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Aline Ramos de Araújo MSc , Geni Rodrigues Sampaio PhD , Lucas Ribeiro da Silva RDN , Vera Lúcia Portal PhD , Melissa Medeiros Markoski PhD , Alexandre Schaan de Quadros PhD , Marcelo Macedo Rogero PhD , Elizabeth Aparecida Ferraz da Silva Torres PhD , Aline Marcadenti PhD

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Effects of extra virgin olive oil and pecan nuts on plasma fatty acids in patients with stable coronary artery disease

Running head: Olive oil, pecan nuts and plasma fatty acids in CAD.

Aline Ramos de Araújo[1], MSc; Geni Rodrigues Sampaio[2], PhD; Lucas Ribeiro da Silva[3], RDN; Vera Lúcia Portal[4], PhD; Melissa Medeiros Markoski[5], PhD; Alexandre Schaan de Quadros[4], PhD; Marcelo Macedo Rogero[2], PhD; Elizabeth Aparecida Ferraz da Silva Torres[2], PhD; Aline Marcadenti[3,4], PhD.

[1]Graduate Program in Nutrition Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). 245 Sarmento Leite Street, 90050-170, Porto Alegre, Rio Grande do Sul, Brazil.

[2]Department of Nutrition, Faculty of Public Health, Universidade de São Paulo (USP). 715 Dr. Arnaldo Avenue, 01246-904, São Paulo, São Paulo, Brazil.

[3]HCor Research Institute, Hospital do Coração (IP-HCor). 250 Abílio Soares Street, 04004-050, São Paulo, São Paulo, Brazil.

[4]Graduate Program in Health Sciences (Cardiology), Instituto de Cardiologia/Fundação Universitária de Cardiologia do Rio Grande do Sul (IC/FUC). 395 Princesa Isabel Avenue, 90040-371, Porto Alegre, Rio Grande do Sul, Brazil.

[5]Graduate Program in Biosciences, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). 245 Sarmento Leite Street, 90050-170, Porto Alegre, Rio Grande do Sul, Brazil.

Corresponding author:

Prof. Dr. Aline Marcadenti

HCor Research Institute, Hospital do Coração (HCor)

Abílio Soares Street, 250, São Paulo, Zip Code 04004-05

São Paulo, Brazil. Phone: +55(11) 3053 6611 – extension 3558

Email: marcadenti.aline@gmail.com

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Highlights

- Healthy diets, pecan nuts and olive oil may enhance certain plasma fatty acids.
- In patients with CAD, 30 mL/day of olive oil increased plasma oleic fatty acid.
- No difference between groups in plasma fatty acids was detected after 3 months.
- Weak correlations between diet, plasma fatty acids and lipid profile were detected.

Abstract

Objective: The primary objective of this study was to determine the effects of a healthy diet supplemented with extra virgin olive oil or pecan nuts on plasma fatty acid (PFA) in patients with stable coronary artery disease (CAD).

Research Methods & Procedures: Patients aged 40 to 80 years were randomized to one of three dietary interventions (allocation ratio 1: 1: 1): healthy diet based on guidelines (control group [CG]), healthy diet supplemented with 30 g/day of pecan nuts (PNG), or a healthy diet supplemented with 30 mL/day of extra virgin olive oil (OOG). PFA were identified at baseline and at the end of follow-up (12 weeks), and correlations between dietary fatty acids intake, PFA, and clinical biomarkers of the lipid profile were also assessed before and after the interventions.

Results: Among 149 participants included in the analysis (CG: n = 43; PNG: n = 51; OOG: n = 55), correlations were observed between food intake, PFA, and lipid profile before and after interventions independent of statins used, but all correlations were considered weak. At the end of the study, the OOG showed increased concentrations of oleic fatty acid independently of the type of statin in use (1.49%, 95% confidence interval 0.08 – 2.89; P = 0.029); however, there were no significant differences between the groups regarding the final mean values of oleic fatty acid or in the other PFAs.

Conclusions: In patients with stable CAD, there were no significant differences in PFA after 12 weeks according to dietary interventions evaluated.

Clinical Trials registration: NCT02202265. First registered on July 28, 2014; Last update on September 10, 2020.

Keywords: Coronary Artery Disease; Diet, Fat-Restricted; Nuts; Olive Oil; Fatty Acids.

Introduction

Coronary artery disease (CAD) is the most common clinical presentation of ischemic heart disease and the leading cause of death and disability worldwide [1]. Atherosclerosis is the primary pathophysiological mechanism for the development of CAD, and the presence of dyslipidemia is closely related to the genesis of the disease [2]. Dietary intake of different lipids can positively or negatively modulate the concentrations of different biomarkers related to the plasma lipid profile [3], indirectly contributing to the formation of atherosclerotic plaque.

Plasma fatty acids (PFA) are also related to different lipoprotein patterns and concentrations of other clinical indicators of the lipid profile such as serum triglycerides (TG) [4], and they are altered in individuals with CAD [5]. PFA may reflect the consumption of different foods—sources of dietary lipids—although the literature is controversial about the correlation between PFA and food intake [6, 7], considering that a series of variables, such as age, sex, genetic factors, lifestyle, metabolism, and absorption, can interfere in this relationship [5]. In addition, exposure to certain drugs such as statins, widely used in the treatment of CAD, can interfere with the concentration of PFA [9, 10].

Nuts and extra-virgin olive oil are foods rich in unsaturated fatty acids (UFA), which is characteristic of the Mediterranean diet. This dietary pattern is considered to be cardioprotective [11], but can be difficult to adhere to in places outside the region bordering the Mediterranean Sea [12]. In addition to contributing to the improvement of diet, the composition of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) characteristic of these foods, together with their antioxidants, are related to a better lipid profile, as has been observed in primary cardiovascular prevention [13, 14].

