Plasma polyunsaturated fatty acid profile is associated with vascular endothelial function in patients with type 2 diabetes

Tomoaki Morioka, Masanori Emoto, Satoshi Imamura, Yoshinori Kakutani, Yuko Yamazaki, Koka Motoyama, Katsuhito Mori, Shinya Fukumoto, Atsushi Shioi, Tetsuo Shoji, Masaaki Inaba

MORIOKA, T., et al. (2018). Plasma polyunsaturated fatty acid profile is associated with vascular endothelial function in patients with type 2 diabetes. *Diabetes & Vascular Disease Research*. Vol.15, Issu.4, pp.352-355. Copyright © 2018 Authors. DOI: 10.1177/1479164118774314.

Short Report

Plasma Polyunsaturated Fatty Acid Profile is Associated with Vascular Endothelial Function in Patients with Type 2 Diabetes

Tomoaki Morioka [1], Masanori Emoto [1], Satoshi Imamura [1], Yoshinori Kakutani [1], Yuko Yamazaki [1], Koka Motoyama [1], Katsuhito Mori [2], Shinya Fukumoto [3], Atsushi Shioi, [4,5], Tetsuo Shoji [4,5], Masaaki Inaba [1,5]

[1]Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan
[2]Department of Nephrology, Osaka City University Graduate School of Medicine, Osaka, Japan
[3]Department of Premier Preventive Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan
[4]Department of Vascular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan
[5]Vascular Science Center for Translational Research, Osaka City University Graduate School of Medicine, Osaka, Japan

Running head: Plasma PUFA profile and FMD in type 2 diabetes
Word count: 1483 words excluding references and tables

Corresponding author: Tomoaki Morioka, MD, PhD
Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
Tel.: +81-6-6645-3806; Fax: +81-6-6645-3808
E-mail: m-tomo@med.osaka-cu.ac.jp

Abstract

Decreased plasma n-3 polyunsaturated fatty acid (PUFA) levels or the n-3/n-6 PUFA ratios are associated with a risk of cardiovascular events. In this cross-sectional study, we measured plasma levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) and investigated the association between the plasma PUFA profile and vascular endothelial function in 396 patients with type 2 diabetes. Endothelium-dependent, flow-mediated dilatation (FMD) of the brachial artery was measured using ultrasonography. Multiple regression analyses, including age, sex, body mass index, and other cardiovascular risk factors, revealed that plasma EPA levels (β = 0.140, p = 0.008) and the EPA/AA ratio (β = 0.127, p = 0.019), but not plasma DHA levels (β = 0.067, p = 0.220) or the DHA/AA ratio (β = 0.034, p = 0.559), were independently and positively associated with FMD. In conclusion, plasma EPA levels and the EPA/AA ratio are independently associated with endothelial function in patients with type 2 diabetes. This study indicates a positive association between EPA, rather than DHA, and endothelial function in type 2 diabetes.

Keywords: polyunsaturated fatty acid; eicosapentaenoic acid; endothelial function; flow-mediated dilatation; type 2 diabetes

Introduction

The consumption of n-3 polyunsaturated fatty acids (PUFAs),[1] or the circulating PUFA profile, including eicosapentaenoic acid (EPA) levels, docosahexaenoic acid (DHA) levels, and the n-3 PUFA/arachidonic acid (AA) ratio,[2, 3] are inversely associated with cardiovascular outcomes. Many clinical trials have also shown that the supplementation of n-3 PUFAs improves vascular endothelial function, a predictor of subclinical cardiovascular outcomes.[4] However, the effect of n-3 PUFA supplementation on endothelial function has been inconsistent in studies on patients with type 2 diabetes,[5, 6] who evidently have endothelial dysfunction.[7] Moreover, to our knowledge, no study has demonstrated the association between the circulating n-3 PUFA profile and endothelial function in patients with type 2 diabetes. Our hypothesis was that endothelial dysfunction is associated with an abnormal plasma PUFA profile in patients with type 2 diabetes.

