Effects of omega-3 fatty acids on endothelial function, arterial wall properties, inflammatory and fibrinolytic status in smokers: A cross over study

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Abstract

Background: Smoking is associated with endothelial dysfunction and arterial stiffness. Supplementation of omega-3 PUFAs is associated with better prognosis. Aim of this study was to evaluate the effects of omega-3 polyunsaturated fatty acids (PUFAs) supplementation on smoking-induced impairment of arterial function.

Methods: We studied the effect of a 12 weeks oral treatment with 2 gr/day of omega-3 PUFAs in 20 healthy smokers on three occasions (day0: baseline, day28 and day84). The study was carried out on two separate arms (omega-3 fatty acids and placebo), according to a randomized, placebo-controlled, double-blind, cross-over design. Measurements were carried out before (pSm), immediately and 20 min after cigarette smoking. Endothelial function was evaluated by flow-mediated dilation (FMD) of the brachial artery. Carotid-femoral pulse wave velocity (PWV) was measured as an index of aortic stiffness and augmentation index (Alx) as a measure of arterial wave reflections. Circulating levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1) were measured.

Results: Compared with placebo, omega-3 PUFAs treatment resulted in a significant improvement in pSm values of FMD (p<0.05), Alx (p<0.001) and PWV (p<0.01). Although, acute cigarette smoking decreased FMD and caused an increase in Alx and PWV, omega-3 PUFAs treatment blunted the acute smoking-induced impairment of FMD (p<0.001), Alx (p<0.05) and PWV (p<0.05) and significantly decreased levels of TNFs (p<0.05) and IL-6 (p=0.01) and increased levels of PAI-1 (p=0.05).

Conclusions: Omega-3 PUFAs improved endothelial function and the elastic properties of the arterial tree in healthy smokers, with a parallel anti-inflammatory effect.

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Continuous variables as mean value±SD

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>27.63 ± 2.65</td>
</tr>
<tr>
<td>Gender (males/females)</td>
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</tr>
<tr>
<td>Body mass index (Kg/m2)</td>
<td>22.58 ± 0.98</td>
</tr>
<tr>
<td>Pack/year index</td>
<td>5.78 ± 0.04</td>
</tr>
<tr>
<td>Aortic systolic pressure (mm Hg)</td>
<td>103.3 ± 7.8</td>
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<tr>
<td>Aortic diastolic pressure (mm Hg)</td>
<td>78.3 ± 9.1</td>
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<tr>
<td>Aortic pulse pressure (mm Hg)</td>
<td>24.6 ± 5.0</td>
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<tr>
<td>Peripheral systolic pressure (mm Hg)</td>
<td>116.5 ± 7.8</td>
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<tr>
<td>Peripheral diastolic pressure (mm Hg)</td>
<td>77.5 ± 9.1</td>
</tr>
<tr>
<td>Peripheral pulse pressure (mm Hg)</td>
<td>39.1 ± 9.0</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>71.6 ± 8.7</td>
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<tr>
<td>AIX (%)</td>
<td>0.70 ± 12.91</td>
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<tr>
<td>PWV (m/s)</td>
<td>5.87 ± 0.63</td>
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<td>FMD (%)</td>
<td>7.27 ± 2.56</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>785 ± 9.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>1798 ± 33.9</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>1082 ± 19.7</td>
</tr>
<tr>
<td>High density lipoprotein (mg/dl)</td>
<td>51.1 ± 11.58</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>742 ± 38.45</td>
</tr>
</tbody>
</table>

#### 2. Methods

**2.1. Study population**

Twenty smokers, aged 27.63 ± 2.65 years old, 7 females 13 males, were included in this double-blind, placebo controlled, cross-over trial. Individual characteristics of the participants are presented in Table 1. All of the subjects were active smokers (smoked > 20 cigarettes per day for > 5 years). They had no evidence of hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, a family history of premature vascular disease, any kind of acute inflammatory-infectious or chronic disease. They also were not taking any regular cardiovascular medications (or growth hormone), antioxidant vitamin supplementation, anti-inflammatory or steroid substances during the past 2 months, or oral contraceptives (female participants). The participants refrained from caffeine, alcohol, smoking and any food for 12 hours before each study. Their body mass index (BMI) was between 20 and 25 kg/m², total cholesterol plasma level was below 200 mg/dl and their clinical examination and electrocardiogram were normal. All measurements were performed at the same place in the morning (09:00 pm).