The intake of nuts and olive oil has been associated with an increase in the concentration of certain PFA [15, 16, 17] in primary prevention, enhancing the beneficial

effects of specific PFA on cardiovascular risk factors related to CAD [18]. However, few clinical studies have been conducted on the relationship between the consumption of UFA-rich foods and concentrations of PFA in secondary cardiovascular prevention. Those that have been conducted have shown controversial results [9, 19].

In addition, the identification of potential correlations between lipid profile and PFA that could be mediated by dietary interventions in patients with CAD would contribute to a better understanding of the relationship between diet and risk factors in this specific population. Thus, this study's primary objective was to determine the effects of a healthy diet supplemented with pecan nuts or extra virgin olive oil on PFA concentrations in patients with stable CAD. As a secondary objective, we evaluated the correlations between dietary intake, PFA, and lipid profile of the participants at the beginning and at the end of the study.

Material and methods

This was a sub-analysis of the GENUTRI study, for which the protocol [20] and the primary outcomes have been published [21]. A randomized clinical trial, in parallel and unicentric, with an allocation rate of 1:1:1, and lasting 3 months was performed at a tertiary referral hospital in Cardiology (Porto Alegre, Brazil). The enrolled participants came from the institution's Hemodynamics Service or were recruited from the general population by public calls.

For this sub-analysis, only participants whose plasma was available and properly stored were used. Informed consent was obtained from all participants before inclusion in the study. The protocol was approved by the Research Ethics Committee of the Institute of Cardiology/University Foundation of Cardiology of Rio Grande do Sul (CAAE No. 26591514.8.0000.5333) and is registered in the ClinicalTrials.gov database under the identification number NCT02202265. The study was conducted between August 2014 and

June 2016, in accordance with the guidelines of Good Clinical Practice and the Helsinki Declaration.

Participants

The study included individuals aged between 40 and 80 years with a previous diagnosis of stable CAD for more than 60 days. The exclusion criteria were psychiatric illnesses, extreme obesity (body mass index [BMI] ≥ 40 kg/m²), a life expectancy of less than 6 weeks, pregnancy or lactation, kidney failure on dialysis, use of a wheelchair, hypothyroidism and uncontrolled hyperthyroidism, congestive heart failure, use of food supplements, chronic use of anti-inflammatories and immunosuppressants, and participation in other clinical studies [20].

Randomization

The block randomization sequence was generated with the website www.randomization.com, and was later organized in sealed and opaque envelopes. Only one researcher had access to the list, who did not interact with the participants. Due to the nature of the proposed intervention, this was an open study; however, the outcome assessors were blinded to the allocation [20].

Interventions

Participants were allocated to one of three groups: a healthy diet, prepared according to the recommendations of the nutritional guidelines at the beginning of the study [20, 21] (control group [CG]); a healthy diet supplemented with 30 g/day of pecan nuts (PNG); or a healthy diet supplemented with 30 mL/day of extra virgin olive oil (OOG). The pecan nut was chosen for its greater availability and accessibility in the region of Brazil where the study

was conducted, and the extra virgin olive oil used in the study was produced from olive trees grown in the same region. Thus, it was decided to offer the study participants two locally available foods as dietary supplements. Patients also received information, provided in the form of a table, about the foods to be avoided, eaten in moderation, and consumed daily, in addition to a folder with general guidelines on healthy eating. The CG was instructed not to consume any oilseeds or olive oil during the study period; the PNG was instructed not to ingest olive oil and oilseeds other than those offered in the study, and the OOG was instructed not to consume oilseeds and any other olive oil that was not offered in the study.

The diets were determined individually according to the participant's needs with regard to maintaining or reducing body weight. There was no isocaloric equivalence between the diets specified for the study groups. All participants received the same dietary pattern as the control group and the patients allocated to the PNG and OOG groups were instructed to add supplements (pecan nuts or olive oil, respectively) to their daily diet, without using them as substitutes for other foods.

Examples of the nutritional composition of the diets of the three study groups, and the fatty acid composition of the supplements offered are published [21] and described in the Supplementary Material (Tables S1 and S2).

Data collection and follow-up

In the first evaluation, the participants were administered a questionnaire on their sociodemographic characteristics, their current and previous clinical/surgical history, their use of medications, and lifestyle variables. The medical diagnoses of dyslipidemia, systemic arterial hypertension and type 2 diabetes mellitus were performed according to clinical guidelines and the study protocol [20]. Body weight (kg), height (cm), and waist

circumference (cm) measurements were obtained, and BMI was calculated and described in kg/m^2.

Food consumption was assessed using a 24-hour dietary recall (24HR) method. The interviews were conducted by trained evaluators, and the calculation of nutrients was carried out using tables that depicted the chemical composition of foods and databases of homemade recipes, with the aid of the Avanutri Revolution® software (Rio de Janeiro, Brazil). The total energy intake (TEI) was defined in kilocalories (kcal), and the macronutrients (carbohydrates, proteins, total fats, saturated fatty acids [SFA], MUFA, and PUFA) were defined based on the percentage of the TEI. Dietary fibers were defined in grams (g), and dietary cholesterol was quantified in milligrams (mg).

Patients were required to fast for 12 hours, in order to determine their lipid profiles. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and TG were evaluated using the enzymatic colorimetric method (Roche modular P Chemistry Analyzer [Roche, Basel, Switzerland]), and high-density lipoprotein cholesterol (HDL-c) was evaluated using the immunoprecipitation method (Roche modular P Chemistry Analyzer [Roche, Basel, Switzerland]). Very low-density lipoprotein cholesterol (VLDL-c) was obtained using the TG/5 formula. Non-HDL cholesterol was calculated from the difference between the TC and the HDL-c. The atherogenic index (AI) was determined by the ratio of non-HDL cholesterol and HDL-c.

Participants were visited monthly for 3 months (12 weeks), and laboratory tests were assessed at baseline and at the last visit.

