Omega-3 fatty acid therapy dose-dependently and significantly decreased triglycerides and improved flow-mediated dilation, however, did not significantly improve insulin sensitivity in patients with hypertriglyceridemia

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ABSTRACT

Background: Experimental studies demonstrate that higher intake of omega-3 fatty acids ($n-3$ FA) improves insulin sensitivity, however, we reported that $n-3$ FA 2 g therapy, most commonly used dosage did not significantly improve insulin sensitivity despite reducing triglycerides by 21% in patients. Therefore, we investigated the effects of different dosages of $n-3$ FA in patients with hypertriglyceridemia.
Methods: This was a randomized, single-blind, placebo-controlled, parallel study. Age, sex, and body mass index were matched among groups. All patients were recommended to maintain a low fat diet. Forty-four patients (about 18 had metabolic syndrome/type 2 diabetes mellitus) in each group were given placebo, $n-3$ FA 1 (O1), 2 (O2), or 4 g (O4), respectively daily for 2 months.
Results: $n-3$ FA therapy dose-dependently and significantly decreased triglycerides and triglycerides/HDL cholesterol and improved flow-mediated dilation, compared with placebo (by ANOVA). However, each $n-3$ FA therapy did not significantly decrease high-sensitivity C-reactive protein and fibrinogen, compared with placebo. O1 significantly increased insulin levels and decreased insulin sensitivity (determined by QUICKI) and O2 significantly decreased plasma adiponectin levels relative to baseline measurements. Of note, when compared with placebo, each $n-3$ FA therapy did not significantly change insulin, glucose, adiponectin, glycated hemoglobin levels and insulin sensitivity (by ANOVA). We observed similar results in a subgroup of patients with the metabolic syndrome.
Conclusions: $n-3$ FA therapy dose-dependently and significantly decreased triglycerides and improved flow-mediated dilation. Nonetheless, $n-3$ FA therapy did not significantly improve acute-phase reactants and insulin sensitivity in patients with hypertriglyceridemia, regardless of dosages.

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1. Introduction

Epidemiological and clinical evidences suggest a significant inverse association between long-term intake of omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and mortality associated with coronary artery disease [1–3]. Thus, consumption of fish or fish-oil may help prevent adverse consequences of coronary artery disease, especially fatal myocardial infarction and sudden cardiac death. Consumption of omega-3 fatty acids causes improvement in many relevant cardiovascular biomarkers including those represented by hypertriglyceridemia [4], vascular dysfunction [5,6], and inflammation [6]. However, recently, the reported beneficial effects of omega-3 fatty acids remain debated. Indeed, a recent meta-analysis stated that omega-3 fatty acids may protect against vascular disease, but the evidence is not clear-cut, and any benefits are certainly not as great as previously believed [7].

Endothelial dysfunction associated with metabolic syndrome and other insulin resistant states is characterized by impaired nitric oxide (NO) bioavailability and release from endothelium [8–10]. This reduces

blood flow and impairs delivery of substrates and hormones to metabolic target tissues. Thus, improvement in endothelial function is predicted to increase sensitivity to metabolic actions of insulin and improvement in insulin resistance. This may be one mechanism by which omega-3 fatty acids decrease the incidence of coronary heart disease. Adiponectin is one of adipokines secreted specifically by adipose cells [11]. In humans, plasma levels of adiponectin are negatively correlated with adiposity and insulin resistance [11] and low levels of adiponectin are a strong and consistent predictor of the onset and prevalence of type 2 diabetes [12].

Omega-3 fatty acids are used to treat patients with hypertriglyceridemia. Experimental studies demonstrate that higher intake of omega-3 fatty acids improves insulin sensitivity [13,14], however, observational studies report that omega-3 fatty acids are associated with modestly higher incidence of type 2 diabetes [15,16]. We reported that omega-3 fatty acid 2 g therapy, most commonly used dosage did not significantly improve insulin sensitivity despite reducing triglycerides by 21% in patients [17]. Therefore, we investigated the vascular and metabolic effects of different dosages of omega-3 fatty acids in patients with hypertriglyceridemia.

