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Omega-3 Levels and Nicotine Dependence: A Cross-Sectional Study and Clinical Trial

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Key Words
Clinical trial · Cross-sectional study · Smoking · Tobacco · Omega-3 · EPA · Docosahexaenoic acid

Abstract
Background: High oxidative stress, which is caused by smoking, can alter omega-3 fatty acid concentrations. Since omega-3 fatty acids play a role in dopaminergic neurotransmission related to dependence, it is important to understand their effects on nicotine dependence. ***Methods:*** This research comprised 2 studies. The first one consisted of a cross-sectional evaluation, in which the levels of the most important omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were compared between smokers and non-smokers in a sample of 171 individuals; of them, 120 were smokers and 51 were non-smokers. The other study was a clinical, double-blind, randomized, placebo controlled, in which 63 smokers received daily treatment with capsules of fish oil (a source of omega-3/3 g/day) or mineral oil (used as placebo, also 3 g/day), taken 3 times a day for 90 days. Each fish oil capsules contained approximately 210.99 mg EPA and 129.84 mg of DHA. The outcome was evaluated by means of psychometric and biological measures as well as self-reports of tobacco use. The evaluations were carried out at the beginning of treatment and once a month thereafter (total of 4 times). ***Outcomes:*** The omega-3 fatty acid lipid profile showed that smokers present lower concentrations of DHA. After treatment, the omega-3 group showed a significant reduction in their levels of dependence. ***Interpretation:*** Smokers showed lower peripheral levels of omega-3, and treatment with the most important omega-3 fatty acids brought about a reduction in nicotine dependence.

Introduction

Many pathologies associated with smoking involve the deleterious effects of free radicals [1]. Polyunsaturated fatty acids (PUFAs) are highly susceptible to the action of free radicals and present dramatic changes in concentration in situations of high oxidative stress [2–7].

Smokers present increased concentrations of lipid peroxidation markers [2, 4, 5]; however, a few studies have evaluated the effects of smoking on the concentrations of omega-3 fatty acids [7, 8]. Simon et al. [8] determined that smokers have lower concentrations of docosahexaenoic acid (DHA) and other PUFAs than non-smokers. In contrast, Pawlosky et al. [7] observed higher plasma PUFA concentrations in smokers than in non-smokers.

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The current treatments available for smoking cessation are nicotine replacement therapy, bupropion and varenicline [9, 10]. Even though they display effectiveness, they also present several side effects, which have led to the search for new treatment approaches [10].

In the nervous tissue, the omega-3 PUFAs participate in the constitution of the neurons cell membranes, influencing directly and indirectly its function [11–14].

Studies with animal models have shown that omega-3 fatty acids deficiency results in structural changes in the nervous tissue, mainly at dopaminergic and serotonergic systems, due to the reduction in the number of neurotransmitter vesicles [12, 15, 16]. Using the same animal model described in the studies mentioned earlier, Chalon [17] showed that after restoration of omega-3 fatty acids in diet, the changes are reversed.

The deficiency of essential fatty acids, omega-3 or 6 subtype, have been associated with impulsivity states and compulsive behaviors [18, 19], affecting the healthiness of the neural systems, especially serotoninergic ones [12, 20].

The increase of omega-3 fatty acids in diet increases the central serotoninergic activity in the prefrontal cortex, thus reducing aggression and impulsivity [12, 20, 21].

As the omega-3 fatty acids deficiency affects dopaminergic and serotoninergic neurotransmission in several systems, including the mesolimbic and mesocortical pathways and since the dopamine release may be compromised, it is possible that the oral omega-3 fatty acid administration increases its bioavailability. Therefore, the concentration may increase in the central nervous system, thus favoring the balance between the involved structures, thereby decreasing the nicotine dependency.

In a recent study conducted by Rabinovitz [22], 48 regular smokers were treated in a double-blind, placebo controlled, clinical trial, with omega-3 fatty acids or placebo during a month. The participants were advised to take 5 capsules a day. The placebo capsules contained a mixture of mineral and soybean oil, and the PUFAs capsule contained eicosapentaenoic acid (EPA; 542 mg) and DHA (408 mg). The subjects were analyzed at the end of a 30-day period, and at the follow-up (30 days after). The results showed that the subjects treated with the PUFAs capsules presented significant lower tobacco craving when compared with the baseline assessment, and with the follow-up. On the other hand, the placebo did not show a significant reduction in the craving after the intervention and at the follow-up. The study also shows that the smokers treated with omega-3 fatty acids significantly reduced the number of cigarettes per day after the 1 month of treatment [22].