Determination of plasma fatty acids

The concentrations of saturated (stearic, myristic, and palmitic), monounsaturated (oleic and palmitoleic), and polyunsaturated (arachidonic; cis-8,11,18 eicosatrienoic;

docosahexanoic; eicosapentaenoic; linoleic; and linolenic) PFAs were evaluated in samples obtained from the Laboratory of Molecular and Cellular Cardiology of the Institute of Cardiology/University Foundation of Cardiology of Rio Grande do Sul, where they were stored at -80ºC. Prior to storage, blood was obtained after a 12-hour fast, collected in tubes containing ethylene diaminetetraacetic acid (1 mg/mL), and centrifuged to obtain plasma (3000 rpm, for 15 minutes, at 4°C). Plasma analyses of fatty acids were performed at the Laboratory of Food Components and Health of the Department of Nutrition, Faculty of Public Health, University of São Paulo.

For the analysis, 100 uL of plasma were used. The PFA preparation and extraction stage consisted of the addition of 1 ml of methanol/chloroform (2:1 v/v), and later, they were converted into methyl esters through the addition of sodium methoxide [22].

The PFA profile was determined on a Shimadzu GC-2010 gas chromatograph equipped with a DB-FFAP capillary column (15 m × 0.100 mm x 0.10 µm; J&W Scientific, Agilent Technologies, Folsom, CA, USA). Hydrogen was used as a carrier gas with a flow rate of 0.27 mL/min, a linear velocity of 35 cm/s and a pressure of 187.8 kPa. The rates of synthetic, nitrogen, and hydrogen air flow were 300, 30, and 30 mL/min, respectively.

The standard used consisted of a mixture of 37 fatty acid methyl esters (FAME 37, code 47885, Sigma Chemical Co., St. Louis, MO, USA). The injection volume was 2 uL, in an automatic AOC 20i injector. Plasma fatty acids were identified by comparing the retention times of the external standard with the samples. The internal standard methyl tricosanoate (C23: 0, T9900, Sigma Chemical Co.) sodium was used for quality control [22].

The integration of the peaks' values was performed by a researcher blinded to the protocol participants, and was reviewed by two other researchers who were aware of the allocation of patients in relation to the study groups. The results were quantified as an area below the peaks and their values were expressed as a percentage of the sample.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 17.0 for Windows (IBM Corp., Armonk, NY, USA). Continuous variables were described as means and standard deviations, and categorical variables were described in absolute numbers and percentages. Comparisons between continuous variables were made using analysis of variance (for parametric distribution) or Kruskal-Wallis test (for non-parametric distribution). Comparisons between proportions were made using Pearson's Chi-square test (evaluation between groups) and McNemar's test (intragroup evaluation). Repeated measurements regarding nutrient intake were assessed according to the treatment group and using general estimation equations (GEE) with normal or gamma probability distribution depending on the distribution of variables, followed by a Bonferroni post-hoc test. All dietary variables were adjusted for TEI by the residual method [23]. Pearson's correlation tests—adjusted for baseline values and statin use—were used to identify correlations between PFA, dietary lipid intake, and clinical markers of the lipid profile. GEE equations were used to assess PFA according to the treatment group and the time (before and after the intervention), adjusted for baseline PFA values and use of statins. A significance level of 5% was considered.

Results

Between August 2014 and January 2016, 370 individuals were screened for the GENUTRI study [21]. Among the 204 participants initially included in the study [20], 149 had plasma available for the evaluation of PFA. Thus, in this exploratory analysis of GENUTRI study, 43 participants were from the CG, 51 from the PNG, and 55 from the

OOG. Details regarding follow-up losses, side effects, adverse events, and adherence rates in the original study have already been published [21].

Table 1 shows the characteristics of the participants at baseline by randomization group. The prevalence of systemic arterial hypertension was lower among patients allocated to the OOG compared with the other two groups (P = 0.005), and there were no significant differences in the other variables between the groups. At the end of the study, there were no differences between the groups in terms of the number of patients who changed the doses or the type of statins (P = 0.41) or other medications (antihypertensive drugs, hypoglycemic agents, anti-platelet aggregators; P = 0.13). Likewise, there were no significant differences in level of physical activity of the participants at the end of the follow-up compared with the beginning of the study (CG: P = 0.48; PNG: P = 0.36; OOG: P = 0.88) (data not shown).

At the beginning of the study, and after adjustments for the type of statin in use, there were negative correlations between the intake of SFA and stearic PFA (r = -0.265, P = 0.001), and dietary cholesterol and eicosatrienoic PFA (r = -0.202, P = 0.014); there was a positive correlation between PUFA intake and arachidonic PFA (r = 0.242, P = 0.003). With regard to PFA and lipid profile at baseline, independent of the use of statins, the following correlations were observed: a positive correlation between total saturated PFA and LDL-c (r = 0.202, P = 0.016); positive correlations between palmitic PFA and TC (r = 0.218, P = 0.009), LDL-c (r = 0.180, P = 0.031), and non-HDL cholesterol (r = 0.205, P = 0.014); a negative correlation between stearic PFA and TG (r = -0.264, P = 0.001); a positive correlation between total MUFA PFA and TG (r = 0.298, P <0.0001) but negative with LDL-c (r = -0.228, P = 0.006); a positive correlation between palmitoleic PFA and TG (r = 0.222, P = 0.008); a negative correlation between oleic PFA and LDL-c (r = -0.243, P = 0.003), but positive with TG (r = 0.289, P 0.0001); a negative correlation between total PUFA PFA and TG (r = -0.230, P = 0.006); a positive correlation between arachidonic PFA and HDL-c (r =

0.178, P = 0.034), and negative with TG (r = -0.266, P = 0.001) and AI (r = -0.188, P = 0.023). Total dietary lipid intake was positively correlated with TC (r = 0.213, P = 0.011), LDL-c (r = 0.167, P = 0.047), TG (r = 0.254, P = 0.002), non-HDL cholesterol (r = 0.253, P = 0.002), and AI (r = 0.280, P = 0.001), and the intake of SFA correlated positively with TG (r = 0.302, P <0.0001) and AI (r = 0.250, P = 0.003) after adjustments for statin use. Despite statistically significant, all these correlations were considered weak.

