) in Mexico City. Recruitment and follow up were conducted between November 2013 and May 2014 and ended at completion of the intended sample size. The intervention included the oral supplementation with 3 capsules of FO (GNC Preventive Nutrition® Triple Strength Fish Oil) per day, each containing 647 mg of eicosapentaenoic acid (EPA) and 253 mg of docosahexaenoic (DHA) (daily intake: 2.7 g/day of DHA and EPA in fish oil). The subjects were asked to consume the capsules with food, *as* it has been shown in previous studies to have the maximum absorption [53].

The primary outcome of the study was changes in triglycerides levels in plasma between baseline and 6 weeks intervention and the secondary outcomes included changes in lipid metabolism, glucose metabolism and inflammatory response markers in plasma between baseline and 6 weeks intervention depending on *PPARα* and *PPARγ2* genotypes.

The study consisted of three visits. Visit 1 (V1, baseline): subjects' body weight and body composition were measured in a bioelectric impedance analyzer In Body 720, height was measured with a wall stadiometer, and waist circumference was measured at midway between the uppermost border of the iliac crest and the lower border of the costal margin. Clinically trained personnel performed all measurements. A dietary analysis was conducted using a validated food frequency questionnaire (SNUT) (long form) [54]. A 20 mL blood sample was drawn under fasting conditions (12 h) by venipuncture for separation of serum, plasma, red blood cells (RBC) and peripheral mononuclear cells using a Vacutainer system (Becton-Dickinson, NJ, USA). Participants received enough capsules supplement for 3 weeks and were asked to avoid changes in their eating and physical activity patterns during the study. Visit 2 (V2, at 3 weeks): the subjects filled and returned the side effects journal and the unused capsules, received the results of the clinical and biochemical parameters analyzed in V1 and discussed them with a nutritionist. They completed a 24-h food recall questionnaire and were provided with FO supplements for the last three weeks. Visit 3 (V3, 6 weeks): subjects were assessed for the same parameters as in baseline, except for the clinical history and SNUT questionnaire. The 24-h recall, physical activity and consumption of medication and/or supplementation questionnaires were collected in all 3 visits. All measures and surveys were conducted by standardized personnel. A series of biochemical and molecular parameters were determined in blood samples collected in V1 and V3.

The sample size was calculated to 200 participants according to the reported frequencies of the studied alleles in Mexican population and taking under consideration the genotype effects in blood lipids following a FO supplementation in previous studies [45,46]. This sample size would be sufficient to identify a small size effect (Cohen's $d=$ 0.25–0.3) of the treatment in each genotype group for the primary outcome, which was the reduction in fasting triglyceride levels from baseline. Considering a dropout rate of 20%, the recruitment aimed for 240 participants.

Compliance to treatment was assessed according to the number of returned capsules and treatment days, and the concentration of twenty-seven fatty acids in RBC membranes analyzed according to the HS-ω-3 index™ methodology *via* gas chromatography at Omegametrix GmGH Laboratory (Germany) [55]. HS-Omega-3 index results are given as EPA + DHA expressed as a percentage of total identified FA after response factor correction (based on correlation curves). The difference in concentration of EPA, DHA and the HS-ω3 index between baseline and after treatment were used as indicators of compliance.

2.2. Biochemical and molecular parameters

Glucose, glycosylated hemoglobin (HbA1c), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides and total cholesterol (TC) were measured in

fasting serum using standard methods. Concentrations of insulin, adiponectin, apelin, interleukin 6 (IL-6) and C reactive protein (CRP) were measured in plasma by DuoSet ELISA methods as described by the manufacturer (R&D Systems, Minneapolis, USA). Glucose and insulin were used to estimate HOMA-IR [glucose (mg) x insulin (mU) /405]. Mononuclear cells were isolated from an EDTA whole blood sample using Ficoll Paque PLUS (GE Healthcare), followed by two washes with PBS and stored at−80 °C for RNA isolation. Buffy coat was separated from an EDTA tube and frozen at−80 °C for isolation of genomic DNA using the Quick g DNA miniprep kit (Zymo Research). The quality control and concentration of the DNA samples were analyzed in a Nanodrop (Thermo Fisher Scientific) and using an ethidium bromide stained gel. Genotypes were determined by allelic discrimination using TaqMan® probes in a Via7 DNA analyzer (Applied Biosystems). The probes for the genotypes were C_1129864_10 (rs1801282) and C_881767_20 (rs1800206). PPARα L162 L were defined as the carriers of the homozygous genotype for the major allele (Leu162Leu) and PPARα X162V as the carriers of the minor allele, both heterozygotes (Leu162Val) and homozygous (Val162Val). Similarly, PPARγ2 P12P were defined as the carriers of the homozygous genotype for the major allele (Pro12Pro) and PPARγ2 X12A as the carriers of the minor allele, both heterozygotes (Pro12Ala) and homozygous (Ala12Ala).

RNA was isolated from mononuclear cells using Trizol reagent (Thermo Fisher Scientific) according to manufacturer's directions. Concentration, purity and integrity of isolated RNA were analyzed in a Nanodrop (Thermo Fisher Scientific) and agarose gel electrophoresis stained with ethidium bromide. A subsample was evaluated in an Agilent 250 bioanalyzer (Agilent Palo Alto, CA, USA). All samples fulfilled the criteria for purity and integrity with a RIN value>8. One μg of RNA was used for the reverse transcription reaction using the First strand cDNA Synthesis kit (Thermo Fisher Scientific). Reactions were conducted with one μL of cDNA reaction in PCR Universal Master Mix, using the TaqMan® PCR program. The analyzed genes were AdipoR1, AdipoR2 and interleukin 6 (IL-6) (catalog numbers: Hs00226105_m1, Hs01114951_m1 and Hs01075666_m1, respectively) using TaqMan® assays (Thermo Fisher Scientific) in a DNA analyzer VIA7. Three internal controls (GAPDH, 18S ribosomal subfraction and beta-actin) were used for normalization. Expression differences in PBMCs between V1 and V3 were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.3. Ethics