2. Methods

2.1. Study population and design

We used a randomized, single-blind, placebo-controlled, parallel study design. Allocation concealment was achieved by using envelopes with the collaboration of a statistician to ensure that investigators were blinded to interventions. Age, gender, and body mass index were matched among all subjects. We recruited patients from a primary care setting in the Vascular Medicine and Atherosclerosis Unit, Cardiology, Gil Medical Center, Gachon University. We excluded patients with moderate or severe hypertension, uncontrolled diabetes (HbA$_{1c}$ > 9%), nephrotic syndrome, hypothyroidism, coronary artery disease, or peripheral vascular disease. No patient had taken any cholesterol-lowering agent, hormone replacement therapy, or antioxidant vitamin supplements during the 2 months preceding study enrollment. Before and during the study period, a dietitian educated patients to maintain a low fat diet. Activity levels of the subjects were not monitored before or during the study. We randomly administered placebo, omega-3 fatty acid 1, 2, or 4 g to 44 patients with primary hypertriglyceridemia (>150 mg/dl), respectively once daily during a 2 month treatment period. Two patients on placebo and one patient on omega-3 fatty acid 2 g withdrew from the study because they moved to other places and dropped out from the study (Fig. 1). A research nurse counted pills at the end of treatment to monitor compliance. Thus, 42 patients on placebo, 44 patients on omega-3 fatty acid 1 g, 43 patients on omega-3 fatty acid 2 g and 44 patients on omega-3 fatty acid 4 g, respectively, finished the study. Baseline characteristics are in Table 1. About 16 patients among each group had metabolic syndrome according to the definition of National Cholesterol

Table 1
Baseline characteristics of the study population.

	Placebo (n = 42)	Omacor 1 g (O1) (n = 44)	Omacor 2 g (O2) (n = 43)	Omacor 4 g (O4) (n = 44)
Risk factors, n (%)				
Current Smoking	6 (14)	7 (16)	7 (16)	8 (18)
Metabolic Syndrome	15 (36)	16 (36)	14 (33)	16 (36)
Diabetes	2 (5)	3 (7)	3 (7)	2 (5)
Medications, n (%)				
β-Adrenergic blockers	10 (24)	13 (30)	11 (26)	12 (27)
Calcium channel blockers	7 (17)	7 (16)	8 (19)	8 (18)

Education Program Adult Treatment Panel III [18]. Some patients were taking beta adrenergic blockers and/or calcium channel blockers to control blood pressure. No additional medications including aspirin or non-steroidal anti-inflammatory drugs were allowed during the study period to avoid confounding effects of other drugs. Calcium channel or beta adrenergic blockers were withheld for ≥48 h before the study. This study was approved by the Gil Hospital Institutional Review Board and all participants gave written, informed consent.

2.2. Laboratory assays and vascular studies

Blood samples for laboratory assays were obtained at approximately 8:00 a.m. following overnight fasting before and at the end of each 2-month treatment period. These samples were immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence.

Assays for lipids, glucose, and plasma adiponectin were performed in duplicate by ELISA (R & D Systems, Inc., Minneapolis, Minnesota), assays for high sensitivity C-reactive protein (CRP) levels by latex agglutination (CRP-Latex(II)®, Denka-Seiken, Tokyo, Japan) and assays for plasma insulin levels by immunoradiometric assay (INSULIN-RIABEAD® II, SRL, Inc., Tokyo, Japan) and assays for ambient glycemia, glycated hemoglobin (HbA$_{1c}$) by high performance liquid chromatography assay (VARIANT II TURBO®, BIO-RAD, Inc., Hercules, California) as previously described [17,19–24]. The interassay and intraassay coefficients of variation for plasma adiponectin were <6%. Quantitative Insulin-Sensitivity Check Index (QUICKI), a surrogate index of insulin sensitivity based on fasting glucose and insulin levels, was calculated as follows (insulin is expressed in μU/ml and glucose in mg/dl): QUICKI = 1 / [log(insulin) + log(glucose)] [25]. Imaging studies of the right brachial artery were performed using an ATL HDI 3000 ultrasound machine (ATL Philips, Bothell, WA, USA) equipped with a 10 MHz linear-array transducer, based on a previously published technique [17,19,20,22–24].