Methods

Study design and participants

In this cross-sectional study, we consecutively enrolled 396 patients with type 2 diabetes who were admitted to the Diabetes Center of the Osaka City University

Hospital between January 2009 and June 2013. Patients who were regularly taking drugs containing n-3 PUFAs were excluded from the present study.

This study was performed in accordance with the Declaration of Helsinki (1975, as revised in 2013). The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (No. 308). All participants provided written informed consent prior to the study.

Measurements

Frozen plasma samples were shipped to SRL (Tokyo, Japan), and EPA, DHA, and AA concentrations were measured using capillary gas chromatography as previously described.[3, 8] We measured FMD and endothelium-independent, nitroglycerin-mediated dilatation (NMD) of the brachial artery using an ultrasound system (UNEXEF; Unex Co. Ltd., Nagoya, Japan). The measurements were performed in a quiet, air-conditioned room at 25.0°C for inpatients who had not consumed any foods, caffeine, or tobacco and had not engaged in exercise for at least 12 hours before the measurements, according to the International Brachial Artery Reactivity Task Force guideline.[9]

Statistical analysis

Correlations were examined using the nonparametric Spearman's rank correlation test. Multiple regression analyses were used to explore the influence of each of the

PUFA levels or the n-3/n-6 PUFA ratio on FMD or NMD. Skewed parameters, such as triglycerides and the plasma PUFA profile, were logarithmically transformed before regression analyses. A p value of <0.05 was considered significant. Statistical analyses were performed using the JMP 10 software (SAS Institute Inc., Cary, NC, USA).

Results

This study's participants included 228 men and 168 women, aged 65 years (median). The median duration of diabetes and the body mass index (BMI) were 11 years and 24.9 kg/m^2, respectively. The median plasma EPA, DHA, and AA levels were 39.0 μg/mL, 94.3 μg/mL, and 138.7 μg/mL, respectively. The median EPA/AA, DHA/AA, and (EPA+DHA)/AA ratios were 0.29, 0.66, and 0.96, respectively. The median FMD and NMD were 5.9% and 14.5%, respectively.

The FMD was negatively correlated with age (ρ = –0.167, p = 0.001), duration of diabetes (ρ = –0.117, p = 0.020), and systolic blood pressure (ρ = –0.179, p < 0.001), and was positively correlated with estimated glomerular filtration rate (eGFR) (ρ = 0.222, p < 0.001). None of the parameters of the plasma PUFA profile were significantly correlated with FMD in unadjusted analyses. To explore the independent association between plasma PUFA levels or n-3 PUFA/AA ratios and FMD, we performed multiple

regression analyses after adjusting for potential confounders. Aside from the traditional risk factors for atherosclerosis, including age, BMI, systolic blood pressure, and glycated hemoglobin (HbA1c) levels, plasma EPA levels and the plasma EPA/AA ratio were found to be independently and positively associated with FMD (Table 1). No significant association was found between FMD and plasma DHA levels, plasma AA levels, the DHA/AA ratio, or the (EPA+DHA)/AA ratio. In contrast, none of the parameters of the plasma PUFA profile were significantly associated with NMD, after adjusting for the same variables as those used in the models for FMD (data not shown).

Discussion

The present study demonstrated that plasma EPA levels and the EPA/AA ratio were positively associated with FMD of the brachial artery in patients with type 2 diabetes. Notably, those associations were independent of the traditional cardiovascular risk factors. Accumulating evidence indicates a beneficial effect of n-3 PUFA supplementation on endothelial function in individuals with cardiovascular disease or its risk factors.[4] Because no study has examined the association between circulating levels of n-3 PUFAs and FMD in patients with type 2 diabetes, this is the first study regarding

an association between the plasma PUFA profile and endothelial function in those patients.