2.4. Statistical analysis

Three (3) analysis populations were defined for the study: (i) Full analysis dataset (FAS) which included all enrolled subjects, except those that did not meet any major inclusion criteria, did not take any dose of the supplement, or did not have baseline data for primary endpoint (genotype or TG assessment), (ii) Per protocol (PP) dataset which included all subjects from the FAS, except those with any major deviation (Visit 3, V3, performed more than 8 days later than 6 weeks, V3 performed more than 8 days earlier than 6 weeks, low supplement compliance defined as number of returned capsules greater than 20% (26 capsules) and consumption of any dietary supplements or any other medication that would affect the study outcomes) and (iii) Safety dataset which included all treated subjects was used for adverse events and concomitant medications analysis. The statistical analysis was performed in both FAS and PP datasets using Stata software. Results from the FAS dataset with the same direction as the results in PP dataset are presented here. The outcomes were assumed to be normally distributed and the studentized residuals of the model correcting for baseline value and the two independent genotypes PPARα (PPARα L162 L or PPARα X162V) and PPARγ2 (PPARγ2 P12P or PPARγ2 X12A) were assessed for normality (residual included in [−2;2] interval and symmetric histogram) and for homogeneity of variance (if Spearman correlation coefficient p-value>0.05, residuals independent from predicted value). If one of the two conditions was not fulfilled, a log-transformation was applied and the studentized residuals of the model log (Visit 3/Visit 1, V3/V1) correcting for log (baseline) value and the two independent genotypes PPARα and PPARγ2 were analyzed the same way. If normality or homogeneity of variance was not found, a non-parametric approach was used (Wilcoxon rank sum test). The change in TG levels between the final visit and the baseline, the primary outcome of the study, was compared using an ANCOVA model adjusting for baseline and including the two independent genotypes PPARα (PPARα L162 L or PPARα X162V) and PPARγ2 (PPARγ2 P12P or PPARγ2 X12A) in order to identify the responders within each gene. A second ANCOVA model was implemented, adjusting on baseline and including a single genotype combination in 4 classes (PPARα L162 L/ PPARγ2 X12A, PPARα L162 L/ PPARγ2 P12P, PPARα X162V/ PPARγ2 X12A, PPARα X162V/PPARγ2 P12P). Interactions between genotype and BMI (normal weight: [18.5–24.9], overweight [25.0–29.9]) and between genotype and gender were investigated. The supplement effects on lipid metabolism (fasting levels of TC, HDL, LDL and fatty acid composition of the erythrocytes), glucose metabolism and insulin resistance (fasting HbA1c, glucose, insulin and adiponectin) and on inflammatory response (fasting level of markers such as CRP and IL-6) were analyzed as the primary outcome.

The identification of the covariance between the fatty acid profiles and changes in TG concentration (Δ) was done by using partial least squares regression (PLSR) [56]. Models were built by regression of the differential fatty acid profiles, obtained by subtraction (V3-V1), onto the ΔTG from same time-points. Dimensionality of the models (number of latent variables) was obtained by internal cross-validation by using random segments. Reliability of the model was assessed by evaluation of the model by CV-ANOVA [57]. Model's regression coefficients and X-loading weights of the selected models were used to identify those fatty acids more relevant to explain changes in TG. Distribution of the values of TG increase recommended constraining the models to the 0–200 mg/L range. As a confirmation on the selection, highlighted compounds were confirmed by univariate regression and correlation coefficients. To better understand the influence of compliance in the study, a second round of the analyses was performed on groups stratified according to high and average compliance rates.

Hardy–Weinberg equilibrium for the genotype frequencies was assessed using a χ^2 test.

3. Results

3.1. Subject characteristics

A group of 253 subjects were assessed for eligibility of which 226 met the major inclusion criteria and were subjected to treatment. From these, 196 participants completed the study and these were considered in the FAS dataset. Thirty participants dropped out before V3 due to adverse effects; were lost to follow up; or they withdrew from the study for unknown reasons (Fig. 1). The most frequent side effects were gastrointestinal (reported by 52% of the FAS participants). No serious adverse effects were reported. Five subjects violated the exclusion criteria as they consumed medications that could interfere with the study outcomes and were removed from the final analysis. The distribution by gender in FAS was as follows: 121 (63.4%) female and 70 (36.6%) male. Overall, the mean (±SD) for age was 26.6 (±6.3) years. Mean values of anthropometric data, body composition, and vital signs at baseline were within normal range (Table 1). Data on estimated macronutrient intake as assessed by the SNUT questionnaire, showed compared to recommendations, a higher intake of lipids (>30% of energy intake), a slightly lower intake of carbohydrates (45% of energy intake), while protein intake was within the recommendation (15% of energy intake). Specifically, the mean total energy intake was mean, ±SD: 1970.6 (±884.1), and the mean%, ±SD of carbohydrates, proteins and lipids in the diet was 47% (±7.3), 15.6 (±3.7) and 37.7 (±6.6). Descriptive values of the analyzed biochemical variables are shown in Table 2. Although the mean values of the phenotypes are within normal range, some risk factors for cardiovascular disease were identified in the population. At baseline, 33% of the participants were overweight (BMI>25) and 15.8% of the women and 7.7% of the men had large waist circumference (>80 cm for female and >90 cm for male). In addition, 28.6% of women and 19.8% of men had low HDL concentrations (<50 mg/dl for females and <40 mg/dl for male), 13% of participants had TG concentrations >150 mg/dl and a low mean (±SD) baseline HS-ω3 index of 4.8% (±1.2).

3.2. Supplement intake and compliance

A 100% consumption of the treatment was equivalent to 126 capsules or 42 days of treatment. Most participants finished the study and returned the remaining capsules (87%); the mean intake (±SD) of the capsules was 72.8% (±13.5) or 42.6 (±3) days of treatment with 4.8 (±5.5) days of interruption. As a measure of compliance, fatty acid analyses in RBC showed significant differences after the intervention in all the analyzed components. The FAS dataset consisted of N=191 and the PP dataset of N=61 subjects. HS-ω3 index significantly increased in both FAS and PP datasets (57.1%, P<.001 and 68.3%, P<.001, respectively). The observed shifts in O3FA were significant and with the same direction in both datasets (Supplemental Fig. 1).
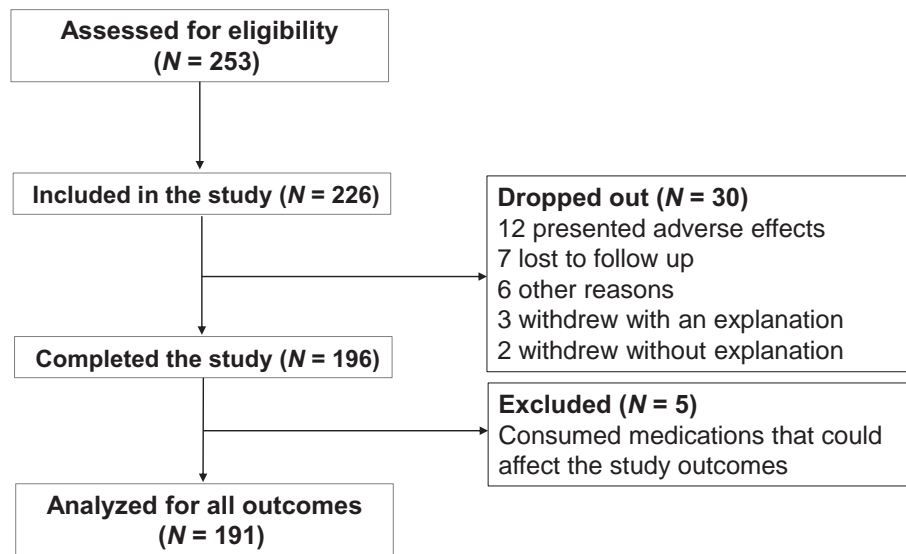
Fig. 1. Study outline.

3.3. PPARs genotype frequency and distribution

The frequency of the analyzed genetic variants among the sample was 11% for the minor allele of *PPARα* L162V, and 19.9% for the minor allele of *PPARγ2* P12A. The distribution of the combined genotypes was as follows: *PPARα* L162 L/*PPARγ2* X12A (n=33), *PPARα* L162 L/*PPAR-γ2* P12P (n=137), *PPARα* X162V/*PPARγ2* X12A (n=5) and *PPARα* X162V/*PPARγ2* P12P (n=16) (Supplemental Table 1). The genotype frequencies were all in Hardy–Weinberg equilibrium (P>.05) and they followed the expected estimated genotype distribution in the Mexican population.