Table 2 shows the changes in the participants' dietary profiles during follow-up by study groups. After 3 months, PUFA intake was significantly higher in the PNG compared with the CG and OOG (P = 0.006). MUFA intake in the OOG was higher compared with the CG at the end of the study (P <0.001), whereas the PNG showed no difference when compared with the CG. There were no significant differences in relation to TEI and other nutrients according to the groups at the end of follow-up.

The initial and final means (crude and adjusted) of the PFAs according to the groups are presented in **Table 3**. After adjusting for baseline values of the respective fatty acid and use of statins, an increase in plasma oleic fatty acid concentrations was observed in the OOG at the end of follow-up (1.49%, 95% confidence interval [CI] 0.08 – 2.89; P = 0.029). However, there were no significant differences between groups when comparing final mean values of oleic fatty acid (difference between CG *vs.* PNG: -0.80%, 95% CI -2.81 – 1.22, P = 1.0; CG *vs.* OOG: -1.82%, 95% CI -3.68 – 0.04, P = 0.062; PNG *vs.* OOG: -1.05%, 95% CI -2.98 – 0.93, P = 1.0). There were also no significant differences in the final means of the other PFAs between the study groups at the end of follow-up.

Table 4 presents the adjusted partial correlation between PFA and lipid profile markers according to the study groups. For this analysis, 3 participants from the CG, 3 from the PNG, and 4 from the OOG were excluded because they did not present the final means of at least one of the markers. The correlations observed at the beginning of the study between

palmitic PFA, TC, LDL-c, and non-HDL cholesterol; stearic PFA and TG; and oleic PFA, LDL-c, and TG were attenuated regardless of the allocated intervention, as well as the correlations observed with the total SFA, MUFA, and PUFA PFAs. The positive correlation between palmitoleic PFA and TG remained in the PNG (r = 0. 370, P = 0.01) and in the OOG (r = 0.292, P = 0.039). Arachidonic PFA remained negatively correlated with TG in the PNG (r = -0.335, P = 0.02) and with AI in the OOG (r = -0.355, P = 0.01).

Discussion

In this study, we observed that a healthy diet supplemented with 30 mL/day of extra virgin olive oil increased the plasma concentrations of oleic fatty acid in patients with stable CAD; however, at the end of the protocol, there was no difference when compared with a healthy diet with or without supplementation of 30 g/day of pecan nut. In addition, correlations were observed between dietary lipid intake, clinical markers of the lipid profile, and PFA before and after interventions, independently of the use of statins.

Previous epidemiological studies have suggested that biomarkers of food intake related to endogenously non-synthesized fatty acids are more suitable for assessing consumption [24]. However, in studies in which food intake is controlled, biomarkers of fatty acids could complement dietary assessment, with the potential to be used in a more quantitative manner [24]. In this sense, the evaluation of the oleic PFA concentration is recognized as a good indicator for identifying the consumption of olive oil [16, 25]. In individuals with hypercholesterolemia without using any lipid-lowering medication, ingestion of 35 to 50 mL/day of extra virgin olive oil, for 4 weeks, correlated with increased concentrations of oleic PFA [15]. In patients with CAD (approximately 82% using statins), the recommendation to follow a Mediterranean diet for 1 year did not change plasma

concentrations of oleic fatty acid [9]. However, unlike the GENUTRI study, in that clinical trial, olive oil was not offered directly to participants.

Although the OOG showed an intragroup difference in relation to the percentage of oleic PFA, there was no difference at the end of the study when compared with a healthy diet with or without supplementation of 30 g/day of pecan nuts. In adults with obesity, the intake of ~ 43 g/day of pecan nuts, for 4 weeks, did not change the concentrations of oleic fatty acid in the plasma membrane of erythrocytes in the intragroup analysis, but it increased significantly compared with the control diet in the comparison between groups [17]. In healthy individuals, the intake of approximately 70 g/day of pecans was associated with an increase in the content of oleic fatty acid in plasma TG [26]. Pecan nuts are rich in MUFA; however, unlike other nuts for which the evaluation of certain biomarkers is already established for the evaluation of consumption [25], it is not known whether oleic fatty acid is the best parameter considering that pecans are rich in a number of other phytochemicals [27]. The amount of nuts offered in our study may not have been sufficient to identify an intragroup difference with respect to plasma concentrations. On the other hand, the MUFA concentration of the foods offered was very similar, which may explain why no difference was identified between the groups at the end of the study.

Statins have an influence on the PFA profile [10]. Simvastatin can directly or indirectly stimulate the activity of delta-5 desaturase, delta-6 desaturase, and elongases, improving the conversion of linoleic fatty acid into arachidonic fatty acid [28, 29] (increasing the conversion rate from 40% to 55% in human leukemic monocytic cells [THP-1]) [28] and the ratios between stearic/palmitic acid, gamma-linolenic/linoleic, and arachidonic/di-homo-gamma-linolenic [30]. On the other hand, high doses of potent statins such as atorvastatin, rosuvastatin, and pitavastatin can reduce plasma concentrations of eicosapentaenoic and docosahexaenoic acids, and this reduction has been found to be positively correlated with the

reduction of LDL-c in patients with CAD [31]. The use of 40 mg/day of simvastatin for 24 weeks was associated with decreased concentrations of alpha-linolenic fatty acid, but with an increase in its plasma metabolic product docosahexaenoic acid, and did not change the plasma concentrations of eicosapentaenoic acid [32].