**2.2. Study design**

At baseline, the participants rested in a quiet dark room for 30 min, under controlled temperature 22–24 °C. Endothelial function as well as the elastic properties of arterial tree were evaluated in all the patients at baseline (pSm), immediately after they smoked a single cigarette (Sm0), as well as 20 min after the cigarette smoking (Sm20). All the subjects smoked one standard cigarette (1.1 mg nicotine, 12 mg tar) during 5 min. Endothelium-independent dilation was estimated at the end of each study. Blood samples were obtained at baseline. After the baseline visit, the patients were randomly allocated into 2 groups to receive an oral treatment with omega-3 PUFAs (dose of two grams, 46% eicosapentaenoic acid – 38% docosahexaenoic acid) or placebo, for a period of 12 weeks. Both the active drug preparation and placebo were prepared as identical formulations (caps) and were administrated once daily.

Follow-up was performed at the end of fourth week and at the end of twelfth week. After a 4 weeks wash-out period, the same patients were called back for the second part of the study. At that time, the same measurements were performed, but those patients who received placebo in the first part of the study now received omega-3 PUFAs, vice versa, in a double-blind cross-over fashion.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in an a priori approval by the institution’s human research committee and each subject gave written informed consent.

**2.3. Evaluation of endothelial function**

Endothelial function was evaluated by estimating the flow mediated dilation (FMD) in the brachial artery, as previously described [13-15]. Endothelium-independent dilation was defined as the %change of vessel diameter from rest to the maximum diameter post-nitrate administration. The repeatability of the technique in our institution for determining FMD was determined according to the Bland-Altman method. The repeatability coefficient, which was calculated as defined by the British Standard Institution, that is, according to the formula: repeatability coefficient = $2 \times \sqrt{(\text{SD}^2 / N)}$ (where N is the sample size and di the difference between the two measurements in a pair), was 5.0%.

**2.4. Evaluation of aortic elastic properties**

Carotid-femoral pulse wave velocity (PWV), which is considered to be an index of aortic stiffness was calculated from measurements of pulse transit time and the distance traveled between 2 recording sites (PWV = distance in meters divided by transit time in seconds) by using a well validated noninvasive device (Sphygmoscor; AtCor Medical) [3] as previously described [16].

**2.5. Measurement of Wave Reflection Indexes**

Augmentation index (AIX) of the central (aortic) pressure waveform was measured as an index of wave reflection. AIX is a composite measure of the magnitude of wave reflection and arterial stiffness, which affects timing of wave reflection. Large values of AIX indicate increased wave reflection from the periphery and/or earlier return of the reflected wave as a result of increased PWV (owing to increased arterial stiffness) and vice versa. Because Aix is influenced by changes in heart rate, it was also corrected accordingly (corrected for a steady heart rate of 75 beats/min-AI75) [17]. AI75 was measured with a validated, commercially available system (Sphygmoscor; AtCor Medical) that uses the principle of planation tonometry and appropriate acquisition and analysis software for noninvasive recording and analysis of the arterial pulse, as previously described [16,18]. Waves of radial pressure were calibrated according to sphygmomanometric systolic and diastolic pressure measured in the brachial artery.

