Effects of Omega-3 Fatty Acids on Serum Lipids and High Sensitivity C Reactive Protein in Cigarette Smokers

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Abstract: The aim of this study was to determine the impact of omega-3 fatty acids on lipid metabolism and low-grade inflammation in cigarette smokers. In a double-blind, placebo-controlled trial, 59 male cigarette subjects were randomly assigned to consume 3 g omega-3 fatty acids or corn oil/day for 8 weeks while continuing to consume their usual diet. High sensitivity C reactive protein (hs-CRP) was determined by ELISA. Fifty-one subjects completed the study. Neither omega-3 fatty acids nor corn oil supplementation had a significant effect on triglyceride, HDL-cholesterol or total cholesterol concentrations. After adjustment for baseline values, fasting LDL-cholesterol concentrations increased 16.7% with omega-3 fatty acids supplementation (p<0.05) in comparison with the change in the corn oil group. The intervention caused no significant changes in serum hs-CRP concentrations. In conclusion a daily intake of omega-3 fatty acids increases LDL-cholesterol by 8% in cigarette smokers and had not effects on serum triglyceride and hs-CRP concentrations.

Key words: Omega 3 fatty acid, cigarette smoking, lipids, hs-CRP

INTRODUCTION

Fish oils are rich sources of the long-chain omega-3 polyunsaturated fatty acids, Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). A meta-analysis of 11 studies involving 16,896 patients found that, compared to a control diet or placebo, a diet enriched with omega-3 fatty acids or the use of supplements was associated with significant reductions in fatal infarction (risk ratio 0.7, 95% CI 0.8-0.8), sudden death (risk ratio 0.7, 95% CI 0.6-0.9) and total mortality (risk ratio 0.8, 95% CI 0.7-0.9) (Bucher et al., 2002). There are well-known mechanisms linking (omega-3) fatty acids and cardiovascular disease, including reduction of serum triglycerides, decreased platelet aggregability and antiarrhythmic effects (Conner, 2000).

The effect of omega-3 PUFAs on plasma lipid and lipoprotein metabolism has been reviewed extensively. In a review of 44 intervention studies that supplemented with a range of 0.5 to 25 g omega-3 PUFA day$^{-1}$ for an average of 6 weeks, it was shown that supplementation had little effect on plasma LDL and HDL-cholesterol concentrations, but that it consistently and significantly reduced plasma triacylglycerol concentrations (Harris, 1989).

Numerous studies from various parts of the world have clearly established that CRP predicts future risk for cardiovascular diseases in apparently healthy persons, independent of established risk factors in the majority of studies (Jialal et al., 2004). In the studies to date, CRP has been shown to predict myocardial infarction, Coronary Artery Disease (CAD) death, stroke, peripheral arterial disease, sudden death, etc. (Ridker, 2003). Thus, the Centers for Disease Control and the American Heart Association have issued a statement recommending that patients at intermediate risk of CAD might benefit from measurement of CRP (Pearson et al., 2003). However, several epidemiological studies have reported that intake of omega-3 polyunsaturated fatty acids or fish oil is inversely associated with serum C-reactive protein concentrations (Lopez-Garcia et al., 2004; Madsen et al., 2000; Niu et al., 2006), but four studies investigating the effects of omega-3 fatty acids on hs-CRP were also inconsistent and non-significant (Balk et al., 2006).

Cigarette smokers have a higher risk of coronary artery disease than non-smokers. Several possible explanations have been offered for this association, including altered blood coagulation, impaired integrity of the arterial wall and changes in blood lipid and lipoprotein concentrations.

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Smoking is associated with an adverse effect on serum lipids (elevated low density lipoproteins and triglycerides and reduced high density lipoproteins) (Craig et al., 1989) and with insulin resistance (Reaven and Tsao, 2003). In addition, free radicals in cigarette smoke damage lipids, resulting in the formation of proatherogenic oxidized particles, specifically oxidized low density lipoprotein cholesterol (Valkonen and Kusni, 1998).