Lower levels of omega-3 fatty acids are related to the dysfunction of the dopaminergic system, including other neurotransmitter systems [15–17]; also, the evidence of compounds present in the cigarette smoke interfere in the lipid profile of PUFA fatty acids [7, 8]. The hypothesis of this study was that smokers have altered concentrations of omega-3 fatty acids, which could result in hypofunctioning of the systems and structures related to dependence. Thus, the objective of this study was to evaluate a possible difference between the levels of the most important omega-3 fatty acids in smokers and non-smokers, and therefore evaluate the effects of daily dietary supplementation with omega-3 fatty acids (fish oil) on nicotine dependence.

Materials and Methods

Experimental Procedures

The research was approved by the Ethics Committee of the Universidade Federal de São Paulo (CAAE: #03850412.2.0000.55.5) and registered in Clinical Trials (#NTC01735279). Data were collected in São Paulo/Brazil between June 2012 and August 2013. All participants signed an informed consent prior to the beginning of the study. This study consisted of 2 experiments: a cross-sectional evaluation and a clinical trial.

Awareness about the recruitment process was created through posters in the University environment and at the Medical Hospital (Hospital São Paulo – SP, Brazil) linked to the University and through small and large newspapers of São Paulo. This specific part of the disclosure was done by the University Press Office. All the posters contained basic information about each study. The participants did not receive any kind of compensation for participation in the study. The only benefit that they received was that they received the results of the tests performed during the study.

Cross-Sectional Study

The cross-sectional study compared a sample of smokers with a sample of non-smokers. The inclusion criteria were healthy individuals between 20 and 60 years old and, in the case of smokers, those who had been smoking an average of 20 cigarettes daily for more than 2 years. The eligibility criterion for non-smokers was those individuals who have never smoked (never-smoker) and the criterion for ex-smokers was those who had given up smoking and had not smoked in the last 10 years. These criteria were checked by posing a direct question to the participants at the screening visit. When the subjects state that he or she was an ex-smoker, a test using the monoximeter (PiCO+ Bedfont Technical Instruments Ltd., Sttingbourne, Kent, UK) was performed to confirm the information. The use of any food supplementation in the last 4 months prior the beginning of study was considered exclusion criteria.

The parameters evaluated were gender, age, height, weight, body mass index (BMI), anxiety and depression symptoms (Beck Anxiety and Depression Inventory – BAI, BDI, respectively), the ingestion of fatty acids through food (Food Log) and concentrations of the most important omega-3 fatty acids, DHA and EPA.

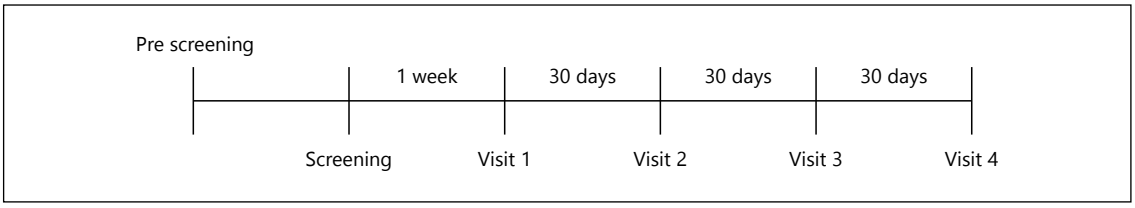

Fig. 1. Flowchart of the clinical trial. The study comprised five sessions (screening and 4 follow-up visits). The pre-screening was based on information given by the participants during a telephone interview; the questions were about smoking habits (brand and cigarettes per day), pre-existence of illness, and the medications in use. During the screening visit, all the procedures were explained and questionnaires were applied to check out the eligible criteria. The intervention totalized 4 visits, separated for 30 days each.

The study comprised 2 appointments: at the first visit, the procedures were explained and the subjects were instructed about the questionnaires used; at the second visit, peripheral blood was collected to measure the omega-3 fatty acids concentrations. These procedures are described in detail in Questionnaires and Biomarkers.

The statistical analysis consisted of an ANCOVA (univariate analysis of covariance) for assessing differences between smokers and non-smokers with regard to omega-3 fatty acid concentrations. We controlled for the following co-variables: level of anxiety and depression, BMI, age and amount (in grams) of PUFAs ingested through food. Height and weight were used to calculate the BMI. Post-hoc Bonferroni tests were used when necessary. The level of significance adopted was $p < 0.05$. STATISTICA version 7 software was used for all analyses.

Clinical Trial

The clinical intervention was a double-blind, placebo-controlled, randomized study that involved 63 volunteers. The inclusion criteria were healthy smokers of both genders, aged between 20 and 60; level of dependence above low (Fagerström test for nicotine dependence – FTND ≥5 points); anxiety below moderate (BAI <16); depression below mild (BDI <19); average motivation to quit smoking (Richmond test – RT >6 points) and schooling higher than the 5th grade of primary sSchool. All the subjects who participated in the omega-3 fatty acid intervention had been regular smokers for more than 2 years, and the average number of cigarettes smoked per day was 20. The exclusion criteria were psychiatric disorders, abusive use of alcohol or other substances, use of any medication and/or clinical condition that interacted with the intervention, and use of any food supplementation for the past 4 months.