**2.6. Biochemical measurements**

Venous blood samples were centrifuged at 3000 rpm and serum/plasma was collected and stored at −80°C until assayed. Serum levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured as well-established inflammatory markers and plasma levels of plasmogen activator inhibitor-1 (PAI-1), as a marker of fibrinolysis system. TNF-α, IL-6 and PAI-1 were measured by commercially available ELISA kits. Biochemical measurements including lipids and glucose levels were measured by using colorimetric enzymatic method in a Technicon automatic analyzer (RA-1000, Dade-Behring Marburg GmbH).

**2.7. Statistical analysis**

All variables were tested for normal distribution of the data. The values of TNF-α, IL-6 and PAI-1 were skewed and they were evaluated with non-parametric test. Normally distributed data were expressed as means ± s.d. or otherwise as median and first and third quartile. The effect of cigarette smoking on arterial wall properties and the impact of the two interventions were evaluated with 2-way repeated-measures ANOVA. In cases in which ANOVA yielded a significant interaction, appropriate post hoc tests were done by comparing the changes between different examined days. Friedman’s test was used to test for differences in biochemical markers between different examined days. Exact values of p < 0.05 were considered statistically significant. All values are expressed as means ±SD or as median and 1st and 3rd quartile. Data analysis was performed with SPSS software, version 13.0.

**3. Results**

**3.1. Effects of omega-3 PUFAs supplementation on FMD**

Omega-3 PUFAs treatment improved FMD in pSm measurements (from 7.27 ± 2.56% day 0 to 8.53 ± 3.55% day 28 to 9.98 ± 5.30% day 84, p = 0.038) while there was not such improvement in FMD values at the placebo arm (from 6.92 ± 1.87% day 0 to 6.71 ± 1.58% day 28 to 6.68 ± 1.40% day 84, p = 0.405) (Fig. 1). At day 0, FMD was significantly decreased immediately after smoking, and remained lower than baseline 20 min after smoking for both omega-3 PUFAs (from 7.27 ± 2.56% pSm to 5.45 ± 2.65% Sm0 to 5.21 ± 2.51% Sm20, p = 0.031) and placebo group (from 6.92 ± 1.87% pSm to 5.17 ± 2.18% Sm0, to 5.31 ± 2.39% Sm20, p = 0.047). Compared to placebo, omega-3 PUFAs treatment prevented the smoking induced decrease in FMD in day 28 (p = 0.05) and 84 (p = 0.001) (Fig. 2). Endothelium-independent dilation remained unchanged in both groups during the study period (p = 0.496) (Fig. 2).

**3.2. Effects of omega-3 PUFAs on PWV/AIX**

AIX pSm values were significantly decreased during omega-3 PUFAs treatment period (from 0.70 ± 12.91% day 0 to −2.16 ± 12.47% day 28 to −3.88 ± 13.82% day 84, p < 0.001) while there was no
such improvement in the placebo group (1.83 ± 11.14% day 0 to 1.88 ± 11.07% day 28 to 11.92 ± 10.37% day 84, p = 0.814) (Fig. 1). At day 0 smoking induced an immediate elevation of AIx, which was attenuated 20 min after smoking in both study groups (Fig. 3). Treatment with omega-3 PUFAs prevented the smoking induced increase of AIx in day 84 compared to placebo (Fig. 3).

During the omega-3 PUFAs treatment period there was a significantly improvement in pSm values of PWV (from 5.87 ± 0.63 m/s day 0 to 5.84 ± 0.64 m/sec day 28 to 5.54 ± 0.76 m/sec day 84, p = 0.007) while there was no such improvement in the placebo arm (from 5.87 ± 0.64 m/s day 0 to 5.84 ± 0.69 m/sec day 28 to 5.74 ± 0.58 m/s day 84, p = 0.065) (Fig. 1). At day 0, smoking induced a rapid elevation of PWV which was attenuate 20 minutes after smoking in both study groups. Omega-3 PUFAs treatment prevented the smoking induced elevation of PWV, after 84 days of treatment (Fig. 3).