3.4. Effects of O3FA on anthropometric characteristics, vital signs, body composition and erythrocyte membrane-associated FA concentration

Body weight, BMI, waist circumference, heart rate and blood pressure did not change significantly following 6 weeks of intervention (Table 1). Body fat and fat mass decreased and fat-free mass increased from baseline but not significantly (Table 1). No effects of the genotypes were observed for any of the anthropometric measurements and vital signs.

As expected, a significant shift in relative (%) composition of fatty acid in erythrocyte membranes was observed after supplementation, including an increase in ω3 polyunsaturated fatty acids (PUFA) and a

decrease in ω6 PUFA (Supplemental Fig. 1). Certain saturated fatty acids, such as stearic acid (C18:0) significantly increased following 6 weeks intervention (1.1%, P<.001, Supplemental Fig. 1). Compared to men, female participants had higher baseline concentration of DHA, alpha-linolenic acid (ALA), total O3 PUFA, HS-ω3 index, and ω3/ω6 ratio (P<.014); and after intervention, women raised their concentration in ω3 PUFA (P=.001), and lowered those of ω6 PUFA (P=.003), more than men (Supplemental Fig. 2). No significant effects of genotypes were detected for erythrocyte membranes FA concentration.

3.5. Effects of O3FA on blood lipids

After the intervention and independently of the genotype, an overall decrease of−5.3% in TG concentrations after adjusting for baseline (mean change, ±SD: −4.1 mg/dL, ±51.7, P=.02) was observed. Interestingly, differences between genotypes were noted (Table 2): *PPARα* X162V carriers achieved a greater - though not significantly different - reduction in TG from baseline compared to *PPARα* L162 L carriers [−19.5%, (mean change, ±SD: −19.5 mg/dL, ±40.0) vs −3.9%, (mean change, ±SD: −2.2 mg/dL, ±52.7), P>.05] (Table 3). For *PPARγ2* X12A carriers, a greater reduction was reported compared to *PPARγ2* P12P carriers [−10.6%, (mean change, ±SD: −14.2 mg/dL, ±41.7) vs

Table 1
Mean and (SD) of the anthropometric and clinical characteristics of the participants at baseline and 6 weeks after intervention with 2.7 g/day fish oil

	Baseline N=191	6 weeks N=191	Change from baseline
Age (years)	26.6 (6.3)		
Gender (F:M)	121:70		
Weight (kg)	64.4 (10.2)	64.4 (10.0)	0.07 (1.1)
Height (cm)	164.4 (8.4)		
BMI	23.7 (2.6)	23.8 (2.6)	0.06 (0.4)
WC (cm)	78.3 (8.1)	78.3 (8.1)	-0.07 (2.9)
% Body fat	28.5 (7.3)	28.1 (7.5)	-0.4 (1.5)
Fat mass (kg)	18.3 (5.4)	18.1 (5.6)	-0.2 (1.1)
FFM (kg)	46.1 (9.1)	46.4 (9.1)	0.3 (1.1)
Heart Rate (beats/min)	65.8 (9.3)	68.0 (11.3)	2.2 (9.5)
SBP (mmHg)	111.1 (10.4)	111.0 (11.1)	-0.1 (9.1)
DBP (mmHg)	67.5 (7.8)	66.6 (8.4)	-0.9 (9.2)

No significant differences were found.(F: female, M: male, FFM: fat-free mass, SBP: systolic blood pressure, DBP: diastolic blood pressure).

Table 2
Mean and (SD) of the biochemical assessment in participants at baseline and 6 weeks after intervention with 2,7 g/day dose fish oil

N=191	Baseline	6 weeks	Change from baseline	%change from baseline
Triglycerides (mg/dL)	97.9 (53.5)	93.8 (57.9)	-4.1 (51.7)	-5.3 [1]
Total cholesterol (mg/dL)	173.0 (31.9)	173.5 (29.4)	0.4 (22.9)	0.5
LDL (mg/dL)	104.5 (26.7)	106.3 (25.6)	1.8 (20.8)	1.9
HDL (mg/dL)	48.8 (1.9)	48.5 (11.7)	-0.3 (8.2)	-0.6
Glucose (mg/dL)	84.7 (6.9)	85.6 (6.9)	0.9 (6.5)	1.2
Insulin (mIU/L)	7.7 (11.3)	7.2 (7.2)	-0.5 (10.3)	-6.1 [1]
HbA1c %	5.1 (0.3)	5.1 (0.3)	-0.07 (0.3)	-1.4 [2]
Adiponectin (μg/mL)	4.1 (4.0)	4.7 (6.5)	0.6 (6.2)	7.9
Apelin (pg/mL)	388.6 (120.3)	424.4 (608.3)	36.8 (604.6)	11.9
CRP (pg/mL)	0.8 (0.9)	0.8 (0.9)	0.04 (0.8)	2.2
IL-6 (pg/ml)	39.2 (109.2)	39.5 (117.6)	0.3 (19.8)	-9.0

[1] P value<0.05.
[2] P value<0.001 (LDL: low density lipoprotein, HDL: high density lipoprotein, HbA1c: glycated hemoglobin, CRP: C-reactive protein, IL-6: interleukin 6).

Table 3
TG levels (mg/dL) expressed in mean (±SD) in each visit and their changes by PPARα
L162V and PPARγ2 P12A genotype groups

Triglycerides (mg/Dl)	Baseline	Week 6	Change from baseline	%change from baseline
PPARα L162 L N=170	97.3 (53.9)	95.1 (60.1)	-2.2 (52.7)	-3.9
PPARα X162V N=21	102.9 (51.1)	83.4 (34.5)	-19.5 (40.0)	-16.2
PPARγ2 P12P N=153	96.7 (50.1)	95.2 (61.3)	-1.5 (53.7)	-3.9
PPARγ2 X12A N=38	102.8 (66.1)	88.6 (41.8)	-14.2 (41.7)	-10.6
PPARα L162 L/ PPARγ2 P12P N=137	97.7 (51.2)	97.4 (63.5)	-0.3 (55.3)	-2.9
PPARα X162V/ PPARγ2 P12P N=16	87.9 (40.4)	75.5 (32.6)	-12.4 (36.8)	-12.6
PPARα L162 L/ PPARγ2 X12A N=33	95.5 (65.1)	85.5 (42.8)	-9.9 (40.1)	-7.9
PPARα X162V/ PPARγ2 X12A N=5	150.8 (55.9)	108.7 (29.9)	-42.1 (45.7)	-26.5

No significant differences were found. (PPARα/γ2 : peroxisome proliferator-activated receptor).

−3.9%, (mean change, ±SD: −1.5 mg/dL, ±53.7), P>.05] (Table 3). Neither gender nor BMI significantly influenced the FO response to the TG reduction (P>.05). DHA and EPA effects were explored both (independently and combined) in relation to the TG reduction. After 6 weeks of intervention, EPA and TG levels significantly inversely correlated (P=.025) after adjusted for baseline levels, indicating that the higher the EPA levels in erythrocyte cell membranes, the lower the blood TG levels (Supplemental Fig. 3). The greatest reduction [−26.5%, (mean change, ±SD: −42.1 mg/dL, ±45.7] was observed in the PPARα X162V/PPARγ2 X12A group, which has the combination of the minor alleles (Table 3). The combined effect of all RBC fatty acid levels on TG was also assessed by multivariate analysis: a number of fatty acids were found to be weakly associated with the TG concentration changes from baseline (data not shown).

We did not observe any significant changes from baseline after intervention in fasting total cholesterol, LDL and HDL concentrations in the overall population (Table 2). However, different effects by genotype were observed, including a significant increase in total cholesterol (P=.032) and LDL-c (P=.021) between PPARγ2 X12A and PPARγ2 P12P in subjects with BMI>25 (Supplemental Fig. 4). Men had significantly lower levels of HDL (mean, ±SD), at both baseline and after the intervention, compared to women (baseline, mean change, ±SD: 44.8, ±8.8 vs 51.1, ±12.9, P<.001 and after 6 weeks, mean change, ±SD: 45.7, ±8.7 vs 50.1, ±12.9, P=.02). Following intervention, the HDL concentrations raised more in men than in women (P=.007).

3.6. Effects of O3FA on glucose metabolism markers

After intervention and independently of genotype, fasting insulin was reduced by 6.1% (mean change, ±SD: −0.5mlU/L, ±10.3, P=.034) and HbA1c by 1.4% (mean change, ±SD: −0.07%, ±0.3, P<.001) (Table 2); this last decrease was even larger in subjects with a baseline HbA1c greater than 5.6% (P<.001). No significant changes were seen for fasting glucose and adiponectin (P>.05). HOMA-IR was borderline significantly reduced in all subjects (mean change, ±SD: −0.1, ±2.2, P=.05). The decrease in fasting insulin was significantly greater in PPARγ2 X12A carriers compared to PPARγ2 P12P individuals [−19.8%, (mean change, ±SD: −2.3mlU/L, ±8.4) vs−2.5%, (mean change, ±SD: −0.1mlU/L, ±10.7), P=.032] (Fig. 3). Also, after intervention PPARγ2 X12A subjects showed a trend in increasing in their fasting adiponectin compared to the PPARγ2 P12P groups (10% higher, mean change, ±SD: +1.6 μg/mL, ±8.1, P=.06). The increase in adiponectin levels was not associated with

the observed decline in insulin levels (Table 2). Changes in fasting adiponectin were not correlated with expression levels of AdipoR1 and AdipoR2 genes (data not shown). Mean apelin levels remained unchanged from baseline in the entire population. Interestingly, only the PPARα L162V genotype groups had a borderline significant difference in apelin change with PPARα X162V achieving a significant reduction in apelin levels compared to PPARα L162 L (P=.05) (Supplemental Fig. 5). BMI, gender and fatty acid levels were not associated with any of the changes in glucose metabolism markers (P>.05).

3.7. Effects of O3FA on inflammatory markers

No significant changes were seen for circulating CRP and IL-6 from baseline in the entire dataset and between the genotype groups (Table 2).

3.8. Results with lower doses of O3FA

The main study outcomes were analyzed separately in the subgroup of subjects (N=116) with lower compliance to the intervention (<80% intake of FO capsules). The mean intake of O3FA from supplementation for these subjects was estimated to be about 1.8 g/day. Reduction in fasting TG and other blood lipids were not achieved in this subset (P=.1), indicating that a higher dose of FO is necessary to achieve the expected changes. However, and very interestingly, both mean fasting insulin and HbA1c were significantly reduced following the intervention (P=.019 and P<.001 respectively), suggesting that a dose of 1.8 g per day of O3FA (−33% than originally intended) can have an effect on glucose metabolism in these subjects (Fig. 2a and b respectively). Genotype effects were examined separately in this population but there was no significant difference in the reduction of fasting triglycerides and insulin from baseline between the different genotype groups. There was, however, a greater reduction in fasting insulin in PPARγ2 X12A carriers compared to PPARγ2 P12P [−17.98% (±33.30) vs 20.61% (±147.39)] (Supplemental Fig. 6) and a reduction of triglycerides in PPARα X162V carriers compared to PPARα L162 L [−2.82% (±32.01) vs 7.33% (±81.03)] (Supplemental Fig. 7).
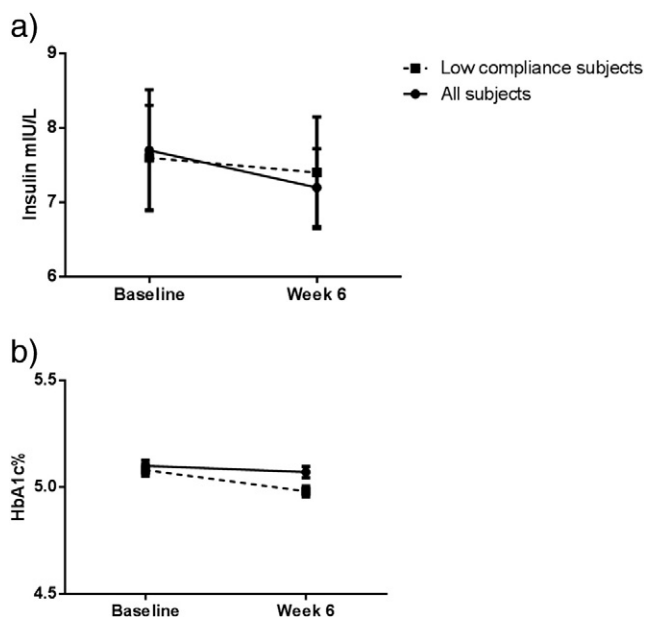

Fig. 2. Mean (SE) insulin (μU/L) and HbA1c (%) in baseline (visit 1) and after 6 weeks of intervention (visit 3) for all subjects (N=191, continuous line) and low compliance subjects (N=116, dashed line).
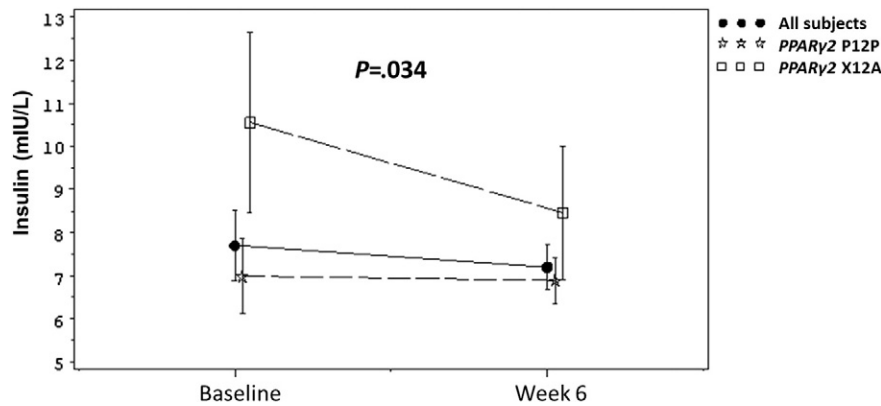
Fig. 3. Mean ($\pm$SD) insulin (mlU/L) in baseline (visit 1) and week 6 (visit 3) for all subjects (N=191), *PPARγ2* P12P carriers (N=153), *PPARγ2* X12A carriers (N=38).