2.3. Statistical analysis

Data are expressed as mean ± SD or median (range: 25%–75%). We used SigmaPlot 11 (SYSTAT SOFTWARE, Inc.). After testing data for normality, we used Student's paired t or Wilcoxon Signed Rank test to compare values between baseline and treatment at
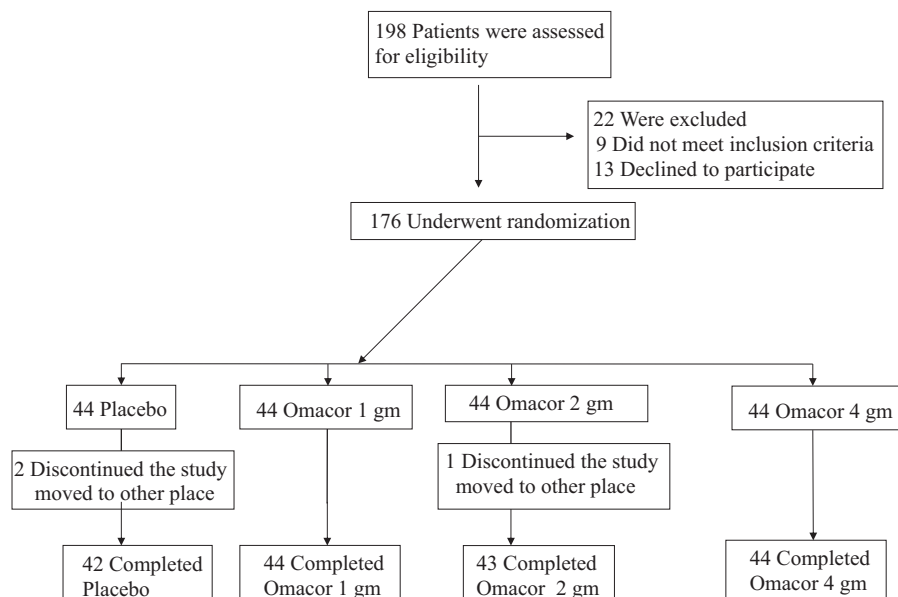

Fig. 1. Flow chart.

2 months, as reported in Tables 2 and 3. We used one way analysis of variance (ANOVA) or Kruskal–Wallis ANOVA on Ranks to compare baseline or treatment effects among treatment groups. Post-hoc comparisons between different treatment pairs were made using the Student–Newman–Keuls multiple comparison procedures or Dunn's method. Pearson or Spearman correlation coefficient analysis was used to assess associations between measured parameters, as reported in Tables 2 and 3. We calculated that 38 subjects would provide 80% power for detecting an absolute increase of 1.6% or greater in flow-mediated dilation of the brachial artery between baseline and omega-3 fatty acid 2 g, with $\alpha = 0.05$ based on our previous studies [17]. The comparison of endothelium-dependent dilation was prospectively designated as the primary end-point of the study. All other comparisons were considered secondary. $P < 0.05$ was considered to represent statistical significance.

3. Results

There were no significant differences between groups for any of the baseline measurements (Tables 2 and 3).

3.1. Effects on lipids

Placebo treatment significantly reduced triglycerides (TG) and TG/high-density lipoprotein (HDL) cholesterol ratio from baseline. Omega-3 fatty acid treatment dose-dependently and significantly reduced TG and TG/HDL cholesterol ratio from baseline. Effects of omega-3 fatty acids on TG levels were significant when compared with placebo treatment ($P < 0.05$ by ANOVA; Fig. 2). Omega-3 fatty acid 2 g and 4 g treatment significantly reduced apolipoprotein AI and non-HDL cholesterol from baseline, respectively. However, omega-3 fatty acid treatment did not significantly change other lipoproteins including total cholesterol, non-HDL cholesterol and HDL cholesterol from baseline. Effects of omega-3 fatty acids on these were not significant when compared with placebo treatment.

3.2. Effects on vasomotor function, high sensitivity C-reactive protein, and fibrinogen

Placebo treatment significantly improved flow-mediated dilator response to hyperemia (FMD) relative to baseline measurements. Omega-3 fatty acid treatment dose-dependently and significantly improved FMD after 2 months of therapy when compared with baseline ($P < 0.001$ by paired t-test) or when compared with placebo treatment ($P < 0.001$ by ANOVA; Fig. 2). Brachial artery dilator responses to nitroglycerin were not significantly different between any of the therapies. Placebo and omega-3 fatty acid treatment did not significantly change high sensitivity CRP and fibrinogen levels relative to baseline measurements except omega-3 fatty acid 4 g increasing fibrinogen levels.

3.3. Effects on adiponectin, glycated hemoglobin, and insulin resistance

Placebo and omega-3 fatty acid treatment did not significantly change insulin or glucose levels from baseline except omega-3 fatty acid 1 g increasing insulin levels. However, the effects of omega-3 fatty acid treatment on fasting insulin and glucose levels were not significant when compared with placebo treatment (Fig. 3). We observed significant inverse correlations between baseline adiponectin and baseline insulin levels ($r = -0.338$, $P = 0.028$ before placebo) and significant correlations between baseline adiponectin levels and baseline QUICKI ($r = 0.451$, $P = 0.003$ before placebo; $r = 0.314$, $P = 0.038$ before omega-3 fatty acid 1 g).

