Biomedicine & Pharmacotherapy 68 (2014) 1071–1077

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Omega-3 fatty acids improve postprandial lipemia and associated endothelial dysfunction in healthy individuals – a randomized cross-over trial

Toru Miyoshi [a,*], Yoko Noda [a], Yuko Ohno [a], Hiroki Sugiyama [a], Hiroki Oe [b], Kazufumi Nakamura [a], Kunihisa Kohno [a], Hiroshi Ito [a]

[a] Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[b] Center of Ultrasonic Diagnostics, Okayama University Hospital, Okayama, Japan

ARTICLE INFO

Article history:
Received 12 September 2014
Accepted 16 October 2014

Keywords:
Omega-3 fatty acid
Triglyceride
Endothelial dysfunction

ABSTRACT

Background: Postprandial elevation of triglycerides impairs endothelial function and contributes to the development of atherosclerosis. We investigated the effects of omega-3 fatty acids on postprandial endothelial function and lipid profiles.
Methods: Healthy volunteers [10] were given supplementation at 4 g/day omega-3 fatty acids (or were not treated) for 4 weeks in a randomised crossover study. Postprandial levels of various lipids were monitored and endothelial function assessed by brachial artery flow-mediated dilation during fasting and after a standard cookie test.
Results: Omega-3 fatty acids reduced postprandial endothelial dysfunction compared with the control diet (flow-mediated dilation at 4 h = -0.5 ± 1.2 *vs.* $-2.0 \pm 1.6\%$, $P = 0.03$). Postprandial levels of triglycerides, apolipoprotein B-48, and remnant lipoprotein-cholesterol increased in untreated subjects, peaked at 2–4 h, and returned to baseline at 8 h, whereas low-density lipoprotein-cholesterol levels did not change. Supplementation with omega-3 fatty acids significantly suppressed postprandial elevation of triglycerides (incremental area under the curve = 220 ± 209 vs. 374 ± 216 mg/h/dL, $P = 0.04$) and remnant lipoprotein-cholesterol (incremental area under the curve = 21.7 ± 13.8 vs. 13.3 ± 12.9 mg/h/dL, $P = 0.04$). Supplementation with omega-3 fatty acids significantly suppressed the increase in triglyceride content in chylomicrons as well as in very-low-density lipoproteins from baseline to 4 h after the cookie test.
Conclusion: Omega-3 fatty acids significantly decreased postprandial triglyceride elevation and postprandial endothelial dysfunction, suggesting that omega-3 fatty acids may have vascular protective effects in postprandial state.

1. Introduction

Epidemiologic studies have shown that non-fasting postprandial triglyceride (TG) concentrations is independent of traditional risk factors for coronary artery disease [1,2]. Postprandial lipemia is characterized with TG-rich lipoproteins which include partially catabolized chylomicrons containing ApoB48 and/or increased hepatic production of very low density lipoproteins (VLDL) containing ApoB100 [3]. TG-rich lipoproteins are highly atherogenic and contribute to the development of coronary artery disease [4]. Postprandial lipemia is involves in the production of proinflammatory cytokines and oxidative stress, which induce endothelial dysfunction even in healthy normolipidemic individuals [5,6]. Therefore, identification of therapeutic approaches that can lower postprandial concentrations of serum lipids is intriguing.

It is well established that supplementation with omega-3 fatty acids (n3-FA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are abundant in fatty fish, can improve aberrant lipid profiles [7,8]. Dietary supplementation with n3-FA has been consistently shown to reduce serum TG concentration [9]. The TG-lowering effect of n3-FA has been suggested to be a consequence of a decrease in the very-low-density lipoprotein (VLDL) fraction [10]. EPA and DHA may have a similar effect on the postprandial secretion of intestinal chylomicron [11]. However, there have been limited studies addressing the effect of n3-FA directly on postprandial lipemia [12,13]. Therefore,

* Corresponding author. Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan. Tel.: +81 86 235 7351; fax: +81 86 222 7353.
E-mail address: miyoshit@cc.okayama-u.ac.jp (T. Miyoshi).

http://dx.doi.org/10.1016/j.biopha.2014.10.008
0753-3322/

the aim of this study was to investigate the effects of n3-FA on postprandial levels of serum TG-rich lipoproteins and postprandial lipemia-induced endothelial dysfunction.

2. Methods

2.1. Participants and design

This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (Okayama, Japan). This study was conducted according to the principles expressed in the declaration of Helsinki, and registered at UMIN Clinical Trials Registry (UMIN000012556). The authors confirm that all ongoing and related trials for this drug/intervention are registered.

This was a randomised, cross-over study. All procedures were carried out at Okayama University Hospital. Participants were recruited through clinical research sites and community outreach between February 2013 and May 2013. After the nature and possible consequences of the study was explained, all participants provided written informed consent before screening and study enrolment. Eligible subjects were healthy adults ranging in age from 20 years to 85 years. The study consisted of two 4-week crossover supplementation periods in which one group of five participants (chosen randomly) was administered 4 g/day of n3-FA (including 1.9 g of EPA and 1.5 g of DHA), whereas the other group of five participants did not receive n3-FA. In the second 4-week period, the groups were reversed, i.e., the previous untreated group received n3-FA supplementation (Fig. 1). There was a 4-week period between the two phases during which neither group received n3-FA. All participants underwent pre-study medical examinations and interviews questioning their medical history. To exclude glucose intolerance, the 75-g oral glucose tolerance test was also carried out. None of the 10 volunteers had hypertension, impaired glucose tolerance, dyslipidemia, smoking or cerebrovascular/cardiovascular disease. Impaired glucose tolerance was defined as 2-h glucose levels of 140–199 mg/dL after glucose tolerance test, [14] and dyslipidemia was defined as one or more of the following criteria in the fasting state: serum triglyceride $\geq$ 150 mg/dL, or LDL cholesterol $\geq$ 140 mg/dL [15].