3.9. Effects on gene expression in PBMC

In the present study, no difference between baseline and after six weeks of treatment was observed in the RNA abundance of *AdipoR1*, *AdipoR2* and *IL-6* in PBMC, neither in the whole sample set, nor according to genotype groups under fasting conditions (data not shown). These observations are concordant with the non-significant differences observed in circulating IL-6 and adiponectin.

4. Discussion

In our study, as expected, the concentration of O3FA increased in erythrocyte membranes after a six-week FO supplementation. This change is consistent with other studies with similar dose [7,31,32] and time [7]. Baseline mean values $\pm$SD of HS-ω3 index in the present study (4.8% $\pm$1.2) were below the recommended range (8–11%) [55,58]. Our findings are concordant with previous investigations that reported low intake of these fatty acids [51] and low content of O3FA in RBC [59] in the Mexican population. After intervention, the HS-ω3 index significantly increased to almost recommended range (7.5% $\pm$ 1.3), regardless of the PPAR genotype. Relative values of ω6 PUFA, such as arachidonic acid (C20:4 ω6), were significantly decreased after intervention, regardless of PPAR genotype. The increase in O3FA in cell membranes seems to influence the synthesis of lipid mediators and preclude the conversion of arachidonic acid in prostaglandins, thromboxanes and leukotrienes and produce instead less potent mediators [5,38]. The parallel reduction of arachidonic acid and increase of EPA in cell membranes (mononuclear cells) has been previously described after consumption of FO, and it is dose-dependent [33].

Undoubtedly, the most consistent effect of FO intake is its beneficial effect of lowering TG, especially in hypertriglyceridemic patients [4–7,21]. Multiple reports support this finding in a variety of human studies (*e.g.* healthy, hypertriglyceridemic, obese, diabetic subjects) with diverse intervention characteristics (time and dose). Studies conducted in healthy subjects have found an overall decrease of circulating TG by 16 to 45% [7,37,43,60]. This effect is believed to be mediated by multiple mechanisms, including the decrease of non-esterified fatty acids (NEFA), the reduction in the expression and activity of transcription factors such as *HNF4α* [60] and SREBP1c in liver, which reduces the expression of lipogenic enzymes [60]. A concomitant activation of *PPARα* may contribute to this effect by the increase in fatty acid oxidation [9,19,21,24]. The changes in the expression of the above mentioned transcription factors may be mediated by the increase in AMPK activity [61]. Although the suppressive effect of O3FA on TG is supported by multiple investigations, it is important to point out the wide variation observed in the

response to FO intake [62,63]. Studies have reported up to 29% of non-responders to treatment with O3FA [7]. There is considerable evidence that this response may be influenced by numerous gene variants [7–9,64,65–69]. The primary outcome of the present study was the change dependent on the genetic variants *PPARα* L162V and *PPARγ2* P12A in fasting plasma TG concentration between baseline and 6 weeks of intervention. Previous studies have shown that the L162V polymorphism in PPARα is associated with differences in gene expression and response to the consumption of high levels of PUFA. Association studies have demonstrated that the *PPARα* 162 V variant is associated to TG concentrations [66,67]. The L162V polymorphism has been shown to affect transactivation efficiency *in vitro* after activation by fibrates. A human study using 5 g of FO during 6 weeks found that carriers of both *PPARα* alleles showed a highly similar decrease in TG between the L162V allele groups, although TG concentrations were slightly different at baseline [68]. Other studies showed that variation in the *PPARγ2* gene influences the physiological responses following intake of O3FA. A study conducted by Lindi et al. [8] showed that the *PPARγ2* P12A polymorphism influenced the variability in the change of TG, after supplementation with O3FA. Rudkowska et al. showed that the *PPARα* L162V may exert its effects on TG *via* altering the transcription rate of lipoprotein lipase [68]. Some of these studies may have had insufficient statistical power to demonstrate a significantly different effect between genotype groups, or the baseline blood lipids and O3FA levels may play role in these gene/diet interactions [69]. In the present study carriers of the *PPARα* X162V and *PPARγ2* X12A variants showed a larger decrease after treatment. Both of these genetic variants have been associated with differences in the activity of the encoded protein PPARα and PPARγ2 and variation in metabolic traits [8,9,23,26–30,66,67], and the independent effect of the analyzed genotypes on the response to intervention with O3FA has been tested before. Indeed, both polymorphisms have been described as "diet-dependent metabolic sensors"; in diets low in PUFA, carriers of the minor alleles have higher blood lipids compared to carriers of the major alleles [9,27]. In our study, levels of PUFA in erythrocytes at baseline are lower than recommended and we observed a clear trend for an increased beneficial effect in specific genotypes. The previous supports that carriers of the minor alleles of *PPARα* L162V and *PPARγ2* P12A with similar lifestyle characteristics as the Mexican population in this study could particularly benefit by increasing their PUFA intake in their diet.

The secondary objectives of the study included the assessment of the bioefficacy of O3FA supplementation, dependent on *PPARα* L162V and *PPARγ2* P12A genetic variants, on glucose metabolism and inflammatory response, assessing also the link to insulin resistance. Interestingly, novel effects of *PPARα* L162V and *PPARγ2* P12A following FO supplementation were observed for glucose metabolism

markers. Our findings revealed a significant improvement in HbA1c and fasting insulin after 6 weeks of intervention. The PPARγ2 P12A variant was shown to associate with the responses in insulin: carriers of the minor allele had higher fasting insulin levels at baseline and achieved a greater reduction after 6 weeks compared to wild-type allele carriers. These observations are supported by the fact that the PPARγ2 P12A variant reduces the transcription of PPARγ2, expressed in adipose tissue and is known to be involved in the modulation of insulin sensitivity, and that the PPARγ2 P12A variant has been associated with a lower risk for T2D mellitus [27,28]. There were no significant effects for PPARα L162V and glucose markers in the present study, perhaps not surprisingly as this locus appeared previously to have no impact on T2D and prediabetes phenotypes [70]. Importantly, in our study lower intake levels of O3FA (1.8 g/day) compared to the intended dose (2.7 g/day) seemed to be sufficient to induce significant changes in these biomarkers, suggesting that lower doses of FO need to be further investigated for the potential to improve glucose management and prevention of T2D. Overall, greater effects in glucose metabolism have been reported in subjects with milder metabolic abnormalities than in subjects with overt diabetes, in which the effects of O3FA intake on glucose metabolism seem to be less clinically significant [11,12,71,72]. Effects on adiponectin and apelin did not contribute to explain insulin and HbA1c reduction in our study, indicating that the observed effect on glucose metabolism is not mediated by these adipokines; other mechanisms, such as changes in insulin signaling, may be exerting the observed modulation in insulin sensitivity. Our findings support that moderately elevated levels of O3FA in the diet may be a preventive step for T2D for some individuals. In light of the technological challenges to add sufficient doses of O3FA in nutraceuticals and the compliance issues related to FO taste and gastrointestinal symptoms, exploring using low doses of O3FA is pertinent.