The participants were not asked to try quit smoking during the intervention. The subjects were instructed that they could smoke prior to the appointments, but they were refrained from smoking for 30 min, under the supervision of the experimenter, while the assessments were performed.

Subjects were assigned to 2 groups that received either capsules of mineral oil (placebo) or fish oil (source of omega-3). The capsules were supplied monthly, which enabled us to evaluate the volunteers' evolution and the side effects.

We chose fish oil as a source of omega-3 because there is plenty of EPA and DHA in that substance [23]. As for the dose, we based it on the studies of Rusca et al. [24], which demonstrated the incorporation of those compounds into cell lysis with the daily supplementation of 3 g. Additionally, according to the FDA, doses up to 3 g of omega-3 (marine origin) daily are considered effective [25].

The capsules used in the clinical trial were fish oil 1,000 mg taken as 3 daily doses and placebo capsules 1,000 mg – mineral oil + food dye #2 (simulating the colour of essential fatty acids), also in 3 daily doses. The capsules from fish and mineral oil were identical to look at and were given in identical bottles.

Fish and mineral oil capsules of the study were purchased from Relthy Laboratories Company, Brazil. Each fish oil capsule contained 210.99 mg of EPA and 129.84 mg of DHA. The participants were instructed to ingest the capsules during the meals.

The compliance of the intervention was analysed by (1) control form – the subjects were asked to fill a form after every capsule intake, and they were asked to return these form to the investigators very month; and (2) by the number of forgotten capsules – the subjects were asked to return the dispenser box every month so the investigators could verify the number of left capsules.

The assignment of volunteers into the 2 groups was conducted in a manner so that the number in each group was equal. Randomization was performed by a person not linked to the project and consisted of a random draw between the 2 possible study treatments. All the procedures of allocation and assignment of the participants were double-blinded and they remained blind until the end of the data collection.

The study comprised 5 sessions (screening and 4 follow-up visits). See figure 1 for more detailed information. The psychometric questionnaires used were BAI and BDI; FTND; RT and questionnaire of smoking urges (QSU). Those procedures were applied every visit. We also used a cigarette diary (self-report of consumption) and a Food Log (because omega-3 fatty acids are only obtained through food). Those last questionnaires were obtained from the participants at the visits number 2, 3 and 4. The biological markers used were plasma omega-3 fatty acids [26], serum cotinine [27]; the biological samples were collected at the first and last visits.

In order to calculate the sample size, we used the software G*Power 3.1.3, estimating the sample value through the use of an analysis of variance (ANOVA) for repeated measures (2 × 4). The minimum size effect was 25%, beta error minimum was 80%, and the level of significance (alpha error) was 5%. Therefore, we estimated the sample size at a minimum number of 35 volunteers.

The treatment effect was evaluated by an ANOVA for repeated measures (2 × 4). The dependent variables were the psychometric measures (level of dependence and craving), and the biological ones (concentration of serum cotinine and plasma levels of EPA

and DHA). We utilized the covariants levels of anxiety, depression and motivation; age; smoking diary, and daily ingestion of PUFAs through food.

We utilized the Intent to Treat (ITT) Analyses Using the Last Observation Carried Forward Method (ITTA-LOCF), since there were several dropouts, a common fact in this kind of study [28, 29]. The ITT allows for a realistic evaluation of the benefits of a proposed intervention. In the case of ITTA-LOCF, the last value collected for dropout patients was computed as their final value. This increases the power of the statistical test (the sample N increases). However, it also increases the number of people who did not have a hypothetical effect of the medication, reducing the effect expected from the intervention (increasing beta error).

Questionnaires

The BAI, BDI, FTND, QSU and RT are self-report questionnaires [30–34], which evaluate anxiety and depression; the level of nicotine dependence, craving of nicotine dependents (smoking urges), and motivation to quit smoking, respectively. The cigarette diary used consists of a self-report of the number of cigarettes smoked daily. The monthly consumption was calculated and used as one parameter for cigarette consumption.

The Food Log consists of a record of all the foods and drinks ingested during a day, which the volunteers were instructed to do on 3 non-consecutive days in the week prior to the follow-up sessions. If the subject does not fill this record correctly (with all the information of the food and beverage he or she took), it is impossible to calculate the amount of compounds of interest. The data were analyzed using the online version of the Avanutri program, which measures the amount of PUFAs ingested, in grams, according to Brazilian tables of centesimal composition of foods [35] and information from food manufacturers.

Biomarkers

To quantify serum cotinine concentrations, the enzyme-linked immunosorbent assay was used. We used a spectrophotometer with filters of 450 and 630 nm, and the software GraphPad Prism, version 5.0 to calculate the concentrations. The concentrations of EPA and DHA were assessed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). This procedure required no fasting.