Placebo and omega-3 fatty acids did not significantly change plasma adiponectin levels, insulin sensitivity (determined by QUICKI), or HbA$_{1c}$ levels relative to baseline measurements except omega-3 fatty acid 2 g decreasing adiponectin levels and 1 g decreasing insulin sensitivity. However, the effects of omega-3 fatty acid treatment on these were not significant when compared with placebo treatment (Fig. 4).

Table 2
Effects of placebo or Omacor on lipids and endocrine parameters in patients with hypertriglyceridemia.

	Placebo (n = 42)		Omacor 1 g (O1) (n = 44)		Omacor 2 g (O2) (n = 43)		Omacor 4 g (O4) (n = 44)		Global ANOVA
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	
Age	54 ± 9		55 ± 9		54 ± 9		55 ± 8		0.944
Sex (M:F)	23:19		22:22		23:20		23:21		
BMI	26.50 ± 2.72	26.45 ± 2.71	26.32 ± 3.20	26.35 ± 3.20	26.51 ± 2.63	26.43 ± 2.61	26.18 ± 3.21	26.09 ± 3.19	0.959
Lipids (mg/dl)									
Total cholesterol	201 ± 29	196 ± 31	197 ± 29	193 ± 32	195 ± 31	194 ± 31	198 ± 30	188 ± 32	0.403
Triglycerides	281 ± 63	247 ± 102*	286 ± 73	229 ± 99*	267 ± 118	203 ± 106+	287 ± 73	191 ± 117‡†	0.021
LDL cholesterol	111 ± 34	109 ± 34	109 ± 32	110 ± 33	109 ± 33	113 ± 32	110 ± 33	109 ± 33	0.743
Apo B	107 ± 20	107 ± 23	105 ± 19	103 ± 19	107 ± 22	108 ± 20	107 ± 21	103 ± 18	0.358
HDL cholesterol	42 ± 8	43 ± 7	41 ± 8	43 ± 9	43 ± 7	42 ± 8	40 ± 7	40 ± 8	0.050
Apo A-I	131 ± 15	133 ± 17	128 ± 16	130 ± 17	132 ± 17	126 ± 14*	128 ± 17	125 ± 17	0.096
TG/HDL ratio	7.0 ± 2.4	5.9 ± 2.9*	7.3 ± 2.6	5.6 ± 2.8‡	6.4 ± 2.9	5.2 ± 3.1‡	7.3 ± 2.4	5.0 ± 3.2‡	0.152
Non-HDL	159 ± 27	153 ± 29	157 ± 27	150 ± 31	153 ± 29	153 ± 29	157 ± 28	148 ± 29*	0.263
Vasomotor									
FMD (%)	5.98 ± 1.42	6.31 ± 1.56‡	6.04 ± 1.52	7.61 ± 1.68‡†	5.82 ± 1.54	7.64 ± 1.74‡†	6.03 ± 1.39	8.37 ± 1.51‡†	<0.001
NTG (%)	16.23 ± 3.19	16.59 ± 3.26	16.25 ± 3.34	16.61 ± 4.14	16.44 ± 3.18	16.78 ± 3.83	16.05 ± 2.96	16.45 ± 4.11	0.989
Inflammation									
hsCRP (mg/l)	1.15 (0.50–1.75)	0.95 (0.48–1.93)	1.05 (0.43–1.68)	0.65 (0.33–1.38)	1.00 (0.50–2.10)	0.80 (0.40–1.70)	1.05 (0.53–1.70)	0.85 (0.60–1.68)	0.401
Fibrinogen (mg/dl)	355 ± 69	348 ± 83	353 ± 65	348 ± 62	354 ± 80	359 ± 70	349 ± 61	370 ± 60*	0.070
Insulin resistance									
ADP (μg/ml)	2.6 (1.9–3.8)	2.7 (1.9–4.1)	2.9 (1.5–4.2)	2.3 (1.7–4.1)	2.6 (1.8–4.5)	2.3 (1.7–4.3)*	2.6 (1.5–3.6)	2.2 (1.6–3.5)	0.375
Insulin (μU/ml)	7.4 (5.5–13.7)	9.3 (5.8–13.5)	7.3 (5.1–11.1)	9.7 (5.9–13.8)*	10.1 (6.4–12.8)	7.8 (6.0–12.0)	7.3 (4.2–10.2)	8.5 (5.6–11.1)	0.068
Glucose (mg/dl)	101 ± 15	101 ± 13	98 ± 15	99 ± 14	102 ± 18	99 ± 12	96 ± 15	98 ± 11	0.397
QUICKI	0.35 ± 0.03	0.34 ± 0.04	0.35 ± 0.03	0.34 ± 0.03*	0.34 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.35 ± 0.03	0.222
HbA$_{1c}$ (%)	5.97 ± 0.65	5.94 ± 0.63	5.93 ± 0.62	5.97 ± 0.55	5.94 ± 0.63	5.98 ± 0.61	5.95 ± 0.33	6.01 ± 0.51	0.389