3.3. Effects of omega-3 PUFAs supplementation on inflammatory process, plasminogen activator inhibitor-1 and lipids levels

Treatment with omega-3 PUFAs resulted in a stepwise decrease in pSm levels of TNF-α at day 28 and 84 (p = 0.013) as it show in Table 2 while there was no difference in baseline levels of TNF-α in placebo treated group (Table 2) (Fig. 4). Omega-3 PUFAs treatment cause also a stepwise decrease in pSm levels of IL-6 (p = 0.01) (Table 2) while placebo did not cause significant changes in pSm values of IL-6 (Fig. 4). Omega-3 PUFAs treatment cause also a significant increase in pSm values of PAI-1 (p = 0.050) while placebo did not change pSm values of PAI-1 as it shown in Table 2 and Fig. 4.

There were no significant changes in serum total cholesterol levels, in triglycerides levels, in HDL cholesterol levels and in serum glucose levels between day 0, day 28 and day 84 neither in the Omega-3 PUFAs treatment group, neither in the placebo group as it is shown in Table 2.

4. Discussion

In the present study we found that omega-3 PUFAs treatment resulted in a significant improvement in endothelial function and arterial stiffness in smokers. Acute cigarette smoking decreased FMD values and caused an increase in AIx and PWV. Moreover omega-3 PUFAs treatment blunted the acute smoking-induced impairment of FMD, AIx, PWV and significantly decreased levels of TNF-α, and IL-6 and increased levels of PAI-1.
4.1 Omega-3 PUFAs, smoking and arterial function

It has been previously reported that smoking leads to endothelial dysfunction, partly by increasing oxidative stress status [19, 20]. Reactive oxygen species depress NO synthesis through their inhibitory effect on endothelial receptors for acetylcholine and other vasodilators, while they directly react with NO to form peroxynitrite, decreasing in this way NO bioavailability [21-23]. Moreover, free radicals, especially superoxide anion, modulate the tone of vascular smooth muscles directly by acting on the smooth muscle cells and reduce the half-life of PGI2 and NO [24]. Reduced production or decreased half-life of NO and/or increased production of superoxide anion promotes endothelial dysfunction, increases peripheral vascular resistance, and eventually elevates blood pressure [24]. Impaired endothelial function leads to decreased forearm hyperemic response and flow-mediated dilatation of the brachial artery [9] in smokers as previously reported.

In recent years, growing evidence links the intake of omega-3 PUFAs with an improvement in endothelial function [25, 26]. The mechanisms by which omega-3 PUFAs might influence endothelial function are likely to be multiple and complex [27]. It is known that omega-3 PUFAs act via incorporation into cellular phospholipids. This incorporation results in a concomitant reduction of n-6 PUFAs suggesting that a specific ratio of n-3 to n-6 fatty acids is important.
in improving endothelial function [8]. The mechanistic basis for the improved endothelium-triggered relaxation with omega-3 PUFAs include the suppression of thromboxane A2 or cyclic endoperoxides, a reduced production of cytokines, the augmented endothelial synthesis of nitric oxide, an improvement of vascular smooth muscle cell sensitivity to nitric oxide, and a reduced expression of endothelial adhesion molecules [8,24,27].

In the present study we found that FMD values were significantly improved during the treatment period in the omega-3 PUFAs treatment group (Figs. 1 and 2). At day 0, FMD was significantly decreased in both groups immediately after smoking. In contrast, at day 28 and 84, compared to placebo, treatment with omega-3 PUFAs blunted the acute smoking-induced decrease in endothelial function. Omega-3 PUFAs may improve endothelial function in our study possibly by decreasing the elevated oxidative stress in smoking. This has resulted in the recovery of endothelial synthesis of NO and PGI2 and in the improvement of vascular smooth muscle cell sensitivity to NO.