In the present study, no response to treatment was observed in primary pro-inflammatory markers, such as IL-6 and C-reactive protein. Previous studies have reported similar findings in healthy middle-aged subjects, with no significant changes in circulating indicators of inflammation including cytokines, chemokines, or cell adhesion molecules [10,38]. The generally healthy state, young age and absence of smoking in the study population may explain the lack of significant changes on the analyzed inflammatory markers.

The effect of the O3FA intake on circulating lipids other than TG (total cholesterol, and bound to specific lipoproteins) has been controversial. Numerous human studies have been conducted using a wide range of O3FA doses with different combinations of fatty acids and treatment times, across different groups of participants, although few of them have tested the effect of specific genotypes. Our study showed that in subjects with BMI>25 carrying the PPARγ2 12A allele, TC and LDL-c are significantly increased following the 6-weeks intervention. These observations are concordant with results seen by Itariu et al. [73].The authors reported differences in the concentration of TC, LDL-c and ApoB related to variation in PPARγ2, after treatment with O3FA (eight weeks, 3.36 g/d of EPA and DHA). Interestingly their study population consisted of severely obese subjects, which in combination with our finding may suggest that these responses may be BMI-dependent. In the present study the pattern of the observed

differences in total cholesterol between PPARγ2 genotypes seemed to be explained by increase in LDL-c, given that changes in HDL-c were not significant and do not appear to contribute to the TC concentration changes. The findings on HDL-c circulating levels in the present study contrast with observations reported by Lee et al. [4], and Carvajal et al. [6]. The first study found an overall favorable shift in TC, LDL-c, TG and HDL in diabetic subjects and the second reported similar beneficial effects in normolipemic, hypertriglyceridemic and hypocholesterolemic Mexican subjects, with concomitant increase in HDL-c. They used a similar dose of O3FA as the present study during only four weeks, in older participants. No genetic effects were tested in these studies. The effect of O3FA intake on HDL-c has been inconsistent across investigations, with responses that include a small increase in HDL-c levels or in some cases, inconsistent sex-dependent effects [24] or genotype-dependent [24,73,74] effects. Studies conducted in in vitro models suggest that treatment with EPA and DHA may decrease the expression of ApoA1 and ABCA1 transport, which would decrease the synthesis of HDL-c [75]. An ex vivo study conducted in human macrophages showed increase of ApoA1 expression after exposure to O3FA [26]. Thus, the effect of O3FA on HDL-c levels remains controversial and requires further investigation.

In order to integrate the described results of our study, the most relevant findings of genotype-related effects are summarized in Table 4.

It is important to acknowledge that our study has some limitations. First, it did not have the power to identify significant effects on TG between all examined genotype groups. The frequency of the PPARα 162 V variant was very low so there were only 5 subjects in this study with the combination of minor alleles of both genotypes. The clear trends observed in the different TG reduction between the genotype groups, supports the need to confirm the effect size of the treatment by genotype in larger intervention studies. Second, it only included the investigation of the role of two genotypes in the O3FA-induced physiological effects; however, other genotypes within the same or other genes may also have an important effect on the response to the intervention. A more comprehensive genome-wide approach is warranted to reveal changes in pathways, including those dependent of or interacting with PPAR that were not investigated in the present study, as proposed in previous studies [7,74,76,77]. Another limitation of the study is the number of inflammation-related markers and transcripts that were analyzed. Measures of other molecules influenced by O3FA, such as other cytokines and marine fatty acid derivatives, and related transcripts is recommended. Findings of the present study may not be applicable to older participants, or subjects with chronic diseases. Finally, another limitation was the low compliance to the treatment (<80%); in this case we consider that the presence of gastrointestinal symptoms reported by > 50% of the participants precluded these participants from completing the treatment. Other studies have reported minor transient gastrointestinal symptoms administering a similar source and dose of O3FA [26]. Interestingly, measuring an objective indicator of the changes induced by the treatment such as the relative fatty acid composition in RBC, we found no significant differences in the levels of FA between the FAS and the PP datasets. This observation may be part of the explanation why regardless of this study limitation, we identified that some of the study outcomes, such as HbA1c and fasting insulin, appeared to be

Table 4
Summary of genotype-related effects

Phenotype	Overall genotype independent effect	Genotype effect
Triglycerides	−5%	5 fold ↓ in carriers of the combined minor alleles compared to overall effect
Total cholesterol	NS	↑in PPARγ2 P12A carriers with BMI>25
LDL-cholesterol	NS	↑in PPARγ2 P12A carriers with BMI>25
Insulin	−6%	8 fold ↓ in carriers of PPARγ2 P12A compared to wild type

significantly reduced even in the subpopulation with the lowest compliance.

Nevertheless, a strength of our study is that it included the interrogation of several biomarkers for cardiometabolic health in a young Mexican population providing insight for potential preventive measures. The Mexican population has a high prevalence of dyslipidemia, obesity and metabolic syndrome and according to the results of the present study, the intake of O3FA may contribute to improve some risk factors for cardiovascular disease in this population. The impact of an increase in the intake of these nutrients on cardiovascular events and other related diseases should be assessed. Future studies need to confirm the effects of the individual genotypes and their combinations in larger populations or subjects at risk for cardiovascular disease and T2D. Lower doses of FO, as well as other sources of O3FA need also to be investigated for their responses to the improvement of glucose metabolism markers, thus contributing to the use of O3FA as a preventive approach against T2D and cardiovascular disease.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2017.02.002.

Acknowledgements

References

[1] Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci (Lond) 2009;116(1):1–16.

[2] Clarke SD. The multi-dimensional regulation of gene expression by fatty aids: polyunsaturated fats as nutrient sensors. Curr Opin Lipidol 2004;116:1–16.

[3] Ellulu MS, Khaza'ai H, Abed Y, Rahmat A, Ismail P, Ranneh Y. Role of fish oil in human health and possible mechanism to reduce the inflammation. Inflammopharmacology 2015;23(2–3):79–89.

[4] Lee TC, Ivester P, Hester AG, Sergeant S, Case LD, Morgan T, et al. The impact of polyunsaturated fatty acid-based dietary supplements on disease biomarkers in a metabolic syndrome/diabetes population. Lipids Health Dis 2014;13:196.

[5] Calder PC. N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83(Suppl. 6):1505S–19S.

[6] Carvajal O, Angulo O. Effect of n-3 polyunsaturated fatty acids on the lipidic profile of healthy Mexican volunteers. Salud Publica Mex 1997;39(3):221–4.

[7] Rudkowska I, Guenard F, Julien P, Couture P, Lemieux S, Barbier O, et al. Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid supplementation. J Lipid Res 2014;55(7):1245–53.

[8] Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K, et al. Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. Mol Genet Metab 2003;79(1):52–60.

[9] Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, et al. The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annu Rev Nutr 2011;31:203–34.

[10] Pot GK, Brouwer IA, Enneman A, Rijkers GT, Kampman E, Geelen A. No effect of fish oil supplementation on serum inflammatory markers and their interrelationships: a randomized controlled trial in healthy, middle-aged individuals. Eur J Clin Nutr 2009;63(11):1353–9.

[11] Akinkuolie AO, Ngwa JS, Meigs JB, Djousse L. Omega-3 polyunsaturated fatty acid and insulin sensitivity: a meta-analysis of randomized controlled trials. Clin Nutr 2011;30(6):702–7.

[12] Lorente-Cebrian S, Costa AG, Navas-Carretero S, Zabala M, Martinez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Physiol Biochem 2013;69(3):633–51.

[13] Foulon T, Richard MJ, Payen N, Bourrain JL, Beani JC, Laporte F, et al. Effects of fish oil fatty acids on plasma lipids and lipoproteins and oxidant-antioxidant imbalance in healthy subjects. Scand J Clin Lab Invest 1999;59(4):239–48.

[14] Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. JAMA 2012;308(10):1024–33.

[15] Brady LM, Lovegrove SS, Lesauvage SV, Gower BA, Minihane AM, Williams CM, et al. Increased n-6 polyunsaturated fatty acids do not attenuate the effects of long-chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians. Am J Clin Nutr 2004;79(6):983–91.

[16] Ramel A, Martinez A, Kiely M, Morais G, Bandarra NM, Thorsdottir I. Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. Diabetologia 2008;51(7):1261–8.

[17] Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, et al. Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. J Nutrigenet Nutrigenomics 2013;6(2):73–82.

[18] Fontani G, Corradeschi F, Felici A, Alfatti F, Bugarini R, Fiaschi AI, et al. Blood profiles, body fat and mood state in healthy subjects on different diets supplemented with omega-3 polyunsaturated fatty acids. Eur J Clin Invest 2005;35(8):499–507.

[19] Guri AJ, Hontecillas R, Bassaganya-Riera J. Dietary modulators of peroxisome proliferator-activated receptors: implications for the prevention and treatment of metabolic syndrome. J Nutrigenet Nutrigenomics 2008;1(3):126–35.

[20] Smith CE, Ordovas JM. Fatty acid interactions with genetic polymorphisms for cardiovascular disease. Curr Opin Clin Nutr Metab Care 2010;13(2):139–44.

[21] Nakamura MT, Yudell BE, Loor JJ. Regulation of energy metabolism by long-chain fatty acids. Prog Lipid Res 2014;53:124–44.

[22] Yong EL, Li J, Liu MH. Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms alpha, beta/delta and gamma) and mechanisms of dyslipidemias. Curr Opin Lipidol 2008;19(2):106–12.

[23] Tai ES, Demissie S, Cupples LA, Corella D, Wilson PW, Schaefer EJ, et al. Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham offspring study. Arterioscler Thromb Vasc Biol 2002;22(5):805–10.

[24] Tai ES, Corella D, Demissie S, Cupples LA, Coltell O, Schaefer EJ, et al. Polyunsaturated fatty acids interact with the PPARA-L162V polymorphism to affect plasma triglyceride and apolipoprotein C-III concentrations in the Framingham heart study. J Nutr 2005;135(3):397–403.

[25] Volcik KA, Nettleton JA, Ballantyne CM, Boerwinkle E. Peroxisome proliferator-activated receptor [alpha] genetic variation interacts with n-6 and long-chain n-3 fatty acid intake to affect total cholesterol and LDL-cholesterol concentrations in the atherosclerosis risk in communities study. Am J Clin Nutr 2008;87(6):1926–31.

[26] Rudkowska I, Garenc C, Couture P, Vohl MC. Omega-3 fatty acids regulate gene expression levels differently in subjects carrying the PPARalpha L162V polymorphism. Genes Nutr 2009;4(3):199–205.

[27] Stumvoll M, Wahl HG, Loblein K, Becker R, Machicao F, Jacob S, et al. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 gene is associated with increased antilipolytic insulin sensitivity. Diabetes 2001;50(4):876–81.

[28] Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. Am J Epidemiol 2010;171(6):645–55.

[29] Mohamed Youssef S, Mohamed N, Afef S, Khaldoun BH, Fadoua N, Fadhel NM, et al. Interaction effects of the Leu162Val PPAR alpha and Pro12Ala PPAR gamma 2.Gene variants with renal function in metabolic syndrome population. PPAR Res 2013;2013:329862.

[30] Aldhoon B, Zamrazilova H, Aldhoon Hainerova I, Sramkova P, Spalova J, Kunesova M, et al. Role of the PPARalpha Leu162Val and PPARgamma2 Pro12Ala gene polymorphisms in weight change after 2.5-year follow-up in Czech obese women. Folia Biol 2010;56(3):116–23.

[31] Pipingas A, Cockerell R, Grima N, Sinclair A, Stough C, Scholey A, et al. Randomized controlled trial examining the effects of fish oil and multivitamin supplementation on the incorporation of n-3 and n-6 fatty acids into red blood cells. Nutrients 2014;6(5):1956–70.

[32] Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P, et al. Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population. J Nutr Biochem 2013;24(1):54–61.

[33] Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KW, et al. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am J Clin Nutr 2006;83(2):331–42.

[34] Rizza S, Tesauro M, Cardillo C, Galli A, Iantorno M, Gigli F, et al. Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. Atherosclerosis 2009;206(2):569–74.

[35] Phang M, Linczv LF, Garg ML. Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. J Nutr 2013;143(4):457–63.

[36] Guebre-Egziabher F, Rabasa-Lhoret R, Bonnet F, Bastard JP, Desage M, Skilton MR, et al. Nutritional intervention to reduce the n-6/n-3 fatty acid ratio increases

adiponectin concentration and fatty acid oxidation in healthy subjects. Eur J Clin Nutr 2008;62(11):1287–93.

[37] Cazzola R, Russo-Volpe S, Miles EA, Rees D, Banerjee T, Roynette CE, et al. Age- and dose-dependent effects of an eicosapentaenoic acid-rich oil on cardiovascular risk factors in healthy male subjects. Atherosclerosis 2007;193(1):159–67.

[38] Yusof HM, Miles EA, Calder P. Influence of very longchain n-3 fatty acids on plasma markers of inflammation in middle-aged men. Prostaglandins Leukot Essent Fatty Acids 2008;78(3):219–28.

[39] Weaver KL, Ivester P, Seeds M, Case LD, Arm JP. Chilton FH effect of dietary fatty acids on inflammatory gene expression in healthy humans. J Biol Chem 2009; 284(23):15400–7.

[40] Hlais S, El-Bistami D, El RB, Mattar MA, Obeid OA. Combined fish oil and high oleic sunflower oil supplements neutralize their individual effects on the lipid profile of healthy men. Lipids 2013;48(9):853–61.

[41] Kaul N, Kreml R, Austria JA, Richard MN, Edel AL, Dibrov E, et al. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. J Am Coll Nutr 2008;27(1):51–8.

[42] Sanders TA, Gleason K, Griffin B, Miller GJ. Influence of an algal triacylglycerol containing docosahexaenoic acid (22: 6n-3) and docosapentaenoic acid (22: 5n-6) on cardiovascular risk factors in healthy men and women. Br J Nutr 2006; 95(3):525–31.

[43] Mann NJ, O'Connell SL, Baldwin KM, Singh I, Meyer BJ. Effects of seal oil and tuna-fish oil on platelet parameters and plasma lipid levels in healthy subjects. Lipids 2010;45(8):669–81.

[44] Mori TA. Dietary n-3 PUFA and CVD: a review of the evidence. Proc Nutr Soc 2014; 73(1):57–64.

[45] Plourde M, Vohl MC, Vandal M, Couture P, Lemieux S. And Cunnane, S.C plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men. Br J Nutr 2009;102:1121–4.