Approximately 5 ml of peripheral blood was processed to determine the plasmatic concentrations of EPA and DHA (10 min at 4°C and 3,000 rpm). The preparation of plasma samples included protein precipitation by acetonitrile, followed by liquid–liquid extraction using a solution of hexane/dichloromethane. The samples were resuspended in the mobile phase and filtered [36]. The equipment used was the Shimadzu 10 ADVP and the mass spectrometer the MicroMass® model QuatroMicro. The unit of measurement of the EPA and DHA fatty acids was nanogram per milliliter (ng/ml).

Results

Cross-Sectional Study

It was an exploratory study and the sample size for the groups was not previously determined. This study aimed at recruiting the largest possible number of participants in both groups. At the end of the recruiting period, the number of smokers interested in the study was bigger than non-smokers. The sample comprised 171 volunteers – 120 smokers and 51 non-smokers. Although the number of individuals in each group was not the same, the statistical analyses were controlled for the following variables: gender, age, BMI, levels of anxiety and depression, and ingestion of PUFAs through food (table 1).

Both groups presented mild anxiety and depression, with no differences detected between the groups ($p_{anxiety} > 0.05$; $p_{depression} > 0.05$). The non-smokers were younger than the smokers ($F_{(1,161)} = 10.4$, $p = 0.001$).

The number of Food Logs completed properly was much lower than the amount of participants. The non-smoker group had the highest rate in this regard (proper filling), since 84% (38 of 45 volunteers) completed the questionnaire. On the other hand, in the smoking group, only 24.3% of subjects (29 of 120 volunteers) completed the questionnaire. Even with a small number of properly filled Food Logs, and the difference between the groups, statistical analyses were performed using these data as a covariant, and no difference was found (based on these analyses, the smokers and non-smokers ate the same amount of food rich in PUFAs, $F_{(1,65)} = 0.09$, $p = 0.76$).

Group effect was not observed for EPA concentrations, even when the statistical model controlled for anxiety ($F_{(1,159)} = 0.91$, $p = 0.34$), depression ($F_{(1,159)} = 0.58$, $p = 0.44$), age ($F_{(1,158)} = 1.36$, $p = 0.24$), BMI ($F_{(1,157)} = 1.26$, $p = 0.26$) and ingestion of PUFAs ($F_{(1,64)} = 3.38$, $p = 0.07$; fig. 2).

The DHA concentration, however, showed a group effect, even when we controlled for anxiety ($F_{(1,161)} = 7.00$, $p < 0.01$), depression ($F_{(1,161)} = 6.26$, $p = 0.01$), age ($F_{(1,160)} = 7.14$, $p < 0.01$), BMI ($F_{(1,159)} = 8.14$, $p < 0.01$) and ingestion of PUFAs ($F_{(1,64)} = 4.78$, $p = 0.03$). Non-smokers presented higher concentrations of DHA (mean ± SD 1,194.0 ± 821.2 ng/ml) than smokers (mean ± SD 830.4 ± 722.9 ng/ml; fig. 2).

Clinical Trial

The total sample comprised 63 subjects, of which 31 were assigned to the omega-3 group and 32 to the control group. Out of the 39 subjects who completed the study, 21 belonged to the omega-3 and 18 to the control group (table 2).

The fish and mineral oil was well tolerated and no adverse or side effects were reported. There were no cases of intolerance or allergy to the supplementation formula used. There was no difference between the adherences of the 2 groups.

Table 1. Descriptive data for the comparative study between smokers and non-smokers. Data regarding age, height, weight, BMI and levels of anxiety and depression were used to match the sample. Data are presented as mean ± SD

Sample characterization	Non-smokers		Smokers		p
	n*	mean ± SD	n	mean ± SD	
Age, years	44	37.6±12.0	119	43.9±10.6	**
Weight, kg	42	69.6±13.1	119	69.8±14.2	–
Height, m	42	1.66±0.99	119	1.66±0.08	–
BMI, kg/m^2	43	25.1±4.2	119	25.02±4.3	–
Anxiety index (BAI)	45	8.1±8.3	119	10.4±8.5	–
Depression index (BDI)	45	7.5±7.0	119	12.0±8.4	–
PUFA ingestion, g	38	9.4±5.1	29	9.8±4.3	–
Omega-3 lipid profile					
EPA	51	112.1±94.7	118	98.1±96.8	–
DHA	51	1,194.0±821.2	120	830.4±722.9	†

* The difference in the number of valid data used for the analysis is due to the lack of complete information obtained from the volunteers.

** The only significant difference found in the sample characterization was that the non-smokers were younger than the smokers ($p = 0.001$). No significant difference was found in terms of weight, height, BMI, anxiety and depression indices, and ingestion of PUFAs through diet. Not significant ($p > 0.05$).

† Compared to the lipid profile of omega-3 series fatty acids, there was a difference between the DHA concentration of smokers and non-smokers ($p = 0.03$). As regards the EPA concentrations, no difference was found ($p > 0.05$).

