Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

A fish-based diet intervention improves endothelial function in postmenopausal women with type 2 diabetes mellitus: A randomized crossover trial

Keiko Kondo[a], Katsutaro Morino[a,*], Yoshihiko Nishio[b], Motoyuki Kondo[a], Keiko Nakao[a], Fumiyuki Nakagawa[a,c], Atsushi Ishikado[a,d], Osamu Sekine[a], Takeshi Yoshizaki[a], Atsunori Kashiwagi[a], Satoshi Ugi[a], Hiroshi Maegawa[a]

[a] Department of Medicine, Division of Endocrinology and Metabolism, Shiga University of Medical Science, Shiga, Japan
[b] Department of Diabetes and Endocrine Medicine, Division of Human and Environmental Sciences, Kagoshima University, Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[c] Osaka Laboratory, JCL Bioassay Corporation, Osaka, Japan
[d] R&D Department, Sunstar Inc, Osaka, Japan

ARTICLE INFO

Article history:
Received 22 October 2013
Accepted 8 April 2014

Keywords:
Diet
n-3 PUFA
Intervention studies
Endothelial function
Diabetes mellitus

ABSTRACT

Objective. The beneficial effects of fish and n-3 polyunsaturated fatty acids (PUFAs) consumption on atherosclerosis have been reported in numerous epidemiological studies. However, to the best of our knowledge, the effects of a fish-based diet intervention on endothelial function have not been investigated. Therefore, we studied these effects in postmenopausal women with type 2 diabetes mellitus (T2DM).

Materials/Methods. Twenty-three postmenopausal women with T2DM were assigned to two four-week periods of either a fish-based diet (n-3 PUFAs ≧ 3.0 g/day) or a control diet in a randomized crossover design. Endothelial function was measured with reactive hyperemia using strain-gauge plethysmography and compared with the serum levels of fatty acids and their metabolites. Endothelial function was determined with peak forearm blood flow (Peak), duration of reactive hyperemia (Duration) and flow debt repayment (FDR).

Results. A fish-based dietary intervention improved Peak by 63.7%, Duration by 27.9% and FDR by 70.7%, compared to the control diet. Serum n-3 PUFA levels increased after the fish-based diet period and decreased after the control diet, compared with the baseline (1.49 *vs.* 0.97 *vs.* 1.19 mmol/l, $p < 0.0001$). There was no correlation between serum n-3 PUFA levels and endothelial function. An increased ratio of epoxyeicosatrienoic acid/dihydroxyeicosatrienoic acid was observed after a fish-based diet intervention, possibly due to the inhibition of the activity of soluble epoxide hydrolase.

Abbreviations: ADMA, asymmetric dimethylarginine; ANOVA, analyses of variance; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; eNOS, endothelial nitric oxide synthase; EPA, eicosapentaenoic acid; FBF, forearm blood flow; FDR, flow debt repayment; GPR, G-protein coupled receptor; 4-HHE, 4-hydroxy hexenal; 4-HNE, 4-hydroxy nonenal; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MCP-1, monocyte chemotactic protein-1; NOS, nitric oxide synthase; 8-OHdG, 8-hydroxydeoxyguanosine; PUFA, polyunsaturated fatty acid; RH, reactive hyperemia; SBP, systolic blood pressure; sEH, soluble epoxide hydrolase.

* *Corresponding author at:* Department of Medicine, Division of Endocrinology and Metabolism, Shiga University of Medical Science, Tsukinowa, Seta, Otsu, Shiga 520-2192, Japan. Tel.: +81 77 548 2222; fax: +81 77 543 3858.
E-mail address: morino@belle.shiga-med.ac.jp (K. Morino).

http://dx.doi.org/10.1016/j.metabol.2014.04.005

Conclusions. A fish-based dietary intervention improves endothelial function in postmenopausal women with T2DM. Dissociation between the serum n-3 PUFA concentration and endothelial function suggests that the other factors may contribute to this phenomenon.

1. Introduction

The beneficial effects of dietary fish intake on coronary heart disease, sudden cardiac death, and all-cause mortality in the general population have been discussed for many decades [1]. A negative correlation between the dietary intake of fish and cardiovascular risk has been noted [2–4]. In diabetic women, a higher consumption of fish has been associated with a lower incidence of coronary heart disease and total mortality [5].

Although the mechanisms underlying the beneficial effects of dietary fish intake remain unclear, n-3 polyunsatulated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), might be important factors.

Endothelial dysfunction is a major contributor to the pathogenesis of cardiovascular disease. Many studies have indicated that endothelial dysfunction is caused by smoking, obesity, dyslipidemia, hypertension, and type 2 diabetes mellitus [6–9]. Supplementation of n-3 PUFAs has been shown to improve endothelial function in patients with hypercholesterolemia [10], peripheral arterial disease [11], acute myocardial infarction [12], and type 2 diabetes mellitus [13,14]. Few reports have sought to evaluate if a fish-based diet intervention, instead of n-3 PUFA supplements, can improve endothelial function directly.

Eicosanoids are generated from other n-3 or n-6 PUFAs and control multiple functions, including inflammation, thrombosis and endothelial function [15]. Increasing evidence has revealed that epoxyeicosatrienoic acids (EETs) contribute to endothelium-dependent vasodilatation [16]. Soluble epoxide hydrolase (sEH), which converts EETs to dihydroxyeicosatrienoic acid (DHET), is a potential pharmacological target of endothelial dysfunction [17]. It has been reported that plasma EETs levels and the EET/DHET ratio decreased in patients with hypertension [18]. In addition, one recent study showed that fish oil supplementation increased the levels of some eicosanoids in young, healthy volunteers [19]. However, the mechanism by which a fish-based dietary intervention changes the circulating eicosanoids in subjects with type 2 diabetes mellitus is not known.

This study had two aims. First, we examined the effects of a fish-based dietary intervention on endothelial function in postmenopausal women with type 2 diabetes mellitus. Second, we sought to analyze the relationships among endothelial function, n-3 PUFAs, and the concentrations of their metabolites.

2. Materials and Methods

2.1. Study population

Twenty-three postmenopausal type 2 diabetic women were recruited (mean age, 69.7 ± 6.6 years; mean BMI, 22.5 ± 3.1 kg/m^2) at Shiga University of Medical Science Hospital. Individuals taking pioglitazone, EPA, or fish oil supplements; those undergoing insulin treatment; and those with poor glycemic control ($HbA_{1c} \geqq 8.4\%$ [68.3 mmol/mol]), high fish intake (frequency of fish intake ≧ seven times per week), fish allergy, smoking, or excessive alcohol ingestion were excluded.

The nature and potential risks of the study were explained to all participants, and written informed consent was obtained. The study was performed in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of Shiga University of Medical Science. The study was registered at UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index.htm) with the Identification No. UMIN000002277.

2.2. Study design

The study included two experimental periods in a randomized crossover design. Participants were randomly assigned to start with either the fish-based diet or the control diet for 4 weeks. Eleven participants (fish-first group) started with the fish-based diet, and 12 participants (control diet-first group) started with the control diet for 4 weeks. After the first experimental period, the participants crossed over to the other experimental diet for an additional 4 weeks.

Participants were instructed to take more than 3.0 g/day n-3 PUFA derived from fish (e.g., Pacific saury, salmon, sardines, etc.) during the fish-based diet period. Conversely, during the control diet period, participants were instructed to avoid fish intake, particularly n-3 PUFA-rich fish. The intake of total energy was 126–135 kJ per kg ideal body weight throughout the study period. Participants were advised by a dietician to modify their habitual diet qualitatively before each diet period. At baseline and at the end of each experimental period, a dietician provided participants with written and verbal instructions regarding the completion of a 3-day dietary record with a digital photograph of each meal. Participants also kept a food diary to record their daily fish intake. Nutritional intake, including intake of n-3 PUFA, was calculated using food composition tables specific for the Japanese people (Eiyoukun, ver5.0, Kenpakusha, Tokyo, Japan) [20]. Participants were advised on maintaining a constant physical activity level throughout the study. They underwent a day of testing at baseline and after 4 weeks of each diet intervention. Tests included anthropometric measurements, blood samples, and body composition measurements after a 10–16 h overnight fast. No medications were allowed to be changed throughout the study period.

2.3. Weight and body composition

Body weight was recorded with an electronic scale without shoes and with light clothing weighing a maximum of 0.1 kg.