However, the effects of marine omega-3 polyunsaturated fatty acids and antioxidants on the oxidative modification of LDL were studied in male smokers (Brude et al., 1997) but to the best of our knowledge, no study has been conducted to examine the independent effects of omega-3 fatty acids on lipid profile in smokers. The aim of this study was to determine the effects of omega-3 fatty acids on lipid metabolism and hs-CRP in male smoker subjects.

MATERIALS AND METHODS

This study was conducted in July 2005. Men aged 40-75 years who were smokers for at least one year were recruited from the general community by media advertising. Entry criteria included: smoking at least 10 cigarettes per day, body mass index (in kg m⁻²) <35, no special diet, no previous medical history of thyroid, liver, renal and chronic inflammatory diseases. None of the subjects were regularly taking lipid-lowering, beta- adrenergic antagonist and thiazide diuretic drugs. All subjects also did not take fish oil supplements and did not drink ethanol. Subjects were excluded if they had a recent history (within 3 months) of heart disease, angina, or major surgery; had a recent history (within 6 months) of myocardial infarction or stroke, had significant liver or renal disease (plasma creatinine >130 μmol L⁻¹), macroproteinuria, or symptomatic autonomic neuropathy; or regularly used nonsteroidal anti-inflammatory drugs. Fifty nine of the 100 subjects screened satisfied the entry criteria. The study was approved by the ethics committee of Bushehr University of Medical Sciences and all subjects gave written consent.

Intervention: Baseline measurements were collected during a 3 week period, during which all subjects maintained their usual diet. They were then randomly assigned to one of 2 groups after stratification by sex, age and body mass index. The subjects consumed 3 g fish oil-fortified drink 3 g omega-3 fatty acids [3 g EPA (2 g) +DHA/d (1 g)] or corn oil/d for 8 weeks while continuing to consume their usual diet. Subjects and investigators were blinded to the treatment. The fish oil-fortified drink was provided by Nooshdaroodarya Company (www.nooshdaroodarya.com).

Subjects were instructed not to change their usual diets, level of physical activity, or other lifestyle factors throughout the intervention period. Before the baseline period, a dietitian gave written and verbal instructions to the subjects on how to keep accurate dietary records, including how to weigh or measure foods. A 3 day dietary record (2 week days and 1 weekend day) and a lifestyle questionnaire including history of illness, medications and physical activity were completed at baseline and after the intervention period. Physical activities of participants were evaluated by a questionnaire based on the International Physical Activity Questionnaire, 2005. Epic-Norfolk Nutritional methods 24 h diet recall was used; the dietary records were analyzed by using N-3 software (LH’s sfx: 2.10L, 1991. Yoshi). Weight, height, waist and hip circumferences of subjects were measured by digital scale and non-stretchable meter. Weight, changes in physical activity and medication and any illness were recorded each week during baseline and at weeks 4 and 8 of the intervention.

Biochemical measurements: Fasting blood samples were taken at baseline and the end of the 8 week study, all samples were promptly centrifuged, separated and analyses were carried out at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, The Netherlands). Serum total cholesterol and HDL-cholesterol were measured using a cholesterol oxidase phenol aminoantipyrine and triglycerides using a glycerol-3 phosphate oxidase phenol aminoantipyrine enzymatic method. Serum LDL-cholesterol was calculated using the Friedwald formula. Serum lipids were measured within 12 h of collection with an automated Technicon Axon Analyzer (Bayer Diagnostics, Sydney, Australia) by using an enzymatic method. Measurement of CRP by a high-sensitivity CRP assay, CRP HS ELISA (DRG International, Inc. USA) was done. The minimum detectable concentration of the CRP HS ELISA assay was estimated to be 0.1 mg L⁻¹. Additionally, the functional sensitivity was determined to be 0.1 mg L⁻¹ (as determined with inter-assay % CV<20%).

Statistical analysis: A two-tailed t-test was used to compare the mean values across groups. Significance of differences within groups was calculated by Paired t-test. p<0.05 was considered statistically significant. We found that log transformation of CRP gave a better fit to a Gaussian distribution. The geometric mean for CRP was defined as the arithmetic mean of the log-transformed data raised to the power of 10. Statistical analysis was performed with an IBM computer using the SPSS 9.05 statistical software package (SPSS Inc., Chicago, IL).
RESULTS

Fifty one of the 59 randomly assigned subjects completed the study. Five subjects were withdrawn because of compliance failure. Two subjects were withdrawn because of immigration and one was withdrawn because of an illness unrelated to the protocol. The characteristics of the patients confirmed that the groups were well matched for all entry criteria (Table 1) and there were no significant differences between the groups in either the type or number of antihypertensive, or oral hypoglycemic medications. The antihypertensive medications taken by the subjects were angiotensin-converting enzyme inhibitors (13%), calcium channel blockers (9%), angiotensin II receptor antagonists (9%), alpha-blockers (3%) and non thiazide diuretics (4%).

There were no significant differences between the groups in total energy intake, macronutrient intake, body weight, number of cigarette at baseline and no significant changes took place during the intervention (data not shown). Medication doses and physical activity were unchanged during the intervention in each group.

Serum lipids and hs-CRP: Fasting triglyceride concentrations at baseline were < 500 mg dL⁻¹ in each of the groups, implying reliable assessment of LDL cholesterol with the use of the Friedwald formula. There were no significant differences between the groups at baseline in any of the variables.

Table 1: Characteristics of participants in the 2 groups at baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Omega-3 fatty acid (n = 27)</th>
<th>Placebo (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.30±8.8</td>
<td>45.00±9.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.11±4.2</td>
<td>24.82±4.19</td>
</tr>
<tr>
<td>WHR</td>
<td>0.80±0.01</td>
<td>0.91±0.01</td>
</tr>
<tr>
<td>Number of cigarettes day⁻¹</td>
<td>17.50±6.7</td>
<td>19.90±10.6</td>
</tr>
<tr>
<td>Pack years</td>
<td>23.20±10.5</td>
<td>23.40±10.9</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (%)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Calcium channel blocker (%)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Angiotensin II receptor antagonist (%)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Non thiazide diuretics (%)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>α-blockers (%)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>BMI: Body Mass Index; WHR: Waist Hip Ratio. Values are expressed as Mean±SD unless otherwise denoted</td>
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</table>

Table 2: Fasting serum lipids, high sensitivity CRP (hs-CRP) at baseline and post intervention in the 2 groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Omega-3 fatty acid</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg dL⁻¹)</td>
<td>237.8±300.74</td>
<td>272.6±213.75</td>
</tr>
<tr>
<td>TC</td>
<td>255.8±39.65</td>
<td>288.8±63.89</td>
</tr>
<tr>
<td>HDL-C</td>
<td>45.2±15.47</td>
<td>40.2±6.95</td>
</tr>
<tr>
<td>LDL-C</td>
<td>135.2±50.49</td>
<td>201.5±48.36†</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>11.48±7.23g</td>
<td>11.19±3.26</td>
</tr>
</tbody>
</table>

TG: Triglyceride; TC: Total Cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; *, p<0.05 vs baseline in paired t-test; †, p<0.05 vs placebo via t-test; g: Geometric mean

Neither omega-3 fatty acids nor corn oil supplementation had a significant effect on triglyceride, HDL-cholesterol or total cholesterol concentrations. But LDL-cholesterol concentration increased significantly in omega-3 group after the intervention (p<0.05). After adjustment for baseline values, fasting LDL-cholesterol concentrations increased 16.7% with omega-3 fatty acids supplementation (p<0.05) in comparison with the change in the corn oil group.

At baseline, hs-CRP was higher in the placebo group than in the omega-3 group, but this difference was not significant (p>0.05). Neither omega-3 fatty acid nor corn oil had an effect on hs-CRP concentration (Table 2).