Tables 3 and 4 present the data related to the parameters during the intervention. All the data were tested, and presented normality and homogeneity. The 1-way ANOVA with the screening data showed no difference between the groups as regards age ($F_{(1,58)} = 0.37$, $p = 0.55$), weight ($F_{(1,57)} = 0.75$, $p = 0.39$), height ($F_{(1,57)} = 3.04$, $p = 0.09$) and BMI ($F_{(1,57)} = 0.00$, $p = 0.98$). The chi-square test (χ^2) did not identify a significant association between schooling and gender between the groups ($\chi^2_{(2)} = 5.74$, $p = 0.95$, $\chi^2_{(2)} = 7.37$, $p = 0.95$, respectively).

The groups also did not present significant differences prior to the study (anxiety: $F_{(3,58)} = 0.23$, $p = 0.63$; depression: $F_{(3,58)} = 0.55$, $p = 0.46$; dependence level: $F_{(1,58)} = 0.003$, $p = 0.95$; motivation to stop smoking: $F_{(3,58)} = 0.16$, $p = 0.70$; smoking urges: $F_{(1,58)} = 0.007$, $p = 0.79$; (cotinine): $F_{(1,54)} = 1.88$, $p = 0.18$).

Psychometric Evaluation

The analysis of anxiety, depression and motivation levels during the treatment showed no differences between the groups (anxiety: $F_{(3,174)} = 1.30$, $p = 0.28$; depression: $F_{(3,174)} = 0.37$, $p = 0.77$; motivation: $F_{(3,174)} = 0.10$, $p = 0.96$).

Analyzing the dependence levels, there was a significant group-time interaction ($F_{(3,162)} = 3.09$, $p = 0.03$). The

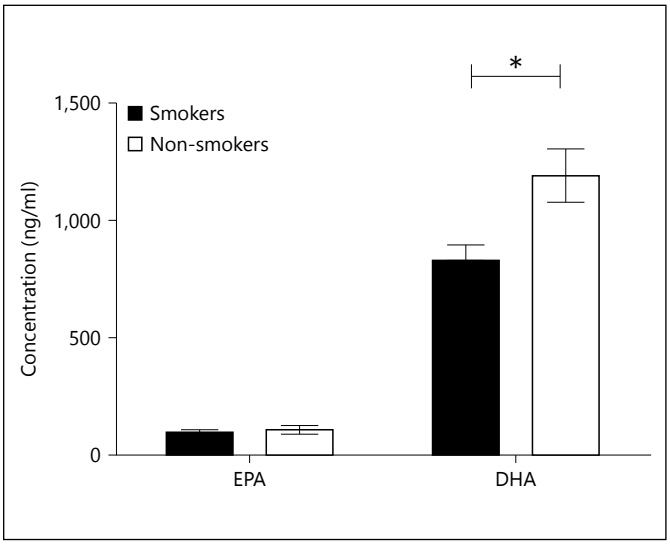

Fig. 2. Comparison between smokers and non-smokers regarding their omega-3 fatty acid profile. In the comparison of EPA and DHA concentrations between smokers and non-smokers, the statistical model considered dummy variables the levels of anxiety, depression, BMI, age and the ingestion of PUFAs through diet. There was no difference as regards the EPA fatty acid concentration ($p > 0.05$). However, the non-smokers (white bar, $n = 51$) presented higher concentrations of DHA fatty acid (* $p = 0.03$) than the smokers (black bar, $n = 120$). Considering that the model that includes the ingestion of fatty acids through diet as a dummy variable has the higher p value = 0.03, this one will be considered for data presentation.

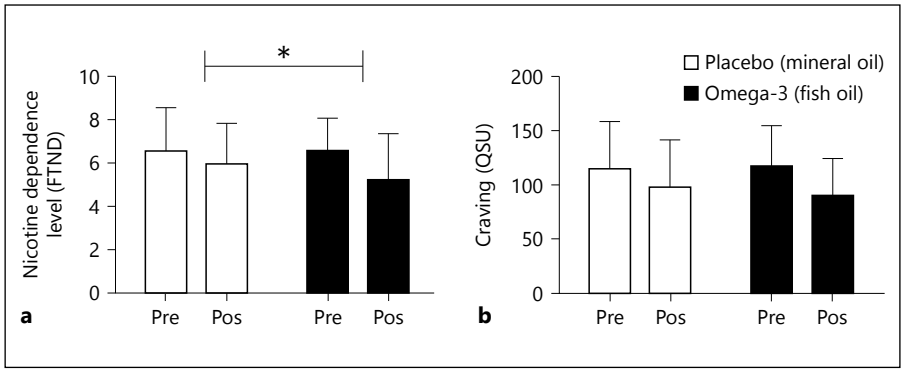

Fig. 3. Comparison between placebo and omega-3 groups regarding their psychometric evaluation. When comparing psychometric data, dependence level (FTND) and QSU, the analysis was controlled for anxiety, depression, motivation and age. **a** Analyzing the dependence level between the 2 groups, we found a significant interaction (* p = 0.03) of the treatment and the omega-3 group (black bar, n = 31). **b** Analyzing the urge to consume cigarettes between the 2 groups, no difference was found as regards the group-time interaction.