The pre-specified primary outcome measure was the difference in the decrease in flow-mediated dilation (FMD) after the cookie test. Secondary outcome measures were the difference in lipids profiles after the cookie test between the n3-FA group and control group.

2.2. Cookie test

A cookie test was carried out after overnight fasting as previously described. [16] One carton of cookies contained 75 g carbohydrate, 28.5 g fat, and 8 g protein for a total of 592 kcal (Saraya Corp., Osaka, Japan) [17]. Participants were given an amount of cookies equivalent to 30 g fat/m^2 body surface. Blood samplings were performed during the fasting state before cookie ingestion and 2, 4, 6, and 8 h thereafter. Participants were not allowed to eat anything else for 8 h after eating the cookie.

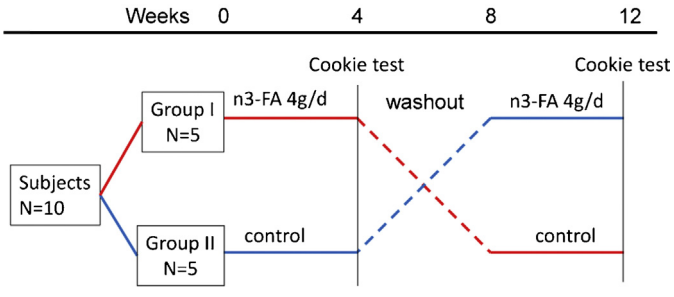

Fig. 1. Flow chart of our crossover trial.

2.3. FMD and NMD measurements

FMD and nitroglycerin-mediated dilation (NMD) which is endothelium-independent dilation were determined during the fasting state and 4 h after ingestion. FMD and NMD measurements were performed with specialized machine (Unex Co. Ltd., Nagoya, Japan) according to guidelines for ultrasound assessment of FMD in the brachial artery [18]. Briefly, FMD was assessed by measuring the change in right brachial artery diameter after 60 s compared with the baseline diameter after deflation of a cuff that had been placed around the forearm and inflated to 50-mmHg higher than systolic blood pressure for 5 min. Measurements of artery diameter was made continuously from 30 s before to $\geq$ 2 min after cuff release. NMD measurement was performed after sublingual administration of 0.3 mg nitroglycerin. FMD and NMD were expressed as the percentage change from baseline in the brachial artery internal diameter after release of occlusion and after administration of nitroglycerin, respectively. FMD/NMD ratio was calculated by dividing peak %FMD by %NMD [19]. An experienced technician blinded to the allocation measured FMD and NMD and intra- and inter-observer correlation coefficients were considerably high ($>$ 0.9).

2.4. Blood examinations

Certain parameters were measured during fasting before cookie ingestion: serum total cholesterol (Total-C), TG, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), remnant lipoprotein cholesterol (RLP-C), apoB-48, adiponectin, pentraxin 3, hemoglobinA1c, insulin, plasma glucose, and fatty acids including n3-FA (EPA and DHA) and n6-FA (arachidonic acid (AA) and dihomo-γ-linolenic acid (DGLA). These analyses were undertaken by the clinical testing laboratory SRL Co. Ltd. (Tokyo, Japan). Serum TG content in lipoprotein fractions was analysed by Skylight Biotech (Akita, Japan) as described [20]. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma glucose (mg/dL) $\times$ fasting plasma insulin (μU/mL)/405. Serum Total-C, TG, LDL-C, RLP-C, apoB-48, plasma glucose, plasma insulin and pentraxin 3 were measured 2, 4, 6, and 8 h after the cookie load. The incremental area under the curve (AUC) was calculated using the trapezoidal method after correction for baseline values [16].

2.5. Statistical analysis

Sample size was determined based on the estimated FMD reported in a previous study. [21] Mean improvement in postprandial %FMD was 2.7 $\pm$ 2.0% (standard deviation (SD)). To use a paired *t*-test for differences between two interventions, a minimum sample size of 8 participants was required to detect statistical differences in %FMD with a power of 90% and α error of 5%. After accounting for potential dropouts and imperfect compliance, the target sample size was determined to be 10 participants. The normality of the distribution of the variables was assessed by the Shapiro–Wilk test, which demonstrated that apoB-48, insulin, HOMA-IR and pentraxin 3 showed a skewed distribution. We used parametric methods and tests (e.g., mean, standard deviation, paired *t*-test) to analyse normally distributed data. We employed nonparametric methods and tests (e.g., median, inter-quartile range, Wilcoxon signed-rank test) to analyse data that deviated from a normal distribution. Results in the figures are the mean $\pm$ SD.