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

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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 [−0.547mlU/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.

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

Abbreviations: ALA, Alpha-linolenic acid; BMI, Body mass index; CI, Confidence intervals; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; FA, Fatty acids; FAS, Full analysis dataset; HDL, High-density lipoproteins; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance Index; IL-6, Interleukin 6; LDL, Low-density lipoproteins; O3FA, Omega (ω) 3 fatty acids; PP, Per Protocol dataset; PPARα/γ2, Peroxisome proliferator-activated receptor α/γ2; PUFA, Polyunsaturated fatty acids; RBC, Red blood cell; SD, Standard deviation; SE, Standard error; TC, Total cholesterol; TG, Triglycerides; T2D, Type 2 diabetes.

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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