Table 2. Characterization of volunteers in the clinical trial. Data are presented as mean ± SD

Sample characterization	Placebo (mineral oil)		Omega-3 (fish oil)		p*
	n	mean ± SD	n	mean ± SD	
Age, years	32	47.8±11.5	31	46.2±11.1	0.55
Weight, kg	32	70.0±13.2	31	67.7±11.2	0.39
Height, m	32	1.70±0.09	31	1.65±0.09	0.09
BMI, kg/m^2	32	24.3±4.7	31	24.7±3.9	0.98
Gender, M:F	32	7:11	31	8:13	0.95

* Comparison between the 2 groups (placebo and omega-3).

Table 3. Data regarding pre- and post-treatment evaluations in both groups of the volunteers who followed through with the treatment. Data are presented as mean ± SD

Psychometric	Placebo (mineral oil)			Omega-3 (fish oil)			p*
	n	pre	post	n	pre	post	
Anxiety (BAI)	32	13.22±9.27	7.89±7.47	31	12.22±8.66	9.50±9.22	–
Depression (BDI)	32	14.54±9.65	10.85±8.42	31	12.69±9.55	9.09±9.44	–
Dependence (FTND)	32	6.53±2.01	5.96±1.85	31	6.56±1.52	5.22±2.15	¥
Motivation (RT)	32	8.03±1.50	7.93±1.80	31	8.19±1.42	8.25±1.83	–
Craving (QSU)	32	115.07±44.26	97.61±43.65	31	117.87±37.09	90.06±34.26	–
Biomarkers							
Cotinine, ng/ml	30	101.25±65.11	97.85±63.29	29	129.38±85.15	98.54±61.94	–
EPA, ng/ml	30	77.07±67.77	77.40±73.25	30	86.76±111.79	174.87±285.37	–
DHA, ng/ml	29	747.76±794.02	663.97±767.46	30	645.38±757.49	1,004.94±1,484.63	–

* Comparison between the 2 groups (placebo and omega-3 group) by ITT analysis. ¥ p = 0.03. Not significant (p > 0.05).

Bonferroni post-hoc test showed that the group-time interaction occurred only in the omega-3 fatty acid group, between the assessment of the visits 1 and 2 (p = 0.008), visits 1 and 3 (p = 0.001), and visits 1 and 4 (p < 0.001; fig. 3).

As regards the smoking urges, the test showed the effect of time ($F_{(3,162)}$ = 3.83, p = 0.01); however, it did not identify the group-time effect ($F_{(3,162)}$ = 1.19, p = 0.31; fig. 3).

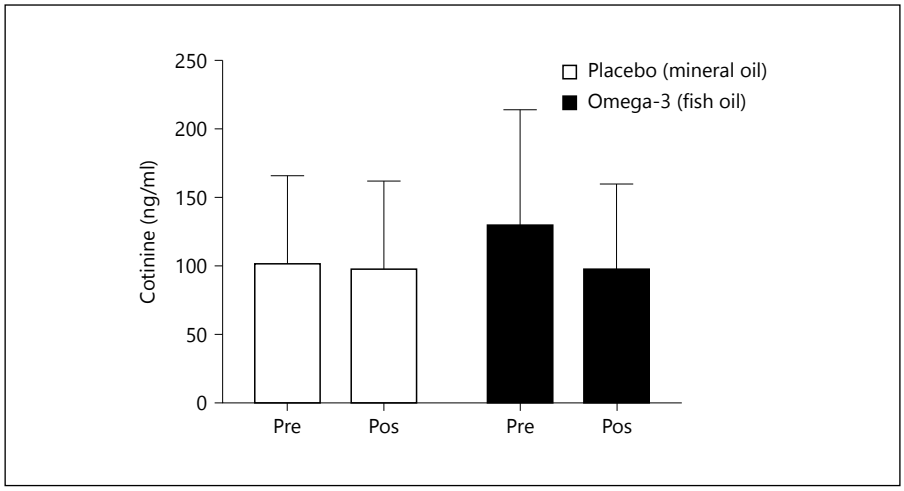

Fig. 4. Comparison between placebo and omega-3 groups as regards the consumption parameters. The concentration of serum cotinine between the 2 groups was controlled by the self-report of consumption during the treatment. No interaction between the treatment and the groups was found ($p > 0.05$).