Data are expressed as means ± SD or median.
There were no significant differences among each baseline value.
*$P < 0.05$, +$P < 0.01$, ‡$P < 0.001$ for comparison with each baseline value.
†$P < 0.05$ for comparison with the value after therapy with placebo.
Global ANOVA indicates group differences.
FMD = flow-mediated dilation, NTG = nitroglycerin-induced dilation, HbA$_{1c}$ = glycated hemoglobin, ADP = adiponectin.
Quantitative Insulin-Sensitivity Check Index (QUICKI) = 1 / [log (insulin) + log (glucose)] [25].

Table 3
Effects of placebo or Omacor on lipids and endocrine parameters in patients with hypertriglyceridemia and metabolic syndrome/type 2 diabetes.

	Placebo (n = 17)		Omacor 1 g (O1) (n = 19)		Omacor 2 g (O2) (n = 17)		Omacor 4 g (O4) (n = 18)		Global ANOVA
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	
Age	53 ± 6		54 ± 10		55 ± 8		54 ± 9		0.877
Sex (M:F)	9:8		9:10		8:9		8:10		
BMI	26.41 ± 2.68	26.42 ± 2.72	26.12 ± 3.05	26.06 ± 2.98	25.89 ± 2.40	25.82 ± 2.44	26.18 ± 2.99	26.06 ± 2.90	0.627
Lipids (mg/dl)									
Total cholesterol	206 ± 37	200 ± 38	193 ± 28	190 ± 36	192 ± 38	196 ± 33	194 ± 28	186 ± 32	0.432
Triglycerides	268 ± 66	243 ± 104	274 ± 70	227 ± 82*	231 ± 119	182 ± 135*	276 ± 56	175 ± 80‡	0.055
LDL cholesterol	110 ± 42	107 ± 42	105 ± 30	108 ± 35	111 ± 39	114 ± 39	112 ± 33	113 ± 31	0.904
Apo B	106 ± 23	110 ± 29	105 ± 23	101 ± 23	107 ± 29	107 ± 17	107 ± 22	105 ± 21	0.576
HDL cholesterol	42 ± 7	43 ± 8	42 ± 9	44 ± 10	43 ± 8	42 ± 8	40 ± 9	40 ± 8	0.445
Apo A-I	136 ± 15	135 ± 18	129 ± 17	134 ± 18	130 ± 13	126 ± 11	127 ± 20	123 ± 18	0.204
TG/HDL ratio	6.7 ± 2.6	5.9 ± 3.1	6.8 ± 2.1	5.4 ± 2.5*	5.5 ± 3.1	4.3 ± 2.8+	7.1 ± 2.0	4.5 ± 2.1‡	0.220
Non-HDL	164 ± 36	157 ± 35	152 ± 27	146 ± 35	149 ± 36	154 ± 29	154 ± 27	146 ± 28	0.222
Vasomotor									
FMD (%)	6.03 ± 1.60	6.37 ± 1.81+	5.76 ± 1.30	7.31 ± 1.30‡†	5.62 ± 1.72	7.39 ± 1.98‡†	5.68 ± 1.11	8.32 ± 1.25††	<0.001
NTG (%)	16.16 ± 3.37	16.35 ± 3.43	15.94 ± 3.32	15.97 ± 4.18	15.98 ± 3.36	15.94 ± 4.40	16.31 ± 3.39	17.25 ± 4.55	0.323
Inflammation									
hsCRP (mg/l)	0.90 (0.30–1.75)	0.70 (0.40–1.25)	1.00 (0.40–1.70)	0.50 (0.30–1.00)	1.10 (0.80–2.30)	1.00 (0.45–2.55)	1.15 (0.60–1.75)	0.85 (0.70–1.43)	0.668
Fibrinogen (mg/dl)	336 ± 68	331 ± 72	343 ± 69	331 ± 49	357 ± 77	366 ± 64	361 ± 56	391 ± 60	0.179
Insulin resistance									
ADP (μg/ml)	3.0 (2.0–4.1)	2.9 (1.8–3.9)	2.1 (1.3–4.0)	2.2 (1.6–3.7)	3.4 (1.4–4.5)	3.1 (1.7–4.2)	2.5 (1.4–3.7)	2.3 (1.6–3.6)	0.656
Insulin (μU/ml)	10.1 (5.5–15.3)	8.6 (5.2–12.4)	6.6 (4.0–8.7)	8.9 (5.1–14.7)	8.3 (6.5–13.1)	7.7 (6.3–13.1)	7.4 (4.6–10.7)	8.6 (5.9–10.7)	0.636
Glucose (mg/dl)	103 ± 19	105 ± 15	95 ± 10	96 ± 13	103 ± 13	104 ± 15	97 ± 10	94 ± 8	0.063
QUICKI	0.34 ± 0.03	0.34 ± 0.03	0.36 ± 0.04	0.35 ± 0.04	0.34 ± 0.04	0.34 ± 0.03	0.35 ± 0.04	0.35 ± 0.03	0.793
HbA1c (%)	5.95 ± 0.49	5.95 ± 0.55	5.79 ± 0.35	5.87 ± 0.40	6.12 ± 0.72	6.16 ± 0.73	5.96 ± 0.30	6.01 ± 0.43	0.649

Data are expressed as means ± SD or median.
There were no significant differences among each baseline value.
*$P < 0.05$, +$P < 0.01$, ‡$P < 0.001$ for comparison with each baseline value.
†$P < 0.05$ for comparison with the value after therapy with placebo.
Global ANOVA indicates group differences.
HbA_{1c} = glycated hemoglobin, ADP = adiponectin.
Quantitative Insulin-Sensitivity Check Index (QUICKI) = 1 / [log (insulin) + log (glucose)] [25].

We investigated whether changes in percent flow-mediated dilator response to hyperemia, plasma levels of adiponectin, insulin, insulin resistance, or HbA_{1c} were related to changes in lipoprotein levels. There were no significant correlations between changes in these parameters and changes in lipoprotein levels following any of the therapies. Further, there were no significant correlations between percent changes in adiponectin levels and percent changes in insulin or percent changes in QUICKI following any of the therapies.

3.4. Effects of therapies in patients with metabolic syndrome/type 2 Diabetes mellitus

We analyzed patients with metabolic syndrome/type 2 diabetes mellitus, as reported in Table 3. Overall, compared with the effects of

each therapy in 44 hypertriglyceridemic patients, we observed similar results in patients with metabolic syndrome/type 2 diabetes mellitus. Omega-3 fatty acid treatment dose-dependently and significantly reduced TG and TG/HDL cholesterol ratio and improved FMD after 2 months of therapy when compared with baseline or when compared with placebo treatment. However, omega-3 fatty acid treatment did not significantly change high sensitivity CRP, fibrinogen, plasma adiponectin levels, insulin sensitivity (determined by QUICKI), or HbA_{1c} levels relative to baseline measurements. Of note, the effects of omega-3 fatty acid treatment on these were not significant when compared with placebo treatment.

4. Discussion

We observed that omega-3 fatty acid therapy dose-dependently and significantly decreased triglycerides and triglycerides/HDL cholesterol
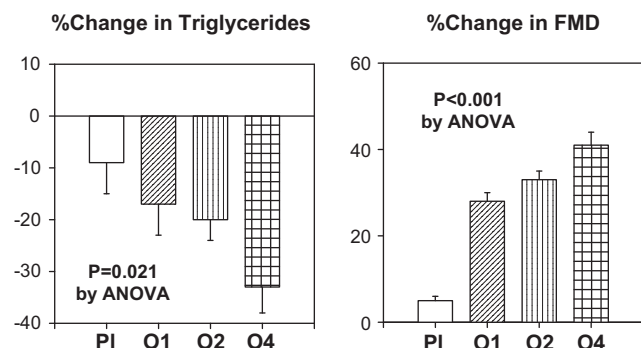

Fig. 2. Omega-3 fatty acid treatment (O1, O2, or O4), dose-dependently and significantly reduced triglyceride levels and improved flow-mediated dilation (FMD) after 2 months of therapy when compared with baseline ($P < 0.001$ by paired t-test) or when compared with placebo treatment ($P = 0.021$ and $P < 0.001$ by ANOVA, respectively). Standard error of the mean is identified by the bars.
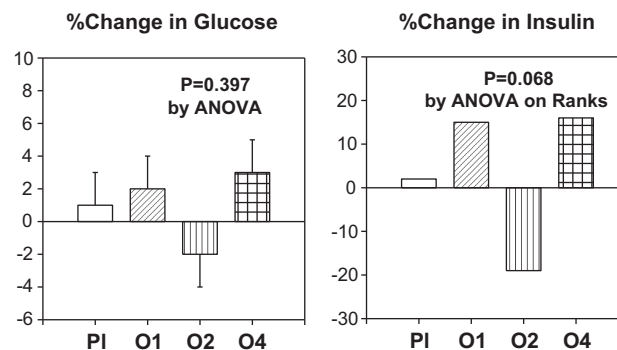

Fig. 3. The effects of omega-3 fatty acid treatment on fasting insulin and glucose levels were not significant when compared with placebo treatment. Median values are used in % change in insulin. Standard error of the mean is identified by the bars.
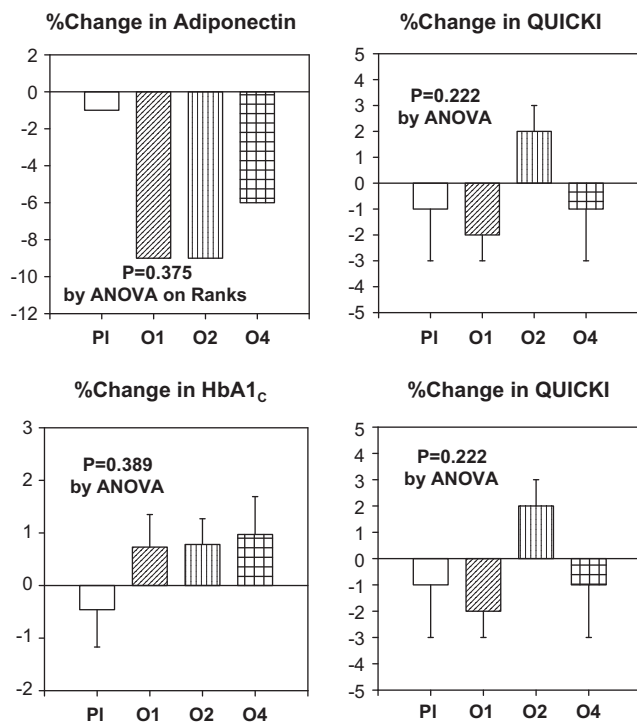
%Change in Adiponectin

%Change in QUICKI

%Change in HbA1$_c$

%Change in QUICKI

Fig. 4. The effects of omega-3 fatty acid treatment on plasma adiponectin levels, insulin sensitivity (determined by QUICKI), or HbA$_{1c}$ levels were not significant when compared with placebo treatment. Median values are used in % change in adiponectin. Standard error of the mean is identified by the bars.

and improved flow-mediated dilation, compared with placebo. Nonetheless, when compared with placebo, omega-3 fatty acid therapy did not significantly change other lipoproteins such as non-HDL cholesterol and HDL cholesterol, high-sensitivity C-reactive protein, fibrinogen, insulin, glucose, adiponectin, glycated hemoglobin levels and insulin sensitivity in patients with hypertriglyceridemia, regardless of dosages. We observed similar results in a subgroup of patients with the metabolic syndrome/type 2 diabetes mellitus.

Several studies reported that omega-3 fatty acids improve flow-mediated arterial dilation [26–28]. This effect of omega-3 fatty acids on endothelial function might be supported by experimental evidences. In the rat fed menhaden oil-rich diets, aortic NO production was increased [29]. EPA also enhanced NO production in cultured human endothelial cells [30] and induced Ca^{2+}-independent activation and translocation of endothelial NO synthase to the cytosol and endothelium-dependent vasorelaxation [31]. In addition, DHA decreased cytokine-induced expression of endothelial leukocyte adhesion molecules and secretion of IL-6 and IL-8 in cultured endothelial cell [32]. However, it remains unclear whether its favorable vasomotor function or anti-inflammatory effects translate to improve insulin sensitivity in patients. For example, our group has demonstrated that statins do not improve but worsen insulin sensitivity in patients despite of improving flow-mediated arterial dilation significantly [19,21,23]. Therefore, a novel point of our current study is to investigate vascular and metabolic phenotype of different dosages of omega-3 fatty acids in patients.

Non-HDL cholesterol is an important one of residual risk factors [33] and has predictive value of cardiovascular events [34,35]. Acute phase reactants such as C-reactive protein have also predictive value of cardiovascular events. However, in the current study, omega-3 fatty acid therapy did not significantly decrease non-HDL cholesterol and HDL cholesterol, high-sensitivity C-reactive protein, fibrinogen, compared with placebo.

When we observed that omega-3 fatty acid 2 g therapy did not significantly improve insulin sensitivity in patients despite reduction of triglycerides and improvement of flow-mediated dilation [17], some

argued high dose may improve [13,14,36]. Therefore, we investigated the effects of low-to-high dose omega-3 fatty acids with a power calculation.

Adiponectin is an adipose-derived factor that augments and mimics both metabolic and vascular actions of insulin [11]. Adiponectin directly stimulates nitric oxide production from endothelium via activation of AMP-activated protein kinase and nitric oxide synthase [37]. Therefore, increasing adiponectin levels is predicted to improve both insulin sensitivity and endothelial function by multiple mechanisms [11]. Regulation of metabolic homeostasis and hemodynamic homeostasis may be coupled by vascular actions of insulin to stimulate production of nitric oxide. Thus, improvements in endothelial function may increase insulin sensitivity while increased insulin sensitivity may improve endothelial function [8,9,38].

QUICKI is a reliable surrogate index for insulin sensitivity that has an especially excellent correlation with the reference standard glucose clamp method in insulin resistant subjects with type II diabetes or obesity [25]. In addition, test characteristics of QUICKI including coefficient of variation and discriminant ratio are significantly better than other simple surrogate indexes and comparable to those of the glucose clamp [39]. A large meta-analysis of insulin resistant subjects demonstrates that QUICKI is among the best surrogate indexes in terms of predictive power for the onset of diabetes [40]. Because measures of insulin resistance were considered secondary in the current study, we used QUICKI to assess insulin sensitivity instead of the reference standard euglycemic glucose clamp technique. Thus, QUICKI is the most extensively validated and accurate surrogate index of insulin sensitivity currently available in humans.

The results of experimental and clinical studies with fish oil and omega-3 fatty acids are controversial. Dietary fish oil increased serum total adiponectin levels in a dietary model of insulin resistance induced by long-term sucrose-rich diet in rats [41]. Experimental studies have demonstrated that dietary fish oils and omega-3 fatty acids increase total adiponectin levels [41–44]. Indeed, one study demonstrated that the G protein-coupled receptor GPR120 is a receptor for omega-3 fatty acids on primary intraperitoneal macrophages and monocytic RAW 264.7 cells and further, activation of GPR120 by omega-3 fatty acids inhibited multiple inflammation cascades in macrophages and reverses insulin resistance in obese mice although this study did not measure adiponectin and acute phase reactant. Since chronic macrophage-mediated tissue inflammation is a key mechanism for insulin resistance in obesity, they fed obese wild type and GPR120 knockout mice a high-fat diet with or without omega-3 fatty acid supplementation. The omega-3 fatty acid treatment inhibited inflammation by observing expression of tumor necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1 and enhanced systemic insulin sensitivity in WT mice by increasing glucose transport and translocation of GLUT4 and enhancing glucose uptake, but was without effect in GPR120 knockout mice [45].

However, other studies are different. EPA significantly decreased adiponectin gene expression and protein secretion in primary cultured rat adipocytes [46]. Omega-3 fatty acids did not significantly increase plasma or high-molecular weight adiponectin levels in overweight-to-moderately obese healthy people [47]. DHA supplementation did not change fasting or postprandial insulin and glucose concentrations and insulin sensitivity, determined by insulin and homeostasis model assessment of insulin resistance (HOMA-IR) in hypertriglyceridemic men [48]. In a meta-analysis of 18 randomized clinical trials, omega-3 fatty acids had no effects on insulin resistance compared to placebo [49]. Instead, some observational studies reported that omega-3 fatty acid or fish consumption was associated with modestly higher incidence of type 2 diabetes [15,16]. In two meta-analyses, fish oil consumption had no overall effects on fasting glucose or HbA$_{1c}$ in patients with type 2 diabetes [50,51]. Overall, it seems that omega-3 fatty acids have no overall effects or increase insulin resistance or diabetes risk, but further investigation is needed.

Metabolic syndrome is associated with atherosclerotic and cardiovascular disease. Patients with metabolic syndrome comprise one of the largest groups of individuals with dyslipidemia and insulin resistance. In the present study, we observed similar results in a subgroup of patients with the metabolic syndrome/type 2 diabetes mellitus.

In addition to epidemiologic studies, recent clinical studies demonstrate that omega-3 fatty acids decreased admission to hospital for cardiovascular reasons and mortality in patients with heart failure [52] or EPA decreased major coronary events, especially non-fatal coronary events, but not sudden cardiac death and coronary death in hypercholesterolemic patients [53]. By contrast, recently published OMEGA and Alpha Omega trial report that low doses of omega-3 fatty acids failed to reduce the rate of major cardiovascular events [54,55].

In summary, omega-3 fatty acid therapy dose-dependently and significantly decreased triglycerides and improved flow-mediated dilation. Nonetheless, omega-3 fatty acid therapy did not significantly improve acute-phase reactants and insulin sensitivity in patients with hypertriglyceridemia, regardless of dosages.

Conflict of interest

None.

Acknowledgment

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