Regardless of the use of statins, we observed significant correlations between food intake, PFA, and lipid profile before the interventions. However, they were considered weak and very weak. In addition to the complex relationships between the use of different statins, availability of dietary substrates (lipids), and possible changes in conversion rates, desaturation and stretching, other issues such as the use of other medications [33], being overweight [33], smoking [34], alcohol intake [35], TEI from carbohydrates in the diet [36], the interaction between dietary lipids and PFA with other dietary components, factors inherent to the individual [8], and endogenous fatty acid synthesis [24] can alter the concentration of PFA, influencing results from epidemiological and clinical studies. Different methods of assessing food consumption may also reflect differences in estimates of dietary intake between studies, reflecting in the results as well.

Statins interact with the diet in modulating clinical markers of the lipid profile [37] and PFA in primary cardiovascular prevention [30], being more effective in the presence of diets with low amounts of dietary cholesterol and SFA and rich in PUFA and dietary antioxidants. Furthermore, in animal models, the activity of the delta 9-desaturase enzyme decreased continuously after the reduction of dietary cholesterol, while the activities of the delta 5- and delta 6-desaturase enzymes increased after 21 days [38]. With the exception of MUFA concentrations, the diets prescribed to the participants in the GENUTRI study were quite similar, especially with regard to low concentration of dietary cholesterol. However, the correlations that remained significant at the end of the study, after the three interventions, were also considered weak, and given that there were no differences between the groups in

the final mean values of PFA, they do not seem to be explained only by supplementation or not with pecan nuts or olive oil. In addition, the same fatty acids can have complex and different biological and metabolic effects on the lipid profile [39]. Furthermore, weight loss interventions with [40] or without [41] UFA supplementation can modulate PFA concentrations, desaturases activity, and lipid profile in overweight individuals; in the GENUTRI study, participants decreased body weight and waist circumference regardless of the allocated intervention [21].

This study had several limitations. As this was an exploratory analysis, there might have been a lack of sampling power to identify any possible effects of the interventions on PFA concentrations. Phenolic compounds and other bioactive substances were not evaluated in the foods offered in the study, making it difficult to make inferences regarding the role of these molecules on the concentration of PFA. Stricter strategies to encourage adherence to dietary interventions were not adopted, with the exception of telephone calls for appointment reminders. The assessment of food consumption was performed using the 24HR method, which might not have provided a reliable estimate of dietary intake due to daily variation and the reliance on the participant's memory. Also, the follow-up time and the amount of food offered might not have been sufficient to identify major differences between the groups. And finally, maybe there were no differences between groups after intervention because control group was a healthy diet and nuts/olive oil may not improve correct nutrition. As for the strengths of this study, in addition to the pragmatic approach that reflected “real life,” we consider the evaluated population to be a strength of the study because, because despite a series of potential interactions between the use of statins (and other drugs), the clinical profile of the participants, and the concentrations of PFA, few studies have been conducted on secondary cardiovascular prevention using extra virgin olive oil or nuts as an intervention.

Conclusions:

In patients with stable CAD, there were no significant differences in PFA after 12 weeks according to a healthy diet supplemented or not with 30 mL/day of extra virgin olive oil or 30g/day of pecan nuts.

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Credit Authors Statement

Aline Ramos de Araújo: data collection; data interpretation; writing - original draft. **Geni Rodrigues Sampaio:** data interpretation; formal analysis; writing - review & editing**. Lucas Ribeiro da Silva:** data collection; writing - review & editing. **Vera Lúcia Portal:** funding acquisition; writing - review & editing. **Melissa Medeiros Markoski:** writing - review & editing. **Alexandre Schaan de Quadros:** writing - review & editing. **Marcelo Macedo Rogero:** data interpretation; writing - review & editing. **Elizabeth Aparecida Ferraz da Silva Torres:** writing - review & editing. **Aline Marcadenti:** conceptualization; data interpretation; formal analysis; methodology; funding acquisition; project administration; supervision; writing - original draft.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Table 1. Baseline characteristics of participants according to study group (mean ± SD, *n* [%]).

	CG (*n* = 43)	**PNG (*n* = 51)**	**OOG (*n* = 55)**
Age (years)	60.7 ±8.8	60.3 ±8.1	57.4 ±6.3
Male sex	29 (67.4)	42 (82.4)	44 (80)
School education (years)	8.9 ±3	9.9 ±3.1	9.9 ±2.7
White ethnicity	40 (93)	49 (96.1)	52 (94.5)
Current or previous smoking	31 (72.1)	28 (54.9)	37 (67.3)
Alcohol abuse	1 (2.3)	4 (7.8)	2 (3.6)
Physical activity level			
Active	16 (37.2)	9 (17.6)	18 (32.7)
Irregularly active	19 (44.2)	23 (45.1)	22 (40)
Sedentary	8 (18.6)	19 (37.3)	15 (27.3)
Body mass (kg)	78.3 ±18.2	83.9 ±16	82.4 ±14.6
BMI (kg/m²)	29 ± 4.4	29.6 ± 5.3	29.3 ±4.1
Waist circumference (cm)	98.7 ±13.6	100.4 ±12.1	100 ±10.7
Dyslipidemia	26 (60.5)	31 (60.8)	30 (54.5)
Diabetes Mellitus	15 (34.9)	16 (31.4)	13 (23.6)
Hypertension	27 (62.8)	38 (74.5)	24 (43.6)
Family history of premature CAD	21 (48.8)	22 (43.1)	23 (41.8)
Previous AMI	41 (95.3)	46 (90.2)	51 (92.7)
Previous PCI	34 (79.1)	45 (88.2)	49 (89.1)
Previous CABG	6 (14)	9 (17.6)	7 (12.7)
Statins (*n* = 136)			
Simvastatin	31 (77.5)	28 (62.2)	38 (74.5)
Rosuvastatin	6 (15)	9 (15.6)	7 (13.7)
Atorvastatin	3 (7.5)	10 (22.2)	6 (11.8)
Total cholesterol (mg/dL)	169.1 ±34.5	163.3 ±33.7	174.8 ±45.2

LDL-cholesterol (mg/dL)	93.6 ±33	88.2 ±24.8	100.4 ±41.7
HDL-cholesterol (mg/dL)	50.1 ±15.8	44.5 ±11.4	45.3 ±10.3
VLDL-cholesterol (mg/dL)	27.2 ±13.8	30.6 ±14.9	31.1 ±17.9
Triglycerides (mg/dL)	136 ±69.1	153.1 ± 74.7	155.6 ± 89.7
Non-HDL-cholesterol (mg/dL)	119.1 ±34	118.8 ±31.5	129.3 ±45.7
Atherogenic index	2.6 ±0.9	2.8 ±1	3 ±1.3

CG: control group; PNG: pecan nut group; OOG: olive oil group; BMI: body mass index; HTN: hypertension; CAD: coronary artery disease; AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CABG: Coronary Artery Bypass Grafting; LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very low-density lipoprotein.
Adapted from Campos VP et al. J Hum Nutr Diet. 2020;33(3):439-450.

Supplementary Material

Table S1. Example of the nutritional composition of a diet prescribed to the study groups.

	CG	PNG	OOG
Total energy, in kcal	2000	2198	2238
Carbohydrate, in % TE	53.91	50.20	48.18
Protein, in % TE	20.73	19.27	18.52
Total fat, in % TE	25.36	30.54	33.30
Saturated fatty acids, in % TE	7.03	7.56	8.44
Monounsaturated fatty acids, in % TE	12.63	17.51	18.68
Polyunsaturated fatty acids, in % TE	5.54	5.57	6.01
Dietary cholesterol (mg)	183	183	183
Dietary fiber (g)	32.3	34.4	32.3

Adapted from Campos VP et al. J Hum Nutr Diet. 2020;33(3):439-450.

CG: control group; PNG: pecan nut group; OOG: olive oil group.

% TE: percentage of total energy.

Table S2. Fatty acid profile (% [g/100 g]) of the pecan nuts and extra virgin olive oil used in the GENUTRI study.

Fatty acid		Pecan nuts	Extra-virgin olive oil
Butyric acid	C4:0	ND	ND
Caproic acid	C6:0	0.10	ND
Caprylic acid	C8:0	0.19	ND
Capric acid	C10:0	ND	ND
Undecanoic acid	C11:0	ND	ND
Lauric acid	C12:0	ND	ND
Tridecanoic acid	C13:0	ND	ND
Myristic acid	C14:0	0.03	0.01
Myristoleic acid	C14:1	ND	ND
Pentadecanoic acid	C15:0	0.01	0.01
10-Pentadecanoic acid	C15:1	ND	ND
Palmitic acid	C16:0	4.90	15.59
Palmitoleic acid	C16:1	0.04	1.93
Margaric acid	C17:0	0.04	0.09
Cis-10-heptadecenoic acid	C17:1	0.05	0.24
Stearic acid	C18:0	2.05	1.45
Elaidic acid	C18:1	ND	ND
Oleic acid	C18:1	48.95	59.98
Linolelaidic acid	C18:2	ND	ND
Linoleic acid	C18:2	3.95	9.36
Arachidic acid	C20:0	0.08	0.30
Gamma linolenic acid	C18:3	ND	0.01
Cis-11-eicosenoic acid	C20:1	ND	0.23
Linolenic acid	C18:3	0.21	0.59
Heneicosanoic acid	C21:0	ND	ND
Cis-11,14-eicosadienoic acid	C20:2	0.32	ND
Behenic acid	C22:0	0.02	0.09
Cis-8,11,14-eicosatrienoic acid	C20:3	ND	ND
Erucic acid	C22:1	ND	ND

Cis-11,14,17-eicosatrienoic acid	C20:3	ND	ND
Arachidonic acid	C20:4	ND	ND
Tricosanoic acid	C23:0	ND	0.32
Cis-13,16-docosadienoic acid	C22:2	ND	ND
Lignoceric acid	C24:0	ND	ND
Cis-5,8,11,14,17-eicosapentaenoic acid (EPA)	C20:5	0.16	0.05
Nervonic acid	C24:1	ND	ND
Cis-docosahexaenoic acid (DHA)	C22:6	ND	ND

Adapted from Campos VP et al. J Hum Nutr Diet. 2020;33(3):439-450.

ND: not detected; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 2. Dietary profile of the participants according to follow-up time and study group (mean ± SD).

	CG (*n* = 43)				PNG (*n* = 51)				OOG (*n* = 55)			
	baseline	**4 weeks**	**8 weeks**	**12 weeks**	**baseline**	**4 weeks**	**8 weeks**	**12 weeks**	**baseline**	**4 weeks**	**8 weeks**	**12 weeks**
TEV (Kcal)	1711 ±691	1483 ±719	1424 ±543	1544 ±763	1784 ±898	1597 ±617	1637 ±472	1661 ±637	2048 ±1405	1644 ±534	1747 ±597	1660 ±566
Carbohydrates (% TE)	49.2 ±10.3	49.3 ±9.8	49.5 ±9.8	49 ±15.6	49.2 ±10.4	48 ±10.8	51.6 ±19.9	51.1 ±27	52.2 ±14.3	48.3 ±9.8	46.7 ±10.6	46.7 ±10.8
Proteins (% TE)	20.5 ±6.3	21.3 ±6.1	19.5 ±5.1	20.5 ±6	19.2 ±4.9	19.2 ±6.1	18.3 ±5.3	18.1 ±5.4	17.4 ±7.2	17.5 ±4.7	18 ±6	16.6 ±5.5
Total fats (% TE)	30.6 ±7.3	29 ±8.4	31.2 ±8.1	30.6 ±12	32.3 ±9.8	34.1 ±9.1	34.8 ±7.7	35.8 ±8.6	31 ±11	34.2 ±8.8	35.4 ±10.5	36.7 ±10
SFA (% TE)	9.2 ±3.6	9.3 ±4.3	8.8 ±3.8	9.7 ±5.3	8.3 ±3.6	9.1 ±5.2	7.6 ±3.2	8.8 ±4.8	8.2 ±4.6	8.6 ±3.3	8.3 ±3.3	8.8 ±3.8
PUFA (% TE)[¶]	4.4 ±2.4	3.7 ±2.6	4.6 ±2.9	**3.9 ±2.5**[a]	4.3 ±3	6.8 ±3.5	6.7 ±2.9	**6.6 ±3.3**[b]	4.1 ±2.8	4.1 ±2.3	3.8 ±2	**4.5 ±2.5**[a]
MUFA (% TE)*	9.2 ±6.8	7.6 ±4.6	7.6 ±3.3	**8 ±5.5**[a]	8.4 ±4.4	11.3 ±5	10.8 ±3.8	**11.6 ±5.1**[a,b]	8.4 ±4.4	13 ±6.6	14 ±7.6	**14.3 ±6.9**[b]
Cholesterol (mg)	203 ±108	197 ±100	183 ±73	241 ±192	182 ±95	185 ±95	183 ±131	178 ±125	157 ±95	177 ±135	196 ±136	182 ±90
Dietary fiber (g)	12.1 ±4.3	13 ±6.3	11.9 ±7.7	12 ±8.6	12.6 ±5.8	14.6 ±6.9	13.8 ±5.8	14.6 ±7.8	14.4 ±9.8	13.7 ±6.3	13.2 ±7	14.6 ±7.8

a, b: different letters indicate differences between-groups at the end of the study (P= 0.006; *P <0.001). Generalized estimating equation (GEE) with normal probability distribution (symmetric variables) and gamma probability distribution (asymmetric variables), followed by Bonferroni test and adjusted for TEV using the residual method. CG: control group; PNG: pecan nut group; OOG: olive oil group; TEV: total energy value; % TE: percentage of total energy; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids.

P-values regarding differences between-groups at the end of the study: TEV: P= 0.67; Carbohydrates: P= 0.30; Proteins: P= 0.54; Total fats: P= 0.38; SFA: P= 0.94; Cholesterol: P= 0.22; Dietary fiber: P= 0.66.

Adapted from Campos VP et al. J Hum Nutr Diet. 2020;33(3):439-450.

Table 3. Initial and final means (crude and adjusted) of plasma fatty acids profile (in %) after 12 weeks of follow-up according to study group.

	CG (*n* = 43)			PNG (*n* = 51)			OOG (*n* = 55)		
	Baseline	**12-weeks***	**Adjusted 12-weeks****	**Baseline**	**12-weeks***	**Adjusted 12-weeks****	**Baseline**	**12-weeks***	**Adjusted 12-weeks****
Estearic (C18:0)	14.84 ±2.59	14.97 ±2.34	14.74 ±1.54	14.60 ±2.65	14.62 ±2.85	14.62 ± 2.68	14.57 ±2.80	14.46 ±2.20	14.49 ±1.66
Miristic (C14:0)	1.19 ±0.97	1.17 ±0.85	1.06 ±0.62	1.23 ±0.77	1.13 ±0.89	1.05 ±0.69	1.32 ±1.04	1.16 ±0.94	0.95 ±0.48
Palmitic (C16:0)	29.21 ±2.16	29.59 ±2.09	29.27 ±1.91	28.53 ±2.68	28.66 ±2.36	28.92 ± 2.04	29.04 ±2.34	28.72 ±2.23	28.53 ±1.88
Total SFA	45.23 ±3.35	45.73 ±2.74	45.30 ±2.27	44.36 ±3.61	44.41 ±3.82	44.74 ±3.79	44.92 ±3.36	44.33 ±3.50	44.13 ±2.61
Oleic (C18:1 n9c)‡	17.76 ±4.28	16.99 ±4.01	16.65 ± 3.04	18.49 ±4.11	18.23 ±4.45	17.45 ± 3.73	**17.08 ±4.16**[a]	18.26 ±4.05	**18.47 ± 3.39**[b]
Palmitoleic (C16:1)	1.09 ±0.83	1.03 ±0.83	1.03 ±0.85	1.25 ±1.05	1.07 ±0.89	0.99 ±0.99	1.09 ±0.86	0.89 ±0.65	0.88 ±0.65
Total MUFA	18.85 ±4.68	18.02 ±4.54	17.68 ±3.42	19.75 ±4.76	19.30 ±4.95	18.42 ±4.10	18.17 ±4.74	19.15 ±4.19	19.43 ±3.69
Arachidonic (C20:4 n6)	11.39 ±3.37	11.32 ±2.91	11.21 ± 2.50	11.26 ±3.15	11.38 ±3.04	11.35 ± 2.62	10.97 ±2.80	10.44 ±2.22	10.67 ±1.79
cis-8,11,18 Eicosatrienoic (C20:3 n3)	2.88 ±0.78	2.97 ±0.74	2.96 ±0.78	2.77 ±0.96	2.75 ±0.89	2.76 ±0.65	2.88 ±0.84	2.92 ±0.87	2.86 ±0.62
Docosahexaenoic (C22:6 n3)	2.08 ±0.54	2.15 ±0.70	2.18 ±0.62	2.23 ±0.77	2.14 ±0.68	2.06 ±0.58	2.15 ±0.86	2.08 ±0.70	2.06 ±0.56
Eicosapentaenoic (C20:5 n3)	0.74 ±0.44	0.73 ±0.29	0.73 ±0.28	0.78 ±0.87	0.67 ±0.32	0.66 ±0.32	0.62 ±0.31	0.65 ±0.34	0.68 ±0.26
Linoleic (C18:2 n6c)	18.46 ±2.79	18.79 ±2.41	19.27 ± 2.61	18.58 ±3.86	19.04 ±3.17	19.29 ± 2.78	19.88 ±3.13	20.08 ±3.07	19.25 ± 2.07
Linolenic (C18:3 n3)	0.37 ±0.33	0.30 ±0.23	0.36 ± 0.37	0.28 ±0.21	0.32 ±0.26	0.44 ±0.43	0.36 ±0.24	0.37 ±0.26	0.39 ±0.27
Total PUFA	35.92 ±4.47	36.25 ±3.71	36.47 ±4.10	35.90 ±4.71	36.29 ±4.20	36.29 ±4.44	36.87 ±4.03	36.53 ±2.89	36.68 ±3.67