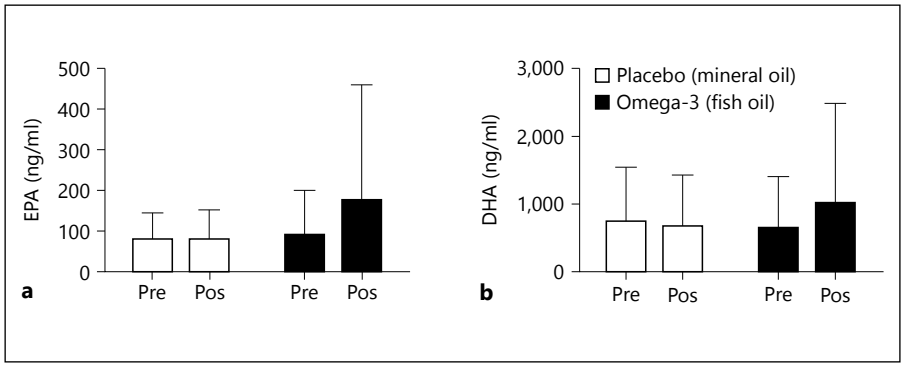

Fig. 5. Comparison between the placebo and the omega-3 groups as regards omega-3 profile. The analysis was controlled by the concentrations of serum cotinine and the amount of PUFAs (in grams) ingested by food during the treatment. **a** Analyzing the comparison between the 2 groups and the treatment as regards the concentration of the EPA fatty acid, no difference ($p > 0.05$) was found. **b** Analyzing the concentrations of the DHA fatty acids, no significant interaction between groups and treatment ($p > 0.05$) was found.

Consumption

The investigation of the smoking diary did not identify the effect of time ($F_{(2,86)} = 0.39$, $p = 0.67$) or group-time interaction ($F_{(2,86)} = 0.07$, $p = 0.93$). And the results of the cotinine concentrations revealed no group-time interaction ($F_{(1,34)} = 2.85$, $p = 0.10$; fig. 4).

Omega-3 Incorporation

First, the consumption of foods rich in PUFAs was evaluated. The number of Food Logs completed with all the required information was lower than the expected. Of the 39 subjects that concluded the study, only 20 of them filled out the Food Log with all the required information for the proper analyses. Of the twenty subjects, 10 of them were from the fish oil group and 10 were from the placebo group. Although the number of questionnaires filled out completely was small, the sample obtained was used as a covariable when we analyzed the incorporation of omega-3 fatty acid.

The study showed that the groups did not differ in food consumption in the first ($F_{(1,20)} = 3.68$, $p = 0.07$) and second ($F_{(1,19)} = 0.89$, $p = 0.36$) months. In the third month, however, the ingestion of PUFAs through diet was different ($F_{(1,17)} = 7.28$, $p = 0.01$); that is, the group treated with omega-3 fatty acids (fish oil) ate foods richer in PUFAs.

The analyses of EPA fatty acid incorporation failed to identify the effect of time ($F_{(1,2)} = 0.55$, $p = 0.53$) or group-time interactions ($F_{(1,2)} = 1.37$, $p = 0.36$). The same occurred in the analyses of the DHA fatty acid incorporation, and no significant time ($F_{(1,2)} = 0.36$, $p = 0.61$) or group-time ($F_{(1,2)} = 3.73$, $p = 0.19$) interaction was found (fig. 5).

Based on the mean values of omega-3 fatty acids incorporation, it is possible to observe that the placebo group did not show variations in the concentrations of DHA and EPA fatty acids. In the omega-3 fatty acid group, however, there was an increase in these fatty acids concentration after the intervention (fig. 5).

Table 4. Description of self-report consumption (smoking diary) and consumption of PUFAs from diet per group. The data are presented in mean ± SD

Cigarette consumption	Control (mineral oil)			Omega 3 (fish oil)			p*
	1st	2nd	3rd	1st	2nd	3rd	
Self-report PUFAs from diet	20.16±8.19	18.10±7.89	18.08±7.94	20.92±15.57	19.07±15.51	19.42±17.04	–
Amount, g	9.09±4.25	8.51±4.41	8.84±3.55	12.63±4.36	10.67±6.01	13.03±3.15	**

* Comparison between the 2 groups (placebo and omega-3). Not significant (p = 0.93).

** On the third month, the omega-3 group ingested more food rich with PUFAs than the placebo group (p = 0.01). In the first ($F_{(1,20)}$ = 3.68, p = 0.07) and second ($F_{(1,19)}$ = 0.89, p = 0.36) months, there were no difference between the groups concerning the amounts (in grams) of PUFAs ingested.

Limitations

In the cross-sectional study, the non-smoker group has fewer participants when compared with the smokers group. Despite the sample size differences over groups (51 non-smokers and 120 smokers), the mean estimation are not jeopardized, even with use of covariates [37].

In the clinical trial study, the number of Food Logs completed with all the required information was lower than the expected. A possible reason for this was the time required to fill it out, since it was necessary to take notes of each and every food/drink ingested, as well as their corresponding amounts. Even with this limitation, the data were used as a co-variable in the statistical analyses.