Contrary to our results, a number of studies performed in patients with diabetes failed to show a beneficial effect of supplementation with n-3 PUFAs on endothelial function.[10, 11] Impaired endothelial function has been documented in patients with type 2 diabetes.[7] Furthermore, a dysregulated plasma PUFA profile was associated with the presence of type 2 diabetes in our recent study.[8] Taken together, the association between the plasma PUFA profile and endothelial function would be complicated in patients with type 2 diabetes. Therefore, our data may indicate that EPA exerts its vasodilatory effect[12] even in patients with type 2 diabetes. It remains unclear why a significant association between the plasma PUFA profile and FMD was found in our study subjects with type 2 diabetes; however, a relatively large number of participants compared to the number in prior studies[10, 11] may be one possible reason.

The present study further showed that plasma levels of DHA were not associated with FMD. Results from limited studies indicate that DHA might be more effective than EPA in improving forearm vascular reactivity and that the vasodilatory effects of EPA are endothelial cell-dependent, while those of DHA are endothelial cell-independent.[12] It needs to be mentioned that none of those previous studies were performed in patients

141 with type 2 diabetes. In our results, neither EPA nor DHA was associated with NMD, an
142 endothelium-independent vasodilatation, whereas only EPA was associated with FMD,
143 potentially highlighting the pivotal role of endothelium-dependent, vasodilatory effects
144 of EPA in type 2 diabetes.

145 This study has several limitations. First, we measured PUFA levels in total plasma
146 lipids, but not in phospholipids from the cellular membrane, which are direct precursors
147 of bioactive eicosanoids. Second, we did not evaluate dietary intake, the use of dietary
148 supplements, or lifestyles of participants, which could have affected vascular function,
149 as well as the plasma PUFA profile. Third, the participants with type 2 diabetes were
150 receiving statin, antihypertensive, and/or anti-diabetic drugs including insulin, which
151 could affect vascular function and related risk factors. Fourth, a relatively small sample
152 size and a single measurement of plasma PUFA could influence the results of the
153 present study. Finally, we did not include non-diabetic controls. Although previous
154 studies in the non-diabetic population have shown inconsistent results, we could have
155 evaluated whether the presence of diabetes modifies the association between circulating
156 PUFAs and FMD using a non-diabetic control group.

157 In conclusion, this study demonstrated that plasma EPA levels and the EPA/AA ratio
158 are independently associated with FMD in patients with type 2 diabetes. Our data

159 indicate a positive association between EPA and endothelial function, an established
160 predictor of cardiovascular disease, and further propose the plasma levels of EPA, rather
161 than those of DHA, as a potential biomarker of vascular health, even in patients with
162 type 2 diabetes who have impaired endothelial function.
163

164 Acknowledgements

165 The authors acknowledge the excellent technical assistance of Ms. Setsuko Arita and
166 Ms. Mika Sakaki of the research laboratory in the Department of Metabolism,
167 Endocrinology and Molecular Medicine, Osaka City University Graduate School of
168 Medicine.
169

170 Declaration of conflicting interests

171 The authors declare no conflicts of interest related to this study.
172

173 Funding

174 This study was supported by a Grant-in-Aid for Scientific Research (No. 20591068)
175 from the Japan Society for the Promotion of Science (to M.E. and K. Mor.).
176

177 ## References

178 1. Mozaffarian D and Rimm EB. Fish intake, contaminants, and human health:
179 evaluating the risks and the benefits. *JAMA*. 2006; 296: 1885-1899.
180 2. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain
181 omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort
182 study. *Ann Intern Med*. 2013; 158: 515-525.
183 3. Shoji T, Kakiya R, Hayashi T, et al. Serum n-3 and n-6 polyunsaturated fatty
184 acid profile as an independent predictor of cardiovascular events in hemodialysis
185 patients. *Am J Kidney Dis*. 2013; 62: 568-576.
186 4. Wang Q, Liang X, Wang L, et al. Effect of omega-3 fatty acids supplementation
187 on endothelial function: a meta-analysis of randomized controlled trials.
188 *Atherosclerosis*. 2012; 221: 536-543.
189 5. McVeigh GE, Brennan GM, Johnston GD, et al. Dietary fish oil augments
190 nitric oxide production or release in patients with type 2 (non-insulin-dependent)
191 diabetes mellitus. *Diabetologia*. 1993; 36: 33-38.
192 6. Sawada T, Tsubata H, Hashimoto N, et al. Effects of 6-month eicosapentaenoic
193 acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability,
194 and concomitant endothelial dysfunction among newly-diagnosed impaired glucose