DISCUSSION

The hypotriglyceridemic effects of omega-3 fatty acids from fish oils are well established. In a comprehensive review of human studies, Harris reported that 4 g d⁻¹ of omega-3 fatty acids from fish oil decreased serum triglyceride concentrations by 25 to 30% (Harris, 1997). Postprandial triglyceridemia is especially sensitive to chronic omega-3 fatty acids consumption, with quite small intake (<2 g day⁻¹) producing significant reductions (Roche and Gibney, 1995). A mechanism to explain the hypotriglyceridemic effects of marine omega-3 fatty acids in humans has not been clarified. Recently, a working model was developed at the gene transcriptional level, which involves four metabolic nuclear receptors (Davidson, 2006). These include liver X receptor, hepatocyte nuclear factor-4 alpha (HNF-4 alpha), farnesol X receptor and peroxisome proliferators-activated receptor (PPARs). Each of these receptors is regulated by sterol receptor element binding protein-1c (SREBP-1c), a hepatic gene transcription factor that stimulates synthesis of the lipogenic enzymes involved in this pathway (Pegorier et al., 2004). Omega-3 fatty acids elicit hypotriglyceridemic effects by coordinately suppressing hepatocellular lipogenesis through reducing levels of SREBP-1c, upregulating fatty oxidation in the liver and skeletal muscle through PPAR activation and enhancing flux of
glucose to glycogen through down regulation of HNF-4 alpha. The net result is the repartitioning of metabolic fuel from triglyceride storage toward oxidation, thereby reducing the substrate available for Very-Low-Density Lipoprotein (VLDL) synthesis (Davidson, 2006).

In this study, the change of TG level in subjects who received omega-3 fatty acids was not significantly different from the change in the control group. Failure of omega-3 fatty acids to significantly lower TG levels in normolipidemic individuals occurred in about half of the placebo-controlled studies reported by Harris (1996). Our subjects were not normolipidemic but they were smokers. The only one study which used smokers in a randomized, double-blind, placebo-controlled trial also failed to show hypotriglycerideremic effect of omega-3 fatty acids (Brude et al., 1997). One possible explanation for the lack of a significant lowering effect of omega-3 fatty acids on TG levels might be the complex effect of cigarette smoking in lipogenesis. Nicotine stimulates the release of adrenaline by the adrenal cortex, leading to the increased serum concentrations of free acid observed in smokers (Kershbaum et al., 1963); free fatty acid is a well known stimulant of hepatic secretion of VLDL and hence TG (Kohout et al., 1971). Therefore, smokers have significantly higher serum concentrations of triglycerides (Craig et al., 1989).

A high dietary intake of omega-3 fatty acids may protect cigarette smokers against chronic obstructive pulmonary disease (Shahar et al., 1994) DHA may have a role in preventing or treating smoking-related chronic obstructive pulmonary disease (Shahar et al., 1999). But present study showed that the hypotriglycerideremic effect of omega-3 fatty acids could not be observed in cigarette smokers. The interaction of smoking and omega-3 fatty acids on triglyceride level could not be explained at the gene transcriptional level. Marian et al. (2006) showed that the percentages of PPAR alpha-positive atherosclerotic macrophages and PPAR alpha-positive cells in the atherosclerotic wall were increased in chronic obstructive pulmonary disease patients compared with control subjects.

Recent evidence suggests that the peroxidation of polyunsaturated fatty acids and subsequent oxidative stress regulates hepatic apolipoprotein B degradation and VLDL production (Pan et al., 2004). According to this mechanism, marine omega-3 fatty acids, as highly unsaturated fats, may undergo extensive peroxidation, which appears to stimulate the degradation of apolipoprotein B, also resulting in the reduction in VLDL secretion (Davidson, 2006). Acute cigarette smoking immediately increases markers of oxidative stress in all models and even results in damage to the cell membrane (van der Vaart et al., 2004). So, it could be hypothesized that smoking-related oxidative damage of target molecules in lipogenesis prevents hypotriglycerideremic effect of omega-3 fatty acids in smokers.

The effect of dietary n-3 fatty acids on plasma cholesterol concentrations is far more variable. In subjects with type IV hyperlipidemia omega-3 fatty acids markedly lowers VLDL cholesterol (VLDL-C) but usually increases LDL-C concentrations at least in the short term. Plasma LDL concentrations are reduced by high doses of n-3 fatty acids in normal persons (Connor et al., 1993; Illingworth and Shmidt, 1993; Worn and Smith, 1959), in those with primary hypercholesterolemia (Illingworth and Shmidt, 1993) and in nonhuman primates (Parks and Rudel, 1980), especially if n-3 fatty acids replaces saturated fatty acids in the diet. The response of serum cholesterol to omega-3 fatty acids appears to depend on several factors, including (i) whether purified n-3 fatty acids are used or marine lipids containing a variety of saturated fatty acids and sterols (ii) whether n-3 fatty acids are taken as a supplement or used to replace other fats in the diet (iii) the level of n-3 fatty acid intake (iv) the underlying lipoprotein phenotype and (v) the specific genotype responsible for a particular lipoprotein phenotype (Vasanadini et al., 2002).

The increased LDL-cholesterol concentration may relate to the hypotriglycerideremic effects of these fatty acids. N-3 fatty acids reduce hepatic VLDL synthesis, VLDL secretion, or both with the result that the smaller VLDL particles formed are more readily converted to LDL than are the larger VLDL particles (Packard et al., 1984). Smaller VLDL particles can also compete with LDL for uptake by LDL receptors (Schmidt et al., 1993).

However, in this study, the hypotriglycerideremic effect of omega-3 fatty acids could not be seen, but we observed that prescription of omega-3 fatty acids increased significantly the serum level of LDL-C of the subjects, in comparison to the control group. Consequently, it would appear that mechanisms other than cholesterol synthesis and absorption are likely to be responsible for increasing in LDL cholesterol. A down-regulation of the LDL receptor has been reported in some but not all studies (Schmidt et al., 1993). Feeding fish oil containing DHA to hamsters (Lindsey et al., 1992) and primates (Strue et al., 1992) results in decreased receptor-mediated clearance of LDL, which could be a result of either decreased binding of LDL receptor. The latter now seems a likely mechanism because, in vitro, DHA down-regulates LDL receptor expression in HepG2 cells (Schechtman et al., 1996). Omega-3 fatty acids may decrease the expression of LDL receptors by down-regulating the transcription or degradation of sterol regulatory element-binding protein. Alternatively, it may lead to the generation of oxysterols within the liver that
stimulate the liver X receptor (Theobald et al., 2004). Further research is needed to clarify the reasons for the increase in LDL cholesterol with intakes of omega-3 fatty acid.

In conclusion, a daily intake of 3 g of omega-3 fatty acids had no benefits for smokers in their lipid profiles. The effect of higher levels of omega-3 fatty acids on serum lipids should be investigated. It is worth mentioning that we did not determine omega-3 fatty acids serum levels in the two groups at the beginning and along the study. It should be taken into consideration that measuring the omega-3 fatty acids concentration in two groups and along the study would make our study more valid and it is the limitation of this study.

Although some epidemiological studies have shown an inverse correlation between dietary fish oil (EPA and DHA) consumption and biomarkers of inflammation (Lopez-Garcia et al., 2004; Madsen et al., 2000; Niu et al., 2006), intervention trials have not yet confirmed these effects (Busu et al., 2006). In this study, the mean CRP change in the fish oil group did not significantly differ from that in the placebo group. Similarly, null effects with EPA and DHA supplementation were seen in healthy subjects (Vega-Lopez et al., 2004; Fujioka et al., 2006), in slightly obese individuals (Jellena et al., 2004), in treated hypertensive type 2 diabetic subjects (Mori et al., 2003), in postmenopausal women (Geelen et al., 2004) and also in patients receiving hemodialysis (Moreira et al., 2007). The currently available data including ours do not support that beneficial effects on CRP are involved in a mechanism explaining the protective effect on heart disease risk of omega-3 fatty acids as present in fish.

Overall, the present study shows that dietary supplementation with omega-3 fatty acids supplements has no effect on serum concentrations of CRP, measured with a highly assay, in smoker subjects.

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