[46] Paradis AM, Fontaine-Bisson B, Bosse Y, Robitaille J, Lemieux S, Jacques H, et al. The peroxisome proliferator-activated receptor alpha Leu162Val polymorphism influences the metabolic response to a dietary intervention altering fatty acid proportions in healthy men. Am J Clin Nutr 2005;81:523–9.

[47] Ulven SM, Myhrstad MC, Holven KB. Marine n-3 fatty acids and gene expression in peripheral blood mononuclear cells. Curr Cardiovasc Risk Rep 2014;8:412.

[48] Afman L, Milenkovic D, Roche HM. Nutritional aspects of metabolic inflammation in relation to health—insights from transcriptomic biomarkers in PBMC of fatty acids and polyphenols. Mol Nutr Food Res 2014;58:1708–20.

[49] Murguia-Romero M, Jimenez-Flores JR, Sigrist-Flores SC, Tapia-Pancardo DC, Jimenez-Ramos A, Mendez-Cruz AR, et al. Prevalence of metabolic syndrome in young Mexicans: a sensitivity analysis on its components. Nutr Hosp 2015;32(1):189–95.

[50] Pedroza-Tobias A, Trejo-Valdivia B, Sanchez-Romero LM, Barquera S. Classification of metabolic syndrome according to lipid alterations: analysis from the Mexican National Health and nutrition survey 2006. BMC Public Health 2014;14:1056.

[51] Ramirez-Silva I, Villalpando S, Moreno-Saracho JE, Bernal-Medina D. Fatty acids intake in the Mexican population. Results of the National Nutrition Survey 2006. Nutr Metab 2011;8(1):33.

[52] Maddison R, Ni Mhurchu C, Jiang Y, Vander Hoorn S, Rodgers A, Lawes CM, et al. International physical activity questionnaire (IPAQ) and New Zealand physical activity questionnaire (NZPAQ): a doubly labelled water validation. Int J Behav Nutr Phys Act 2007;4:62.

[53] Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. Biochem Biophys Res Commun 1988;156(2):960–3.

[54] Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, Willett W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud Publica Mex 1998;40(2):133–40.

[55] Harris WS, Von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? Prev Med 2004;39(1):212–20.

[56] Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of Chemometrics. Chemom Intel Lab Syst 2001;58:109–30.

[57] Eriksson L, Trygg J, Wold S. CV-ANOVA for significance testing of PLS and OPLS® models. In: Reinikainen Satu-Pia, Taavitsainen Veli-Matti, Rantanen Jukka, editors. J Chemometr, 22; 2008. p. 594–600.

[58] von Schacky C. Omega-3 index and cardiovascular health. Nutrients 2014;6: 799–814.

[59] Cuevas-Covarrubias SA, Miranda-Zamora R, Juarez-Oropeza MA, Diaz-Zagoya JC. Comparative analysis of erythrocyte fatty acid composition in a sample of Mexico City children and young adults on a free diet. Arch Med Res 1993;24(4):327–31.

[60] Shearer GC, Savinova OV, Harris WS. Fish oil – how does it reduce plasma triglycerides? Biochim Biophys Acta 2012;1821(5):843–51.

[61] Endo J, Arita M. Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. J Cardiol 2016;67(1):22–7.

[62] Asztalos IB, Gleason JA, Sever S, Gedik R, Asztalos BF, Horvath KV, et al. Effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular disease risk factors: a randomized clinical trial. Metabolism 2016;65(11):1636–45.

[63] Williams CM, Moore F, Morgan L, Wright J. Effects of n-3 fatty acids on postprandial triacylglycerol and hormone concentrations in normal subjects. Br J Nutr 1992;68(3):655–66.

[64] Cormier H, Rudkowska I, Lemieux S, Couture P, Julien P, Vohl MC. Effects of FADS and ELOVL polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation. Genes Nutr 2014;9(6):437.

[65] Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain-PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr 2015;102(2):505–13.

[66] Uthurralt J, Gordish-Dressman H, Bradbury M, Tesi-Rocha C, Devaney J, Harmon B, et al. PPARalpha L162V underlies variation in serum triglycerides and subcutaneous fat volume in young males. BMC Med Genet 2007;8:55.

[67] Sparso T, Hussain MS, Andersen G, Hainerova I, Borch-Johnsen K, Jorgensen T, et al. Relationships between the functional PPARalpha Leu162Val polymorphism and obesity, type 2 diabetes, dyslipidaemia, and related quantitative traits in studies of 5799 middle-aged white people. Mol Genet Metab 2007;90(2):205–9.

[68] Rudkowska I, Caron-Dorval D, Verreault M, Couture P, Deshaies Y, Barbier O, et al. PPARalpha L162V polymorphism alters the potential of n-3 fatty acids to increase lipoprotein lipase activity. Mol Nutr Food Res 2010;54(4):543–50.

[69] Caron-Dorval D, Paquet P, Paradis AM, Rudkowska I, Lemieux S, Couture P, et al. Effect of the PPAR-alpha L162V polymorphism on the cardiovascular disease risk factor in response to n-3 polyunsaturated fatty acids. J Nutrigenet Nutrigenomics 2008;1(4):205–12.

[70] Silbernagel G, Stefan N, Hoffmann MM, Machicao-Arano F, Machann J, Schick F, et al. The L162V polymorphism of the peroxisome proliferator activated receptor alpha gene (PPARA) is not associated with type 2 diabetes, BMI or body fat composition. Exp Clin Endocrinol Diabetes 2009;117(3):113–8.

[71] Flachs P, Rossmeisl M, Kopecky J. The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. Physiol Res 2014;63(Suppl. 1):S93–118.

[72] Giacco R, Cuomo V, Vessby B, Uusitupa M, Hermansen K, Meyer BJ, et al, KANWU Study Group. Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? Nutr Metab Cardiovasc Dis 2007;17(8):572e80.

[73] Itariu BK, Zeyda M, Hchbrugge EE, Neuhofer A, Prager G, et al. Long-chain n-3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. Am J Clin Nutr 2012;96(5): 1137–49.

[74] de Andrade FM, Bulhoes AC, Maluf SW, Schuch JB, Voigt F, Lucatelli JF, et al. The influence of nutrigenetics on the lipid profile: interaction between genes and dietary habits. Biochem Genet 2010;48(3–4):342–55.

[75] Kuang YL, Paulson KE, Lichtenstein AH, Lamon-Fava S. Regulation of the expression of key genes involved in HDL metabolism by unsaturated fatty acids. Br J Nutr 2012;108(8):1351–9.

[76] Baratta R, Di Paola R, Spampinato D, Fini G, Marucci A, Coco A, et al. Evidence for genetic epistasis in human insulin resistance: the combined effect of PC-1 (K121Q) and PPARgamma2 (P12A) polymorphisms. J Mol Med 2003;81(11): 718–23.

[77] Yilmaz-Aydogan H, Kurnaz O, Kurt O, Akadam-Teker B, Kucukhuseyin O, Tekeli A, et al. Effects of the PPARG P12A and C161T gene variants on serum lipids in coronary heart disease patients with and without type 2 diabetes. Mol Cell Biochem 2011;358(1–2):355–63.