* Crude means after 12 weeks of intervention. **Means after 12 weeks adjusted for baseline data and type of statin in use.
‡a,b: different letters indicate a difference between moments intra-group after 12 weeks (*P= 0.029*).
Generalized estimating equation (GEE) with gamma probability distribution, followed by Bonferroni test. CG: control group; PNG: pecan nut group; OOG: olive oil group; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
P-values regarding differences in plasma fatty acids between-groups at the end of the study: Miristic: P=0.44; Palmitic: P=0.19; Palmitoleic: P=0.52; Estearic: P=0.91; Oleic: *P=0.013*[§]; Linoleic: P=0.64; Linolenic: P=0.56; cis-8,11,18 Eicosatrienoic: P=0.77; Arachidonic: P=0.36; Eicosapentaenoic: P=0.76; Docosahexaenoic: P=0.77; SFA: P= 0.10; MUFA: *P= 0.033*[§]; PUFA: P= 0.58.
[§]P for interaction significant due to differences in OOG.

Table 4. Adjusted partial correlation[1] (r) between plasma fatty acids and lipid profile markers at the end of the study according to study groups.

	CG (*n* = 40)						PNG (*n* = 48)						OOG (*n* = 51)					
	TC	**LDL-c**	**HDL-c**	**TG**	**NHDL**	**AI**	**TC**	**LDL-c**	**HDL-c**	**TG**	**NHDL**	**AI**	**TC**	**LDL-c**	**HDL-c**	**TG**	**NHDL**	**AI**
stearic (C18:0)	-0.249	-0.206	-0.006	0.236	-0.299	-0.256	-0.055	0.036	0.029	-0.187	-0.061	-0.092	0.264	0.283*	0.118	-0085	0.242	0.162
Iiristic (C14:0)	0.186	0.121	0.116	0.134	0.176	0.116	-0.60	-0.065	0.102	-0.085	-0.087	-0.153	-0.095	-0.251	-0.068	0.238	-0.095	0.066
almitic (C16:0)	0.034	-0.025	0.171	0.317	-0.053	-0.099	0.166	0.140	0.031	0.224	0.188	0.219	0.252	0.213	0.131	0.060	0.247	0.270
otal SFA	-0.079	-0.109	0.180	0.186	-0.167	-0.195	0.051	0.096	0.063	-0.018	0.053	0.029	0.275*	0.238	0.122	0.035	0.263	0.271
leic (C18:1 n9c)	0.077	0.041	-0.079	0.059	0.113	0.138	-0.101	-0.186	-0.120	0.133	-0.059	-0.039	-0.185	-0.230	-0.136	0.180	-0.141	-0.078
almitoleic C16:1)	0.113	0.065	-0.015	0.276	0.109	0.065	0.123	-0.015	-0.021	0.370*	0.153	0.203	0.124	-0.044	0.322*	0.292*	0.074	0.052
otal MUFA	0.092	0.048	-0.070	0.102	0.123	0.136	-0.069	-0.171	-0.118	0.191	-0.025	0.003	-0.162	-0.218	-0.082	0.200	-0.129	-0.077
rachidonic C20:4 n6)	-0.320*	-0.240	-0.202	-0.114	-0.276	-0.212	-0.161	-0.113	0.116	-0.335*	-0.215	-0.208	-0.215	-0.215	0.245	-0.186	-0.276	-0.355*
s-8,11,18 icosatrienoic C20:5 n3)	0.374*	0.426‡	-0.027	0.249	0.347*	0.314	0.154	0.158	0.296*	-0.034	0.086	-0.023	-0.019	-0.093	0.023	0.062	-0.043	-0.050
ocosahexaenoic C22:6 n3)	-0.051	0.051	-0.238	0.065	-0.015	0.053	-0.162	-0.232	-0.009	-0.144	-0.181	-0.154	-0.138	-0.093	0.165	-0.173	-0.158	-0.231
icosapentaenoic C20:5 n3)	-0.020	0.036	-0.008	0.044	-0.049	-0.067	0.279	0.072	0.332*	0.179	0.202	0.078	-0.236	-0.204	0.232	-0.066	0.276	-0.280*
inoleic (C18:2 6c)	0.144	0.117	0.159	-0.317*	0.132	0.064	0.206	0.335*	-0.124	0.067	0.211	0.248	0.147	0.310*	-0.322*	-0.133	0.209	0.217

inolenic (C18:3 3)	-0.071	-0.040	0.084	-0.222	-0.097	-0.040	0.177	0.195	-0.229	0.244	0.242	0.221	-0.076	-0.036	0.051	-0.037	-0.103	-0.071
otal PUFA	-0.073	-0.033	0.044	-0.271	-0.073	-0.107	0.012	0.098	0.057	-0.216	-0.034	-0.039	-0.110	-0.059	-0.042	-0.195	-0.126	-0.172

TC: total cholesterol; LDL-c: low density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol; TG: triglycerides; NHDL: Non-HDL-cholesterol; AI: atherogenic index; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[1] Adjusted for baseline values of both respective clinical lipid marker and plasma fatty acid, and for statin use. *P <0.05; ‡P <0.01