In addition, we found that during the omega-3 PUFAs treatment period, there was a significantly improvement in PWV and AIx. At day 0, smoking induced a rapid elevation of PWV and AIx, which was attenuate 20 minutes after smoking in both study groups. Omega-3 PUFAs treatment prevented the smoking induced elevation of PWV and AIx, after 84 days of treatment. The favorable effect of omega-3 PUFAs in arterial stiffness is believed to be an effect mediated by the
improvement of endothelial function, since vascular endothelium is a key regulator of the elastic properties of large vessels [2]. Moreover, acute long chain n-3 PUFA-rich meal consumption can improve postprandial arterial stiffness [28] and pulse wave velocity in non-diabetic patients under long-term hemodialysis [29].

4.2. Omega-3 PUFA’s, smoking, inflammatory process and fibrinolytic activity

Inflammatory cytokines such as TNF-α activate endothelial cells to express selectin adhesion receptors that form strong transient bonds to the carbohydrate counter ligands on the surfaces of neutrophils [30]. This transient neutrophil adhesion to the vascular endothelium is termed rolling and is the first step in leukocyte migration across the endothelial wall.

Previous studies showed that omega-3 PUFA’s suppressed production of pro-inflammatory cytokines such as interleukin-1β, interleukin-8, IL-6, and TNF-α [31,32]. Moreover omega-3 PUFA’s enhance the production of antiinflammatory eicosanoids (3-series prostanooids, 5-series leukotrienes) like PGI3, a vasodilator and platelet antiaggre-gator and decrease the production of a range of pro-inflammatory eicosanoids (2-series prostanooids, 4-series leukotrienes). We have previous found that fish intake is associated with decreased levels of proinflammatory markers among healthy adults [33]. Compared to non-fish consumers, those who consumed >300 g of fish per week had on average 33% lower C-reactive protein (CRP), 33% lower IL-6, 21% lower TNF-α, 28% and 4% lower WBC counts. It may be hypothe-sized that fish intake decreases IL-6 synthesis, which then affects CRP production in the liver.

As regarding the effects of smoking on inflammatory process, it has been proposed that smoking activates redox-sensitive inflammatory pathways, i.e. (nuclear factor kappa B transcriptional system), increase the expression of proinflammatory molecules (such as cyto-kines) and triggers atherogenesis [34]. In our study we demonstrat-ed that omega-3 PUFA’s reduced inflammatory status in smokers. More precisely, treatment with omega-3 PUFA’s resulted in a stepwise decrease in serum levels of TNF-α and IL-6 at day 28 and 84. It is reasonable to suggest that omega-3 PUFA’s intake suppresses in-flammation and, thus, produces its beneficial actions in human health.

Regarding the effects of omega-3 PUFA’s on fibrinolytic activity the findings are controversial, depending on study population, dose and duration of the treatment. In the present study, we demonstrated that doses of 2 grams omega-3 PUFA’s for 3 months increase PAI-1 serum levels in smokers. This finding is consistent with previous experimental and clinical studies showing a positive and direct effect of omega-3 PUFA’s on PAI-1 expression [35]. Intake of omega-3 PUFA’s has been associated with increased plasma PAI-1 activity in healthy individuals, in non-insulin-dependent diabetes mellitus patients or patients undergoing coronary bypass surgery [36-38]. Interestingly, in a recent study, PAI-1 concentration and activity increased more following high omega-3 PUFA’s beverage compared with low omega-3 PUFA’s beverage [39].

5. Conclusion

Treatment with omega-3 PUFA’s improved endothelial function and the elastic properties of the arterial tree in healthy smokers, with a parallel antiinflammatory effect. The effect of omega-3 PUFA’s on vascular endothelium and endothelial activation provides a novel mechanism by which omega-3 PUFA’s affects vascular compliance, which requires further investigation. These findings suggest that omega-3 PUFA’s inhibit the detrimental effects of smoking on vascular endothelium. The cardioprotective effects of omega-3 PUFA’s appear to be due not through a single mode of action but to a synergism between multiple, intricate mechanisms involve anti-inflammatory and anti-atherosclerotic effects.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

References