Another limitation of the clinical trial study is that the participants were not asked to guess in which procedure arm they were; so the blinding procedure was not tested. As an attempt to mask the possible cues, such as the fish aftertaste, the subjects were instructed to ingest the capsules with the meals.

There were 24 dropouts across the clinical trial study, 14 from the placebo group and 10 from the omega-3 group. None of the dropouts quoted side effects as the reason for dropping out. Even though the dropouts happened at different times during the study, most of them (11/24) took place in the first month of treatment. Chi-square test did not detect differences between the groups ($\chi^2_{(1)}$ = 0.85, p = 0.10).

Discussion

In this study, smokers showed lower concentrations of DHA than non-smokers. This result agrees with Simon et al. [8], who identified lower concentrations of DHA and other PUFAs, in smokers than in non-smokers. Simon et al. also detected an inversely proportional relationship between the number of cigarettes smoked and the concentration of DHA, heavy smokers (approximately 40 cigarettes/day), showing a decrease of 30% in DHA concentration when compared with non-smokers.

In contrast, our results differ from Pawlosky et al. [38], who analysed the metabolisation kinetics of omega-3 fatty acids in smokers and found that those individuals have higher levels of omega-3 fatty acids available in their plasma than non-smokers as a result of increased metabolisation kinetics.

A possible explanation for these divergent results is that in the study by Pawlosky et al. [38], the volunteers were kept under a controlled diet for a period of 21 days based on the ingestion of foods that alter the synthesis of PUFAs (beef-based diet), which may have increased the synthesis of those compounds, thereby masking potential differences prior to the administration of the study diet. Another factor that may account for the different results is the lack of evaluation of the concentrations of omega-3 fatty acids before the beginning of the diet in the study of Pawlosky et al. [38].

Since the PUFAs are compounds susceptible to peroxidation [2–7], an important fact to consider is the possibility of non-enzymatic oxidation of those by the Reactive Oxygen Species (ROS) [39]. Studies show that smokers have higher rates of ROS and other free radicals [40, 41]. The ROS specimens can attack the fatty acids and induce de formation of the harmful lipids [39]. It is possible that the PUFAs supplementation in smokers results in a potential risk for their health. More studies are necessary to understand this possible relationship.

On the other hand, the clinical trial detected a significant reduction in the level of dependence in the group treated with omega-3 fatty acid, according to FTND [30].

The reduction in the omega-3 group represents a change from moderate to mild dependence, while the level of dependence in the control group remained moderate [30]. When analyzing the consumption indicators (serum cotinine and self-report consumption), we detected no difference between the 2 groups.

In contrast, the Rabinovitz et al. [22] identified a significant reduction on tobacco craving and the number of cigarettes smoked per day, and we could not find any reduction in cigarettes per day nor in craving. One plausible reason for the divergent results is that the Rabinovitz et al. [22] treated the participants for a shorter period of time (30 days), but they used a higher dosage of omega-3 fatty acids (2,710 mg EPA/day and 2,040 mg DHA/day), while in this study we used a different approach (632.97 mg EPA/day and 389.52 mg DHA/day).

The lack of significant difference of the intervention on EPA and DHA fatty acids incorporation could be due to the association of the following aspects: (1) the negative effect of the free radicals and the oxidative stress, caused by the cigarette smoke, on the omega-3 fatty acids [2–7], leading to an impaired absorption of the fatty acids; (2) the use of an inappropriate dosage; although many studies use the 3 g/day dosage, there is no consensus in the literature [22, 42]; and (3) the small period of treatment; considering that smokers could have an impaired absorption of the omega-3 fatty acids [8], an extension of the treatment could have shown an effect.

Reduced peripheral concentrations of omega-3 fatty acids are related to impairment of different physiological systems [13, 14]. It is widely recognized that DHA is one of the main components of the omega-3 in the central nervous system [13, 14] playing a crucial role in the neurotransmission of the dopaminergic system [15–17] and its deficiency is associated with reduced levels of released dopamine [15, 16]. It has been shown that the craving to use a psychoactive substance is triggered by a reduction in the dopamine concentration in the mesolimbic structures [43]. Therefore, the reduced DHA concentrations we identified in smokers may affect the functioning of the dopaminergic system related to compulsion and the perpetuation of dependence [44]. The results of our study suggest that daily supplementation with omega-3 fatty acid might be an adjuvant tool in the reduction of levels of nicotine dependence.

Although this study presents limitations, such as the small sample size and the small number of Food Log filled out properly, it is possible that the use of omega-3 fatty acids, as an addition to the current treatments may be helpful in achieving better rates of success in the treatment of nicotine dependence.

Clinical Trial Information

Ethical approval: CAAE: #03850412.2.0000.55.5; Clinical Trials (#NTC01735279).

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