195 metabolism patients with coronary artery disease. An open label, single blinded,
196 prospective randomized controlled trial. *Cardiovasc Diabetol*. 2016; 15: 121.
197 7. Calles-Escandon J and Cipolla M. Diabetes and endothelial dysfunction: a
198 clinical perspective. *Endocr Rev*. 2001; 22: 36-52.
199 8. Imamura S, Morioka T, Yamazaki Y, et al. Plasma polyunsaturated fatty acid
200 profile and delta-5 desaturase activity are altered in patients with type 2 diabetes.
201 *Metabolism*. 2014; 63: 1432-1438.
202 9. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound
203 assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery:
204 a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*.
205 2002; 39: 257-265.
206 10. Wong CY, Yiu KH, Li SW, et al. Fish-oil supplement has neutral effects on
207 vascular and metabolic function but improves renal function in patients with Type 2
208 diabetes mellitus. *Diabet Med*. 2010; 27: 54-60.
209 11. Woodman RJ, Mori TA, Burke V, et al. Effects of purified eicosapentaenoic
210 acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in
211 hypertensive type 2 diabetic patients. *Atherosclerosis*. 2003; 166: 85-93.
212 12. Kelley DS and Adkins Y. Similarities and differences between the effects of

213 EPA and DHA on markers of atherosclerosis in human subjects. *Proc Nutr Soc*. 2012;

214 71: 322-331.

215

Table 1. Multiple regression analysis for the determinants of FMD in all participants with type 2 diabetes

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Age (years)	–0.209*	–0.183*	–0.168*	–0.219*	–0.185*	–0.208*
Sex (male=1, female=0)	–0.077	–0.086	–0.093	–0.089	–0.095	–0.088
BMI (kg/m²)	–0.116*	–0.121*	–0.130*	–0.102	–0.114*	–0.101
Systolic blood pressure (mmHg)	–0.144*	–0.144*	–0.147*	–0.145*	–0.145*	–0.144*
eGFR (mL/min/1.73 m²)	0.097	0.107	0.107	0.102	0.110	0.109
HbA1c (%)	–0.153*	–0.148*	–0.152*	–0.143*	–0.143*	–0.140*
Log [triglycerides (mg/dL)]	0.032	0.009	0.019	0.030	0.010	0.009
Non HDL-cholesterol (mg/dL)	–0.144*	–0.118	–0.106	–0.117*	–0.098	–0.110
Smokers (yes=1, no=0)	–0.077	–0.067	–0.068	–0.072	–0.064	–0.065
RAS inhibitors (yes=1, no=0)	–0.140*	–0.143*	–0.143*	–0.137*	–0.142*	–0.140*
Statins (yes=1, no=0)	–0.044	–0.030	–0.030	–0.030	–0.022	–0.023
Log [EPA (μg/mL)]	0.140*	–	–	–	–	
Log [DHA (μg/mL)	–	0.067	–	–	–	
Log [AA (μg/mL)]	–	–	0.049	–	–	
Log [EPA/AA]	–	–	–	0.127*	–	
Log [DHA/AA]	–	–	–	–	0.034	
Log [(EPA+DHA)/AA]	–	–	–	–	–	0.094
R^2	0.152*	0.140*	0.138*	0.149*	0.137*	0.143*

Values are standard coefficient determined by multiple regression analysis (β); R^2, coefficient of determination; *, $p < 0.05$. A smoker was defined as a current smoker or an ex-smoker. FMD, flow-mediated dilatation; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; RAS, renin-angiotensin system; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid.