Tohoku J. Exp. Med., 2013, **231**, 63-74

Eicosapentaenoic Acid Improves Glycemic Control in Elderly Bedridden Patients with Type 2 Diabetes

Susumu Ogawa,[1,2] Takaaki Abe,[3,4] Kazuhiro Nako,[1] Masashi Okamura,[1] Miho Senda,[1] Takuya Sakamoto,[1] Sadayoshi Ito[1] and the DIMS study group

[1]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Hospital, Sendai, Miyagi, Japan
[2]Center for the Advancement of Higher Education, Tohoku University, Sendai, Miyagi, Japan
[3]Division of Medical Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Miyagi, Japan
[4]Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are ω3-polyunsaturated fatty acids mainly contained in the blue-backed fish oil, and are effective in decreasing the lipids disorder and the cardiovascular incidence among diabetic patients. Moreover, it has been suggested that EPA and DHA may improve the insulin resistance and glucose metabolism. However, the clinical effects of EPA and DHA on glucose metabolism remain unclear. We aimed to clarify the effects of EPA/DHA treatment on glycemic control in type 2 diabetes mellitus. This study was a multicenter prospective randomized controlled trial involving 30 elderly type 2 diabetic patients on a liquid diet. Their exercises were almost zero and the content of their meals was strictly managed and understood well. Therefore, the difference by the individual's life was a minimum. The subjects were divided into two groups: those receiving EPA/DHA-rich liquid diet [EPA/DHA (+)] or liquid diet lacking EPA/DHA [EPA/DHA (−)]. Changes in factors related to glucose and lipid metabolism were assessed after the three-month study. Serum concentrations of EPA rose in EPA/DHA (+), although the levels of DHA and fasting C-peptide remained unchanged in EPA/DHA (+). In addition, there was a significant decline in the fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), fasting remnant-like particles and apolipoprotein (apo) B in EPA/DHA (+), compared with the values in EPA/DHA (−). EPA/DHA-rich diet might improve glucose metabolism in elderly type 2 diabetic patients on a liquid diet. This phenomenon may be due to the improved insulin resistance mediated by the rise in serum EPA concentrations.

Keywords: arteriosclerosis; docosahexaenoic acid; eicosapentaenoic acid; fasting plasma glucose; insulin resistance
Tohoku J. Exp. Med., 2013 September, **231** (1), 63-74.

Introduction

Although many Americans suffer myocardial infarction, this disease is rare among Alaskan Eskimos, and researchers discovered that eicosapentaenoic acid (EPA), a polyunsaturated fatty acid abundant in sardines, mackerels and other blue-backed fish, may explain this phenomenon (Parkinson et al. 1994). EPA causes a reduction in serum triglycerides (TGs) and it suppresses the progression of arteriosclerosis, and as such, it is also likely to improve glucose metabolism (Lopez-Huertas 2012; Ikeya et al. 2013). Similar effects are apparently exerted by docosahexaenoic acid (DHA) (Horrocks and Yeo 1999), and it has been suggested that the combined use of DHA and EPA may produce a synergistic effect, strongly augmenting the aforementioned effects of these agents alone (Calder 2012; Lopez-Huertas 2012). However, the clinical effects of EPA and DHA on glucose metabolism remain unclear. These phenomena prompted us to test the effects of EPA/DHA on the glucose metabolism of diabetic patients in a clinical setting.

However, the Japanese diet usually involves the consumption of more blue-backed fish than that of Europeans or Americans. Hence, in a study administering EPA/DHA to ordinary Japanese diabetic patients, the difference in the amount of EPA/DHA administered to the experimental and control groups would probably not be particularly significant (Kusunoki et al. 2007). Indeed, as the food intake and physical exercise may differ greatly between diabetic outpatients, a large number of patients would be required to

Received July 12, 2013; revised and accepted September 3, 2013. Published online September 25, 2013; doi: 10.1620/tjem.231.63.
Correspondence: Susumu Ogawa, M.D., Ph.D., Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Hospital, 1-1, Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan.
e-mail: ogawa-s@hosp.tohoku.ac.jp

ensure that the differences in these factors do not influence the results. By contrast, bedridden patients who receive only a liquid diet have a controlled food intake, which allows us to accurately specify the amount of EPA/DHA administered and accordingly, to establish clear differences in the serum EPA/DHA concentrations between the group administered EPA/DHA and the controls. Moreover, there is no need to consider differences in the amount of physical exercise, as this is practically zero in these bedridden patients. Thus, it was thought that by selecting bedridden diabetic patients on liquid diet alone, a meaningful study could be carried out with even a small number of subjects. Therefore, we chose bedridden elderly patients with type 2 diabetes whose nutrition was obtained exclusively through a liquid diet to participate in our study.

We administered two distinct types of liquid diets that have the same carbohydrate content but that differ in the lipid content (one of which was rich in EPA and DHA), and we investigated the changes in the glycolipid metabolism and in arteriosclerotic factors of the patients.

Patients and Methods

This was a multicenter prospective randomized controlled trial targeting elderly type 2 diabetic patients who were exclusively fed by enteral nutrition (liquid diet only administered *via* a tube). The main objective was to assess the changes in fasting plasma glucose (FPG) concentrations caused by a diet rich in EPA and DHA, established as the primary endpoint. However, the effects of this diet on other parameters were also assessed as secondary endpoints, including changes in glycated hemoglobin A1c (HbA1c), fasting serum fatty acid composition, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), apolipoprotein-B (apo-B), apolipoprotein-A1 (apo-A1), plasma adiponectin, tumor necrosis factor α (TNFα), monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6, urinary 8-hydroxydeoxyguanosine (8-OHdG), 2-hour and 4-hour postprandial plasma glucose concentration, and fasting, 2-hour and 4-hour postprandial serum TGs, C-peptide (C-pep), and remnant-like particles (RLPs).

All the subjects were bedridden patients that were not enrolled on any physiotherapy or other programs. Patients whose treatment had been changed recently before the start of the study, or those who were likely to experience changes in their treatment regimen during the study period, were excluded from the study. DIMS was adopted as the liquid diet rich in EPA (25 mg/100 kcal) and DHA (17 mg/100 kcal), while CZ1.5 was adopted as the standard liquid diet that lacks both EPA and DHA [EPA/DHA (−)]. Both DIMS and CZ1.5 are approved liquid diets used in Japan and both of these are products marketed by Clinico Co., Ltd (Tokyo, Japan). DIMS and CZ1.5 are both liquid diet products that contain more or less the same amount of carbohydrates, lipids and protein. The difference is that EPA and DHA are present in DIMS but not in CZ1.5 (Table 1). The more detail description of composition of fatty acids in the test meals is as follows. In DIMS, the contents of fatty acids are caprylic acid (10.0%), capric acid (3.5%), myristic acid (0.5%), palmitic acid (9.5%), palmitoleic acid (0.5%), stearic acid (3.3%), oleic acid (37.0%), linoleic acid (26.0%), α-linolenic acid (8.0%), EPA (1.0%) and DHA (0.7%). In CZ1.5, the contents are caprylic acid (12.0%), capric acid (9.0%), palmitic acid (4.0%), stearic acid (3.0%), oleic acid (37.0%), linoleic acid (27.0%), and α-linolenic acid (8.0%). Thus, CZ1.5 also lacks myristic acid and palmitoleic acid. No subjects in this study had been fed with either of the two diets before they were enrolled onto the trial, and the subjects were randomly assigned to CZ1.5 [EPA/DHA (−)] group or DIMS [EPA/DHA (+)] group. Their diet was switched to the corresponding diet at the start of the study. No major differences were observed in terms of the caloric, carbohydrate, protein or lipid content of the liquid diets that were administered during or prior to commencing the study (1,000.0 ± 211.0 kcal/day in CZ 1.5 group, 1,015.4 ±231.1 kcal/day in DIMS group).

Table 1. Composition of the test meals.

Liquid diet name		DIMS (/100 kcal)	CZ1.5 (/100 kcal)
Volume	(mL)	100	67
Protein	(g)	4.0	4.0
Fat	(g)	2.8	3.0
EPA	(mg)	25	–
DHA	(mg)	17	–
Carbohydrates	(g)	14.3	14.0
Diet fiber	(g)	2.4	1.0
Water	(g)	84	52

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

DIMS and CZ1.5 are liquid diets that contain more or less the same amount of carbohydrates, lipids and protein. They differ in that DIMS contains EPA and DHA, while CZ1.5 does not.

The height and body weight of each subject was measured, and blood samples were drawn, just before they began to receive each diet (= baseline), as well as at the end of the 3-month study. Blood sampling was performed on fasting (0 hour), as well as 2 and 4 hours after meals.

Based on the findings of a preliminary survey, to perform a meaningful study (with a power analysis of α = 5% and power = 80%), and with a change in FPG values between the two study groups and the standard deviation (S.D.) hypothesized as 40 mg/dL, the number of subjects required was 15. The ratio of dropouts and other losses was anticipated to be 30% and thus, 20 subjects per group were required. Accordingly, a total of 40 patients were set as the optimal sample size. Randomization was done by not minimization methods but the usual method of using random sampling numbers.

Plasma MCP-1, IL-6 and TNFα were measured using the MCP-1 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., MN, USA), the IL-6 ELISA kit (R&D Systems, Inc., MN, USA) and the human ultra-sensitive TNFα ELISA kit (BioSource International, California, USA). To measure urinary 8-OHdG, the Highly Sensitive 8-OHdG Check ELISA kit (Japan Institute for the Control of Aging, NIKKEN SEIL Co., Ltd., Fukuroi, Japan) was used.

Blood samples were obtained before breakfast and fasting levels of serum fatty acids, including EPA, DHA and arachidonic acid (AA) were determined. In brief, plasma lipids were extracted using the Folch procedure, and the fatty acids were then methylated with boron trifluoride and methanol, using tricosanoic acid (C23:0) as an internal standard. These methylated fatty acids were then analyzed

by capillary gas chromatography [HP 6890 (Hewlett-Packard) with a flame ionization detector and a DB-WAX 30 m × 0.32 mm ID column].

Apo-A1 and apo-B measurements

A turbidimetric immunoassay (TIA), N-Assay Apo A1-H Nittobo (Nittobo Medical Co., Ltd., Fukushima, Japan) was used to measure apo-A1, and the N-assay TIA Apo B-H Nittobo (Nittobo Medical Co., Ltd., Fukushima, Japan) was used to measure apo-B.

The study fully conformed to the Helsinki Declaration and it was approved by the ethics committee of the Tohoku University Hospital. It was also pre-registered as a randomized controlled trial in the University hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000001645). Tests were only performed after they had been fully explained to the subjects and/or their families, and obtaining their informed consent.

Statistical analysis

Numerical values that showed a normal distribution are presented as the mean ± standard error of the mean (SEM), and those that did not show a normal distribution (such as IL-6 values) are presented as their median value (range). A Student's *t*-test was used to compare the numerical data prior to and after treatment within the same group. As logarithmic-converted values of IL-6 conformed to a normal distribution, such data were used in the analysis instead of the raw data concerning its analysis. The Spearman test was used to examine the significance of the correlation coefficient and multiple regression analysis was used to determine the independent risk factors for the percentage changes in HbA1c and FPG. $p < 0.05$ was designated as the level of statistically significance.

Results

The process of registering the study subjects and allocating them to the two study groups was shown in Fig. 1. Of the 40 subjects registered, 10 dropped out as they were unable to continue the study after the Great East Japan Earthquake that occurred on March 11, 2011. Fifteen patients were therefore allocated randomly to each of the two groups. However, two subjects dropped out of each group due to death through senility or due to major changes in their physical condition (two suffered infection, one suffered myocardial infarction, and the other suffered the recurrence of cerebral infarction). As a result, the final number of subjects analyzed was 13 in each group. Cerebral infarction or cerebral hemorrhage was often cited as the reason the subjects were switched to a liquid diet (cerebral infarction = 8, cerebral hemorrhage = 3, pneumonia = 1, fall and fractures = 4 in CZ group, and cerebral infarction = 8, cerebral hemorrhage = 3, fall and fractures = 4, and Parkinson's disease = 2 in DIMS group). There were two or more causes in some patients. Concomitant hypertension and/or hyperlipidemia, as well as diabetes, was often considered to have caused these diseases (hypertension = 7, hyperlipidemia = 4, chronic renal failure = 2, and dementia = 4 in CZ group, and hypertension = 8, hyperlipidemia = 3, chronic renal failure = 2, and dementia = 3 in DIMS group). The subjects in both groups were being administered a wide range of treatments for diabetes, and no changes were made to their medication regimen, including the dosages, during the study period. None of the sub-

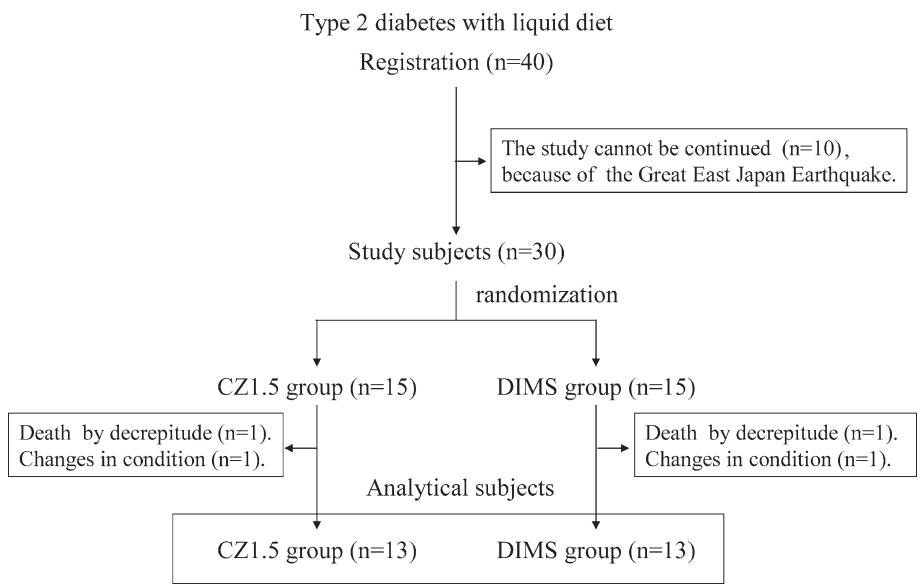

Fig. 1. The flowchart representing the procedure for registration and allocation of the study subjects.
Of the 40 subjects registered, ten dropped out due to being unable to continue the study after the Great East Japan Earthquake (March 11, 2011). CZ1.5 is the EPA/DHA (−) group, DIMS group is the EPA/DHA (+) group. Fifteen subjects were therefore allocated, to each of the two groups (DIMS group; $n = 15$, CZ1.5 group; $n = 15$). However, two subjects dropped out of each group because of death due to old age or major changes in their physical condition (two suffered infection, one suffered myocardial infarction, and one suffered a recurrence of cerebral infarction). As a result, the number of subjects analyzed was 13 in each group. Cerebral infarction and cerebral hemorrhage are often cited as the diseased causing subjects to be switched to a liquid diet.

Table 2. Values of various parameters in the CZ1.5 and DIMS groups prior to and at the end of the study.

Group		Total	CZ1.5			DIMS		
number		26	13			13		
gender	(M/F)	6 / 20	2 / 11			4 / 9		
age	(years)	80.4 ± 8.3	81.2 ± 7.6			79.5 ± 8.6		
			before	after	P1	before	after	P2
BMI	(kg/m^2)	20.1 ± 3.6	20.4 ± 3.6	20.3 ± 3.6	0.15	19.9 ± 4.0	20.1 ± 4.0	< 0.01
TP	(g/dL)	7.2 ± 0.7	7.0 ± 0.7	7.1 ± 0.7	0.20	7.2 ± 0.7	7.0 ± 0.4	0.13
Alb	(g/dL)	3.5 ± 0.4	3.3 ± 0.4	3.4 ± 0.4	0.12	3.7 ± 0.4	3.6 ± 0.4	0.19
eGFR	(mL/min/1.73 m^2)	83.4 ± 39.2	86.3 ± 52.2	86.7 ± 65.2	0.95	80.6 ± 19.1	72.9 ± 23.4 **	0.07
FPG	(mg/dL)	119.7 ± 36.7	104.5 ± 24.5	123.6 ± 29.5	0.02	135.0 ± 40.0	104.5 ± 27.4	< 0.01
C-pep	(μg/mL)	1.3 ± 1.1	1.5 ± 1.1	1.7 ± 1.1	0.17	1.1 ± 1.1	1.3 ± 1.1	0.06
HbA1c	(%)	6.9 ± 1.1	6.7 ± 1.1	7.0 ± 0.7	0.06	7.1 ± 0.7	6.6 ± 0.7	< 0.01
TG	(mg/dL)	132.6 ± 100.1	158.1 ± 126.4	162.9 ± 119.5	0.77	107.1 ± 51.8	119.8 ± 77.4	0.64
TC	(mg/dL)	172.6 ± 48.6	156.8 ± 54.0	159.4 ± 34.6	0.81	188.3 ± 36.4	174.5 ± 30.2	0.06
HDL-C	(mg/dL)	47.1 ± 10.4	47.2 ± 11.5	45.0 ± 13.7	0.16	47.0 ± 9.4	45.6 ± 9.4	0.22
FFA	(mEq/L)	0.7 ± 0.4	0.7 ± 0.4	0.8 ± 0.4	0.26	0.7 ± 0.4	0.7 ± 0.4	0.80
RLP	(mg/dL)	8.6 ± 7.9	8.8 ± 9.7	8.1 ± 6.5	0.60	8.3 ± 5.8	6.6 ± 5.8	< 0.01
apo A1	(mg/dL)	125.3 ± 17.6	125.5 ± 19.4	121.8 ± 22.0	0.24	125.1 ± 15.5	119.9 ± 15.1	0.10
apo B	(mg/dL)	82.5 ± 21.6	75.1 ± 20.2	72.4 ± 21.6	0.11	89.9 ± 19.8	79.5 ± 14.8	0.01
apo B/ apo A1 *		0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.93	0.7 ± 0.1	0.7 ± 0.1	0.06

P1, before vs. after the study in CZ1.5 group; P2, before vs. after the study in DIMS group. Mean ± S.D.

M, male; F, female; BMI, body mass index; TP, total serum protein; Alb, serum albumin; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; C-pep, plasma C-peptide; HbA1c, glycated haemoglobin A1c (NGSP value); TG, serum triglycerides; TC, total serum cholesterol; HDL-C, serum high density lipoprotein cholesterol; FFA, serum free fatty acid; RLP, serum remnant like particle; apo, apolipoprotein.

*The values raised one digit significant digits of apo B / apo A1 in the CZ1.5 and DIMS groups prior to and at the end of the study: 0.66 ± 0.11 (total), 0.60 ± 0.14 (before in CZ1.5 group), 0.60 ± 0.18 (after in CZ1.5 group), 0.72 ± 0.14 (before in DIMS group), 0.67 ± 0.11 (after in DIMS group).

**The eGFR of DIMS group seems to be reduced. It is because there were two DIMS group subjects whose serum creatinine rises, though the precise cause is uncertain. Moderate dehydrations were suspected as the cause, because the examination periods of the two subjects were in the summer. The eGFR of the entire DIMS group is not a significant decrease. ($p = 0.07$)

jects was able to take solid food orally and thus, all of them received only enteral nutrition of a liquid diet through a tube. Moreover, they were unable to walk on their own or to engage in physical exercise. No major differences were observed in terms of the caloric, carbohydrate, protein or lipid content of the liquid diets that had been given to the individuals in the CZ1.5 and the DIMS groups prior to commencing the study.

The values of each parameter at the end of the 3-month study were compared with their corresponding values obtained immediately prior to the study for each subject in the CZ1.5 and DIMS groups (Table 2). There was a significant rise in FPG ($p = 0.02$) and tendency towards a rise in HbA1c ($p = 0.06$) in the CZ1.5 group. Conversely, there was a significant increase in the body mass index (BMI; $p < 0.01$) and a significant reduction in FPG, HbA1c, RLP and apo B ($p < 0.01$) in the DIMS group, in addition to a tendency for estimated glomerular filtration rate (eGFR: $p = 0.07$), TC ($p = 0.06$) and apo B/apo A ratio ($p = 0.06$) to diminish, as well as a tendency for C-pep to increase ($p = 0.06$).

The values of markers of inflammation and oxidative stress were determined prior to and at the end of the study, and the values were compared within the same group (Table 3). While there were no significant changes in any of these markers in CZ1.5 group, the subjects in the DIMS group demonstrated a significant reduction in all these markers except adiponectin ($p = 0.01$ for TNFα, $p = 0.02$ for log IL-6, and $p < 0.01$ for MCP-1 and 8-OHdG).

Metabolic factors were measured 0, 2 and 4 hours after administration of the liquid diet, and the values obtained prior to the initiation of the study were compared with those obtained at the end of the study (Table 4 and Fig. 2). There was a significant rise in the FPG and 2-hour value of C-pep ($p < 0.05$) in the CZ1.5 group, but no significant changes were observed in other factors (Fig. 2A, B, C and D). By contrast, the RLP levels at 0, 2 and 4 hours after administration of the diet were each lower at the end of the study than at the baseline in the DIMS group. However, the postprandial rise in RLPs observed in the baseline measurements were not affected by the 6-month treatment and thus, a decline was only evident in the fasting values (Fig. 2I and

Table 3. Values of inflammatory markers and oxidative stress markers in the CZ1.5 and DIMS groups prior to and at the end of the study.

		Total	CZ1.5		P1	DIMS		P2
			before	after		before	after	
Plasma								
adiponectin	(μg/mL)	15.3 ± 9.4	13.2 ± 10.4	13.7 ± 11.9	0.61	17.4 ± 9.0	19.2 ± 10.4	0.16
TNFα	(pg/mL)	2.9 ± 1.4	2.9 ± 1.8	2.8 ± 1.8	0.83	2.8 ± 1.1	1.8 ± 0.7	0.01
IL-6	(pg/mL)	3.7 (0.4-111.0)	6.3 (1.0-108.0)	6.0 (2.0-17.7)		3.0 (0.4-111.0)	2.1 (0.6-21.6)	
log IL-6	(pg/mL)	0.7 ± 0.7	0.7 ± 0.4	0.8 ± 0.4	0.41	0.7 ± 0.7	0.4 ± 0.4	0.02
MCP-1	(pg/mL)	208.5 ± 82.1	192.1 ± 82.1	193.5 ± 91.8	0.94	224.9 ± 79.2	150.2 ± 41.0	< 0.01
8-OHdG	(ng/mg Cre)	32.0 ± 22.0	23.4 ± 10.8	31.4 ± 14.8	0.10	40.5 ± 26.3	23.3 ± 23.0	< 0.01

P1, before vs. after the study in CZ1.5 group; P2, before vs. after the study in DIMS group. Mean ± S.D.

TNFα, tumor necrosis factor alpha; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; 8-OHdG, urinary 8-hydroxy-deoxyguanosine; Cre, urinary creatinine excretion.

Table 4. Various metabolic factors 0, 2 and 4 hours after administration of each liquid diet.

		CZ1.5			DIMS		
		before	after	P1	before	after	P2
Glucose	(mg/dL)						
0		104.5 ± 24.5	123.6 ± 29.5	0.02	135.0 ± 40.0	104.5 ± 27.4	< 0.01
2		212.0 ± 83.9	220.6 ± 94.7	0.63	238.2 ± 91.1	193.4 ± 60.5	0.06
4		181.2 ± 56.9	168.3 ± 60.1	0.34	191.0 ± 58.0	169.3 ± 55.8	0.22
C-peptide	(μg/mL)						
0		1.5 ± 1.1	1.7 ± 1.1	0.17	1.1 ± 1.1	1.3 ± 1.1	0.06
2		3.7 ± 3.2	4.5 ± 3.2	0.01	4.5 ± 4.7	3.2 ± 2.9	0.25
4		3.9 ± 2.9	4.3 ± 2.9	0.18	3.9 ± 4.0	3.3 ± 2.9	0.27
Triglyceride	(mg/dL)						
0		158.1 ± 126.4	162.9 ± 119.5	0.77	107.1 ± 51.8	119.8 ± 77.4	0.64
2		186.5 ± 132.1	218.8 ± 144.0	0.10	138.7 ± 87.8	130.3 ± 91.8	0.30
4		177.2 ± 145.4	193.9 ± 132.1	0.37	134.4 ± 83.9	125.5 ± 90.7	0.19
Free fatty acid	(mEq/L)						
0		0.7 ± 0.4	0.8 ± 0.4	0.26	0.7 ± 0.4	0.7 ± 0.4	0.80
2		0.4 ± 0.4	0.4 ± 0.4	0.73	0.2 ± 0.0	0.2 ± 0.0	0.75
4		0.4 ± 0.4	0.4 ± 0.0	0.26	0.2 ± 0.0	0.2 ± 0.0	0.39
RLPs	(mg/dL)						
0		8.8 ± 9.7	8.1 ± 6.5	0.62	8.3 ± 5.8	6.6 ± 5.8	0.01
2		9.2 ± 9.7	9.9 ± 6.8	0.80	8.9 ± 6.5	6.7 ± 6.8	0.01
4		10.4 ± 11.2	9.3 ± 6.5	0.56	8.3 ± 6.8	6.7 ± 6.8	0.02

P1, before vs. after the study in CZ1.5 group; P2, before vs. after the study in DIMS group. Mean ± S.D.

RLPs, serum remnant like particles.

J). There was a significant drop in the FPG values in the DIMS group at the end of the 6-month study period, although the fasting C-pep levels remained unchanged (with a tendency to rise; $p = 0.06$). The postprandial rise in plasma glucose and C-pep seen prior to the study in DIMS group was not suppressed at the end of the 6-month study (Fig. 2B and D).

Serum levels of various fatty acids were also determined prior to and at the end of the study (Table 5). In the CZ1.5 group there was a significant rise in linolenic acid ($p < 0.01$) and a significant drop in lignoceric acid ($p = 0.03$), with no significant changes detected in other fatty acids. By contrast, in the DIMS group there was a significant decline in lauric acid ($p = 0.04$), myristoleic acid ($p = 0.02$), oleic acid ($p < 0.01$), γ-linolenic acid ($p = 0.03$), eicosenoic acid ($p = 0.04$), erucic acid ($p = 0.02$), eicosatrienoic acid ($p = 0.01$) and docosatetraenoic acid ($p < 0.01$), as well as a significant rise in EPA ($p < 0.01$), docosapentaenoic acid ($p = 0.01$) and the EPA/AA ratio ($p < 0.01$). The decrease in dihomo-γ-linolenic acid and AA was only marginal ($p =$

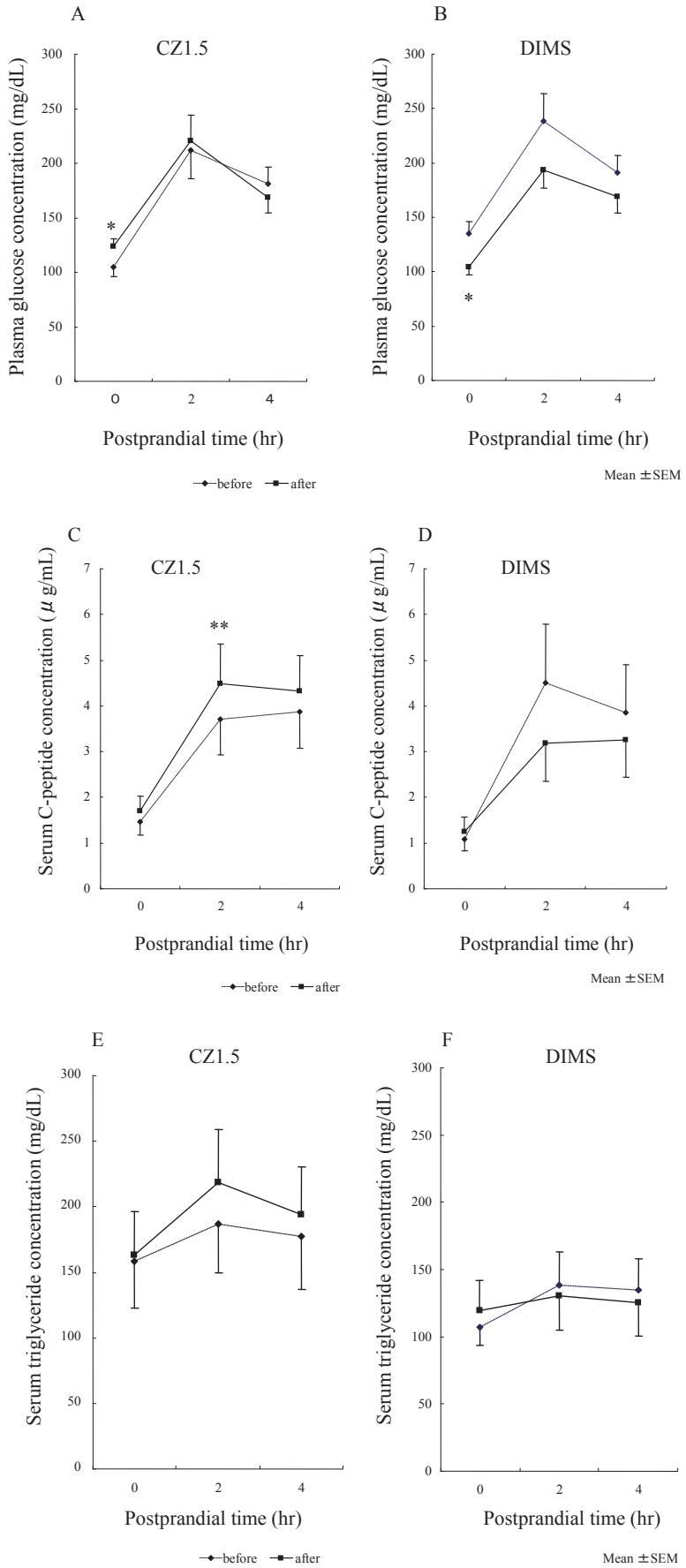
A
CZ1.5
B
DIMS
Plasma glucose concentration (mg/dL)
Postprandial time (hr)
before
after
Mean ±SEM
C
CZ1.5
D
DIMS
Serum C-peptide concentration (μg/mL)
Postprandial time (hr)
before
after
Mean ±SEM
E
CZ1.5
F
DIMS
Serum triglyceride concentration (mg/dL)
Postprandial time (hr)
before
after
Mean ±SEM

Fig. 2.

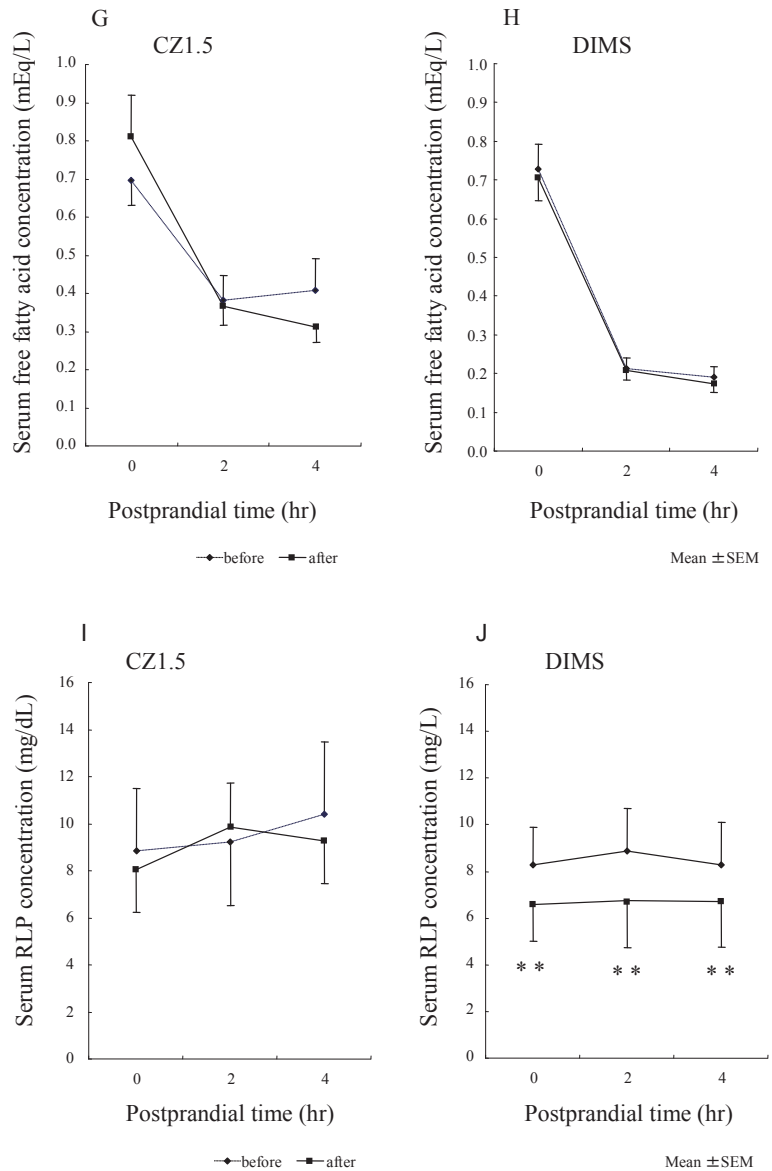

Fig. 2. The changes in metabolic factors after consumption of each liquid diet.
These panels display the values of the various metabolic factors 0, 2 and 4 hours after consumption of each liquid diet, prior to and at the end of the 3-month study. There was a significant rise in the fasting plasma glucose and in the C-pep level 2 hours after consumption at the end of the study in comparison to those values prior to the study in the CZ1.5 group (A and C, $p < 0.05$), although no significant changes were observed in other factors. By contrast, the RLP levels 0, 2 and 4 hours postprandially dropped at the end of the study compared to their respective values prior to the study in the DIMS group. However, the postprandial rise in RLPs that was evident at baseline was not corrected even after the 3-month treatment, resulting in a decline in the fasting values alone (J). There was no change in the RLP levels in the CZ1.5 group at all times and under all conditions analyzed (I). There was a significant drop in FPG (B) but the fasting C-pep levels remained unchanged with a tendency to rise (D, $p = 0.06$) in the DIMS group at the end of the 3-month study period. The postprandial rise seen in plasma glucose and in C-pep in the DIMS group prior to the study was not suppressed after the 3-month study (B and D). The 3-month diet provoked no significant changes in serum levels of triglycerides or free fatty acids at any time point in either of the two groups (E to H). $*p < 0.01$, $**p < 0.05$.

0.06 for both cases). Interestingly, the serum levels of DHA remained unchanged not only in the CZ1.5 group ($p = 0.15$) but also, in the DIMS group ($p = 0.14$).

The comparisons between the delta (absolute) changes and percentage changes of various parameters before and after treatment between the CZ1.5 group and the DIMS group were shown in Table 6. We designated the parameters that showed changes in Tables 1-4 as parameters for analysis, as well as apo A1 and DHA levels. The DIMS group showed significantly larger increases of BMI, EPA, and DHA, and significantly larger reductions of FPG, HbA1c, RLP, apo B, 8-OHdG, TNFα, IL-6, MCP-1, oleic

Table 5. Serum levels of various fatty acids.

		CZ1.5			DIMS		
		before	after	P1	before	after	P2
lauric	(μg/mL)	2.9 ± 34.2	2.0 ± 0.7	0.08	2.8 ± 1.4	1.7 ± 1.1	0.04
myristic	(μg/mL)	28.9 ± 19.1	25.5 ± 17.3	0.30	22.4 ± 12.2	21.1 ± 8.3	0.63
myristoleic	(μg/mL)	2.8 ± 2.9	1.8 ± 1.4	0.16	2.4 ± 1.4	1.1 ± 0.7	0.02
palmitic	(μg/mL)	624.2 ± 324.4	558.4 ± 335.5	0.30	602.4 ± 169.6	556.2 ± 124.9	0.13
palmitoleic	(μg/mL)	94.5 ± 74.5	89.2 ± 59.8	0.56	90.0 ± 46.4	81.9 ± 40.0	0.18
stearic	(μg/mL)	222.3 ± 68.0	176.0 ± 67.0	0.07	200.6 ± 74.2	189.4 ± 57.2	0.49
oleic	(μg/mL)	629.8 ± 356.8	662.2 ± 340.6	0.44	702.0 ± 279.0	591.7 ± 203.4	< 0.01
linoleic	(μg/mL)	787.9 ± 265.0	742.5 ± 273.6	0.71	679.2 ± 158.8	706.5 ± 164.5	0.50
γ-linolenic	(μg/mL)	11.6 ± 6.8	30.6 ± 68.0	0.36	13.5 ± 9.4	10.5 ± 7.9	0.03
linolenic	(μg/mL)	53.1 ± 18.4	69.2 ± 22.3	< 0.01	39.5 ± 18.0	46.5 ± 22.0	0.18
arachidic	(μg/mL)	1.8 ± 0.7	1.7 ± 0.7	0.67	1.6 ± 0.7	1.4 ± 0.7	0.37
eicosenoic	(μg/mL)	6.2 ± 1.8	6.7 ± 2.2	0.36	6.2 ± 2.2	5.4 ± 2.2	0.04
eicosadienoic	(μg/mL)	8.6 ± 2.9	7.5 ± 2.5	0.25	7.1 ± 3.0	7.0 ± 2.2	0.47
eicosatrienoic	(μg/mL)	3.5 ± 2.5	3.2 ± 1.8	0.67	5.4 ± 3.6	3.3 ± 2.9	0.01
dihomo-γ -linolenic	(μg/mL)	44.4 ± 14.4	42.8 ± 19.1	0.54	45.6 ± 15.5	39.5 ± 16.9	0.06
arachidonic	(μg/mL)	109.9 ± 28.8	99.6 ± 45.4	0.47	141.9 ± 49.3	133.0 ± 48.2	0.06
eicosapentaenoic	(μg/mL)	38.8 ± 16.2	35.9 ± 16.9	0.59	33.1 ± 16.9	73.2 ± 21.2	< 0.01
behenic	(μg/mL)	1.5 ± 0.7	2.0 ± 1.1	0.16	1.5 ± 1.1	2.2 ± 1.4	0.24
erucic	(μg/mL)	1.1 ± 0.4	0.7 ± 0.4	0.08	1.6 ± 0.7	0.9 ± 0.7	0.02
docosatetraenoic	(μg/mL)	3.7 ± 1.4	3.6 ± 1.4	0.89	4.9 ± 2.5	3.5 ± 2.2	< 0.01
docosapentaenoic	(μg/mL)	16.9 ± 6.1	17.2 ± 6.1	0.89	19.2 ± 10.1	22.5 ± 11.5	0.01
lignoceric	(μg/mL)	1.1 ± 0.4	0.7 ± 0.4	0.03	0.9 ± 0.4	1.0 ± 0.7	0.84
docosahexaenoic	(μg/mL)	55.4 ± 19.4	46.5 ± 21.2	0.15	66.2 ± 25.6	78.7 ± 28.1	0.14
nervonic	(μg/mL)	2.5 ± 1.1	2.2 ± 1.4	0.65	3.1 ± 1.8	2.7 ± 1.8	0.59
Total	(μg/mL)	2,753.0 ± 946.4	2,803.0 ± 992.5	0.71	2,621.4 ± 776.2	2,580.6 ± 624.2	0.74
EPA/AA ratio		0.3 ± 0.0	0.3 ± 0.4	0.85	0.3 ± 0.0	0.6 ± 0.4	< 0.01

P1, before vs. after the study in CZ1.5 group; P2, before vs. after the study in DIMS group. Mean ± S.D.
EPA, eicosapentaenoic acid; AA, arachidonic acid.

acid, eicosatrienoic acid, and docosatetraenoic acid than the CZ1.5 group.

The comparisons between the correlation coefficients of the percentage changes in the parameters analyzed in Table 6 as well as the EPA/AA ratio and the apo B/apo A1 ratio, and the percentage changes in FPG, between the CZ1.5 group and the DIMS group were shown in Table 7. In the CZ1.5 group, none of the parameters correlated significantly with the percentage change in FPG. In contrast, in the DIMS group, the percentage changes in FPG correlated positively with the percentage changes in HbA1c, 8-OHdG, TNFα, IL-6, MCP-1, and the apo B/apo A1 ratio, and correlated negatively with the percentage changes in apo A1, EPA, DHA, and the EPA/AA ratio. EPA (r = –0.82) showed the strongest correlation with percentage change in FPG, followed by DHA (r = –0.58) and the EPA/AA ratio (r = –0.42).

A multiple regression analysis was performed using the %change of HbA1c as a dependent variable and administration of DIMS, age, gender, %change of BMI and %change of eGFR as independent variables. This analysis found only the administration of DIMS (β = –0.65, p = 0.02) to be an independent factor associated with the %change in HbA1c.

Discussion

The objective of the present study was to clarify whether a diet containing a small amount of EPA/DHA improves glycemic control in patients with diabetes mellitus. Ingestion of such a diet resulted in a rise in the serum EPA concentrations.

EPA/DHA

EPA and DHA alter membrane fluidity, interact with transcription factors such as peroxisome proliferator-activated receptor (PPAR: insulin sensitivity regulator) and sterol regulatory element binding protein (lipids metabolism regulator), and are substrates for enzymes including cyclooxygenase, lipoxygenase and cytochrome P450 (inflammation, platelet aggregation, vasoconstriction regulators). As

Table 6. The comparisons between the delta changes and percentage changes of various parameters before and after treatment between the CZ-1.5 group and the DIMS group.

		Δ change		P1	% change (%)		P2
		CZ1.5	DIMS		CZ1.5	DIMS	
BMI	(kg/m^2)	−0.1 ± 0.2	0.26 ± 0.2	0.01	−0.6 ± 1.2	1.28 ± 0.8	< 0.01
FPG	(mg/dL)	19.2 ± 25.5	−30.5 ± 29.6	< 0.01	22.8 ± 37.3	−20.8 ± 16.2	0.01
HbA1c	(%)	0.3 ± 0.5	−0.5 ± 0.4	< 0.01	5.0 ± 8.6	−7.3 ± 5.4	< 0.01
RLP	(mg/dL)	−0.8 ± 5.3	−1.7 ± 1.9	0.04	8.7 ± 41.4	−21.8 ± 18.8	0.04
Apo B	(mg/dL)	−2.6 ± 5.5	−10.4 ± 11.0	0.01	−3.5 ± 8.9	−10.5 ± 8.1	0.04
Apo Al	(mg/dL)	−3.8 ± 10.7	−5.2 ± 10.2	0.27	−2.9 ± 7.5	−3.8 ± 8.7	0.38
8-OHdG	(ng/mg Cre)	8.0 ± 15.6	−17.3 ± 18.3	< 0.01	51.8 ± 76.9	−44.5 ± 22.3	< 0.01
TNFα	(pg/mL)	−0.1 ± 1.1	−1.0 ± 1.1	0.04	−2.3 ± 44.0	−25.8 ± 51.3	0.02
IL-6	(pg/mL)	−6.1 ± 24.6	−10.6 ± 23.8	0.04	42.2 ± 96.4	−23.9 ± 52.1	0.04
MCP-1	(pg/mL)	1.5 ± 60.9	−74.8 ± 49.5	0.01	5.1 ± 38.3	−27.9 ± 18.5	0.03
Lauric	(μg/mL)	−0.9 ± 1.6	−1.1 ± 1.6	0.76	−14.2 ± 35.4	−24.4 ± 54.1	0.64
Myristoleic	(μg/mL)	−1.0 ± 2.3	−1.3 ± 1.7	0.70	−22.3 ± 58.1	−29.1 ± 59.5	0.68
Oleic	(μg/mL)	32.5 ± 141.2	−110.3 ± 105.5	< 0.01	8.1 ± 22.7	−13.9 ± 10.8	< 0.01
γ-linolenic	(μg/mL)	19.0 ± 68.4	−3.0 ± 4.2	0.28	198.6 ± 679.7	−16.4 ± 25.8	0.29
Eicosenoic	(μg/mL)	0.5 ± 1.8	−0.7 ± 1.1	0.08	12.3 ± 34.5	−10.7 ± 17.0	0.06
Eicosatrienoic	(μg/mL)	−0.3 ± 2.3	−2.1 ± 2.5	0.04	11.5 ± 61.7	−39.5 ± 33.8	0.02
EPA	(μg/mL)	−2.8 ± 17.5	40.2 ± 15.7	< 0.01	−1.4 ± 31.1	201.2 ± 258.5	< 0.01
Erucic	(μg/mL)	−0.4 ± 0.7	−0.7 ± 0.9	0.33	−16.6 ± 54.4	−27.3 ± 62.4	0.67
Docosatetraenoic	(μg/mL)	−0.1 ± 1.5	−1.4 ± 1.2	0.03	2.0 ± 36.6	−28.2 ± 21.1	0.03
Docosapentaenoic	(μg/mL)	0.3 ± 6.8	3.2 ± 3.9	0.24	7.6 ± 40.5	20.0 ± 27.3	0.44
DHA	(μg/mL)	−8.9 ± 20.0	12.5 ± 27.6	0.03	−11.5 ± 36.8	30.9 ± 51.9	0.02

Mean ± S.D.

P1, P2, CZ1.5 group vs. DIMS group.

BMI, body mass index; FPG, fasting plasma glucose concentration; HbA1c, glycated haemoglobin A1c; RLP, remnant like particle; Apo B, apolipoprotein B; Apo A1, apolipoprotein A1; 8-OHdG, urinary 8-hydroxydeoxyguanosine; TNFα, tumor necrosis factor alpha; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acids.

a result, EPA and DHA may improve cardiovascular health by altering lipid metabolism, inducing hemodynamic changes, decreasing arrhythmias, modulating platelet function, improving endothelial function and inhibiting inflammatory pathways (Cottin et al. 2011). Besides these fatty acids, dietary fiber is thought to have the potential to influence the postprandial rise in blood glucose (Sako et al. 2010) and the onset of cardiovascular events (Wallström et al. 2012). The DIMS diet contains 1.4 g/100 kcal more dietary fiber than CZ1.5 does (Table 1), and this may have contributed to the effects of EPA. However, since no improvements in postprandial blood glucose were observed, we conclude that dietary fiber had only a minor influence on blood glucose.

Low levels of EPA and DHA in the blood are associated with the onset of cardiovascular events and indeed, EPA/DHA replacement therapy has been reported to suppress inflammation and inhibit cardiovascular events (Rupp et al. 2004). Alternatively, it has also been noted that even when EPA and DHA are administered, it is only EPA/AA ratio that is associated with the inhibition of cardiovascular events (Rupp et al. 2006). It was recently revealed, however, that similar effects can be obtained by administering DHA alone, and that DHA is strongly linked to the inhibition of cerebral arteriosclerosis (Kim et al. 2012). One mechanism attributed to the anti-atherosclerotic effects of EPA/DHA, such as these highlighted above, is that EPA/DHA are PPAR-gamma (PPARγ) ligands that improve insulin resistance by stimulating PPARγ activity, while they inhibit inflammatory reactions, such as the activation of macrophages and IL-6 secretion, by suppressing nuclear factor kappa B (NF-κB) activity (Li et al. 2005; Draper et al. 2011; Jung et al. 2012). EPA/DHA was recently reported to induce the Nrf2 signaling pathway, which leads to the suppression of oxidative stress, resulting in the inhibition of macrophage activation and of inflammation (Massaro et al. 2008; Wang et al. 2010; Majkova et al. 2011; Takaki et al. 2011; Contreras et al. 2012; Malekshahi Moghadam et al. 2012). EPA is also thought to activate macrophages and to suppress the secretion of MCP-1 and inflammatory cytokines, by modifying the decomposition of very low density lipoprotein (VLDL) in macrophages (Jinno et al. 2011; Majkova et al. 2011). These effects of EPA, improving insulin resistance and suppressing TNFα,

Table 7. The comparisons between the correlation coefficients of the percentage changes in the parameters and the percentage changes in fasting plasma glucose, between the CZ1.5 group and the DIMS group.

	The percentage changes of FPG			
% Change of	CZ1.5 group	P1	DIMS group	P2
BMI	−0.06	0.89	−0.08	0.72
HbA1c	0.14	0.11	0.36	0.02
RLP	0.18	0.09	0.08	0.65
ApoB	0.19	0.08	0.17	0.09
ApoA1	0.04	0.70	−0.37	0.02
8-OHdG	−0.04	0.43	0.24	0.04
TNFα	−0.09	0.31	0.33	0.02
IL-6	−0.14	0.15	0.40	0.01
MCP-1	−0.11	0.12	0.35	0.02
Lauric	0.30	0.06	0.11	0.24
Myristoleic	0.09	0.38	−0.04	0.38
Oleic	−0.31	0.06	0.01	0.85
γ-linolenic	0.15	0.07	−0.24	0.09
Eicosenoic	−0.02	0.79	0.10	0.18
Eicosatrienoic	−0.34	0.05	0.32	0.05
EPA	−0.19	0.09	−0.82	< 0.01
Erucic	−0.27	0.07	−0.21	0.15
Docosatetraenoic	−0.06	0.68	0.10	0.17
Docosapentaenoic	−0.07	0.57	−0.06	0.65
DHA	0.03	0.73	−0.58	< 0.01
EPA/AA ratio	0.01	0.91	−0.42	< 0.01
ApoB/ApoA1 ratio	−0.14	0.19	0.33	0.01

BMI, body mass index; HbA1c, glycated haemoglobin A1c; RLP, remnant like particle; Apo B, apolipoprotein B; Apo A1, apolipoprotein A1; 8-OHdG, urinary 8-hydroxydeoxyguanosine; TNFα, tumor necrosis factor alpha; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acids.

MCP-1 and IL-6, were confirmed in our study, and the suppression of these cytokines is likely to be one of the reasons for the reduction in insulin resistance. The suppression of macrophage-inflammation by EPA is thought to be more significant than that of DHA (Mickleborough et al. 2009), although the converse has also been reported (Weldon et al. 2007). In our study, EPA was significantly more powerful than DHA, and we assume this to be in part due to the increase of EPA in the blood associated with the administration of the DIMS diet while the concentrations of DHA remained unchanged. In other words, as the doses of EPA (254 mg/day, on average) and DHA (173 mg/day, on average) administered in our study were substantially below the recommended dosage, DHA might have failed to reach its effective blood concentration (Mullen et al. 2010). Here, DHA is of particular interest. This parameter showed no significant changes between before and after treatment, even in the DIMS group. Unlike the changes seen in the CZ1.5 group, DHA increased, relatively speaking, in the DIMS group, even when compared with the CZ1.5 group. In the DIMS group, however, the percentage change in DHA showed a correlation with percentage change in FPG. This showed a negative correlation. DHA did not end up showing a statistically significant increase, since some patients saw their serum DHA levels increase while others saw them decrease, before and after treatment with DIMS. However, the DIMS group had a greater number of subjects with elevated serum DHA levels and a greater rate of increase than the CZ1.5 group. It appears that, in the DIMS group, FPG decreased more markedly in subjects who saw their DHA increase sharply than those who did not. EPA increased significantly as a result of DIMS treatment, and the rate of increase correlated the most strongly with FPG's rate of improvement. Therefore, the increase in serum EPA levels may have been involved the most closely with a reduction in FPG, with DHA playing an auxiliary role. The dosages used on this occasion, moreover, elevated the serum EPA level, but may have been insufficient to elevate the serum DHA level. Researchers suggest that DHA may increase blood EPA concentrations, and that the effects of EPA and DHA on the blood vessels differ (Kelley et al. 2008; Cottin et al. 2011; Kelley and Adkins 2012; Mozaffarian and Wu 2012). Thus combined use of DHA with EPA may induce synergistic effects, augmenting the

favorable effects exerted by EPA when administered alone (Mozaffarian and Wu 2012).

An EPA/DHA-mediated rise in adiponectin levels may be linked to lower insulin resistance (Mohammadi et al. 2012), although in our study the levels of adiponectin remained unchanged. This may be due the high levels of adiponectin before the start of the study as the subjects recruited were extremely thin.

Apo B/apo A1 ratio

A rise in the apo B/apo A1 ratio is a risk factor for intracranial arteriosclerosis (Park et al. 2011) and it is also a risk factor for developing retinopathy (Sasongko et al. 2011). In addition, the apo B/apo A1 ratio is a predictor of future increases in insulin resistance in non-diabetic individuals (Sierra-Johnson et al. 2007), and it is an independent risk factor for cardiovascular diseases (Thompson and Danesh 2006).

EPA/DHA is reported to decrease RLPs (Nakamura et al. 1998; Kelley et al. 2008). As a decrease in RLPs induces decreases in large VLDL and small dense low-density lipoprotein (Kelley et al. 2007), not only reducing the number of lipoprotein particles but also, altering the quality of the particles caused by the acceleration of the breakdown of TGs may be involved in the rise in the apo B/apo A ratio caused by EPA/DHA administration (Cottin et al. 2011; Jinno et al. 2011; Kelley and Adkins 2012). However, it is difficult to distinguish which is the cause and which is the effect, the suppression of the apo B/apo A1 ratio or the effects of improving FPG. These two phenomena may be strongly related through a common mechanism to improve insulin resistance. Moreover, it was also recently suggested that EPA/DHA may reinforce hypoglycemic actions by increasing the secretion of glucagon-like peptied-1 from the digestive tract (Hirasawa et al. 2005; Senmaru et al. 2012) The percentage changes in the apo B/apo A1 ratio correlated positively with the percentage change in FPG (Table 7). Essentially, however, it was a correlation not with increased apo B ($r = 0.17$, $p = 0.09$), but between decreased apo A1 ($r = –0.37$, $p = 0.02$) and increased FPG. Patients whose FPG had dropped sharply as a result of improved insulin resistance may be liable to see their apo A1 (HDL-C) levels rise.

Limitations

The biggest problem with this study is the very small sample size and thus, it will be necessary to carry out further studies on a larger scale.

Acknowledgements

The authors acknowledge the editorial assistance and clinical support of Miss Manami Simizu for their help with preparing the references and for their expert assistance with the management of blood and urinary samples. We would like to express our sincere gratitude to all the staff at the facilities for the elderly who assisted us with this study. And we acknowledge the clinical support of the DIMS group for expert management with health care support and for the case's registrations.

The DIMS study group

Satoko Akai, Masao Ando (Kanagami Hospital), Osamu Akaishi, Takashi Akaishi (Akaishi Hospital), Mikihito Ishiki (Special nursing home for aged Koujyuen, Iwate Prefectural Takata Hospital), Akihiro Kawauchi (Ikebukuro Hospital), Sumiko Kijima (Abe Hospital), Masayuki Onishi (Niigata Minami Hospital), Yoichi Otsuka (Tomiya Central Hospital), Kazuhiko Sato (Ohizumi Memorial Hospital), Ryouichi Torisu (Kodaira Chuo Rehabilitation Hospital), Mitihiro Tsumori (Yamane Hospital Misumibunin), and Yuko Yamane (Yamane Hospital).

Sources of Funding

This work was supported by a 21st Century Center of Excellence Program Special Research Grant from the Ministry of Education, Sports and Culture, a research grant for cardiovascular research (13C-5) from the Japanese Ministry of Health, Labor and Welfare, Longitudinal/Cross-Sectional Studies to Generate Evidence for the Diagnosis/Management of Metabolic Syndrome for Health Guidance, and Longitudinal/Cross-Sectional Studies to Generate Evidence for Diagnosis/Management of Metabolic Syndrome in Governmental Health Check-up and Guidance System.

Author Contribution

S.O. planned the design and conducts of the study, collected data, interpreted data and wrote the manuscript, contributing to the discussion. K.N., M.O., M.S., and T.S. contributed to the discussion and DIMS group collected data. T.A. and S.I. contributed to the discussion and revised the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

Calder, P.C. (2012) The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Mol. Nutr. Food Res.*, **56**, 1073-1080.

Contreras, G.A., Mattmiller, S.A., Raphael, W., Gandy, J.C. & Sordillo, L.M. (2012) Enhanced n-3 phospholipid content reduces inflammatory responses in bovine endothelial cells. *J. Dairy Sci.*, **95**, 7137-7150.

Cottin, S.C., Sanders, T.A. & Hall, W.L. (2011) The differential effects of EPA and DHA on cardiovascular risk factors. *Proc. Nutr. Soc.*, **70**, 215-231.

Draper, E., Reynolds, C.M., Canavan, M., Mills, K.H., Loscher, C.E. & Roche, H.M. (2011) Omega-3 fatty acids attenuate dendritic cell function via NF-κB independent of PPARγ. *J. Nutr. Biochem.*, **22**, 784-790.

Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S. & Tsujimoto, G. (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.*, **11**, 90-94.

Horrocks, L.A. & Yeo, Y.K. (1999) Health benefits of docosahexaenoic acid (DHA). *Pharmacol. Res.*, **40**, 211-225.

Ikeya, Y., Fukuyama, N., Kitajima, W., Ogushi, Y. & Mori, H. (2013) Comparison of eicosapentaenoic acid concentrations in plasma between patients with ischemic stroke and control subjects. *Nutrition*, **29**, 127-131.

Jinno, Y., Nakakuki, M., Kawano, H., Notsu, T., Mizuguchi, K. & Imada, K. (2011) Eicosapentaenoic acid administration attenuates the pro-inflammatory properties of VLDL by decreasing its susceptibility to lipoprotein lipase in macrophages. *Athero-*

sclerosis, **219**, 566-572.
Jung, U.J., Torrejon, C., Chang, C.L., Hamai, H., Worgall, T.S. & Deckelbaum, R.J. (2012) Fatty acids regulate endothelial lipase and inflammatory markers in macrophages and in mouse aorta: a role for PPAR*γ*. *Arterioscler. Thromb. Vasc. Biol.*, **32**, 2929-2937.
Kelley, D.S. & Adkins, Y. (2012) Similarities and differences between the effects of EPA and DHA on markers of atherosclerosis in human subjects. *Proc. Nutr. Soc.*, **71**, 322-331.
Kelley, D.S., Siegel, D., Vemuri, M., Chung, G.H. & Mackey, B.E. (2008) Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. *J. Nutr.*, **138**, 30-35.
Kelley, D.S., Siegel, D., Vemuri, M. & Mackey, B.E. (2007) Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am. J. Clin. Nutr.*, **86**, 324-333.
Kim, Y.J., Kim, O.Y., Cho, Y., Chung, J.H., Jung, Y.S., Hwang, G.S. & Shin, M.J. (2012) Plasma phospholipid fatty acid composition in ischemic stroke: importance of docosahexaenoic acid in the risk for intracranial atherosclerotic stenosis. *Atherosclerosis*, **225**, 418-424.
Kusunoki, M., Tsutsumi, K., Nakayama, M., Kurokawa, T., Nakamura, T., Ogawa, H., Fukuzawa, Y., Morishita, M., Koide, T. & Miyata, T. (2007) Relationship between serum concentrations of saturated fatty acids and unsaturated fatty acids and the homeostasis model insulin resistance index in Japanese patients with type 2 diabetes mellitus. *J. Med. Invest.*, **54**, 243-247.
Li, H., Ruan, X.Z., Powis, S.H., Fernando, R., Mon, W.Y., Wheeler, D.C., Moorhead, J.F. & Varghese, Z. (2005) EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. *Kidney Int.*, **67**, 867-874.
Lopez-Huertas, E. (2012) The effect of EPA and DHA on metabolic syndrome patients: a systematic review of randomised controlled trials. *Br. J. Nutr.*, **107**, S185-194.
Majkova, Z., Layne, J., Sunkara, M., Morris, A.J., Toborek, M. & Hennig, B. (2011) Omega-3 fatty acid oxidation products prevent vascular endothelial cell activation by coplanar polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.*, **251**, 41-49.
Malekshahi Moghadam, A., Saedisomeolia, A., Djalali, M., Djazayery, A., Pooya, S. & Sojoudi, F. (2012) Efficacy of omega-3 fatty acid supplementation on serum levels of tumour necrosis factor-alpha, C-reactive protein and interleukin-2 in type 2 diabetes mellitus patients. *Singapore Med. J.*, **53**, 615-619.
Massaro, M., Scoditti, E., Carluccio, M.A. & De Caterina, R. (2008) Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. *Prostaglandins Leukot. Essent. Fatty Acids*, **79**, 109-115.
Mickleborough, T.D., Tecklenburg, S.L., Montgomery, G.S. & Lindley, M.R. (2009) Eicosapentaenoic acid is more effective than docosahexaenoic acid in inhibiting proinflammatory mediator production and transcription from LPS-induced human asthmatic alveolar macrophage cells. *Clin. Nutr.*, **28**, 71-77.
Mohammadi, E., Rafraf, M., Farzadi, L., Asghari-Jafarabadi, M. & Sabour, S. (2012) Effects of omega-3 fatty acids supplementation on serum adiponectin levels and some metabolic risk factors in women with polycystic ovary syndrome. *Asia Pac. J. Clin. Nutr.*, **21**, 511-518.
Mozaffarian, D. & Wu, J.H. (2012) (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J. Nutr.*, **142**, 614S-625S.
Mullen, A., Loscher, C.E. & Roche, H.M. (2010) Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages. *J. Nutr. Biochem.*, **21**, 444-450.
Nakamura, N., Hamazaki, T., Kobayashi, M., Ohta, M. & Okuda, K. (1998) Effects of eicosapentaenoic acids on remnant-like particles, cholesterol concentrations and plasma fatty acid composition in patients with diabetes mellitus. *In Vivo*, **12**, 311-314.
Park, J.H., Hong, K.S., Lee, E.J., Lee, J. & Kim, D.E. (2011) High levels of apolipoprotein B/AI ratio are associated with intracranial atherosclerotic stenosis. *Stroke*, **42**, 3040-3046.
Parkinson, A.J., Cruz, A.L., Heyward, W.L., Bulkow, L.R., Hall, D., Barstaed, L. & Connor, W.E. (1994) Elevated concentrations of plasma omega-3 polyunsaturated fatty acids among Alaskan Eskimos. *Am. J. Clin. Nutr.*, **59**, 384-388.
Rupp, H., Rupp, T.P., Wagner, D., Alter, P. & Maisch, B. (2006) Microdetermination of fatty acids by gas chromatography and cardiovascular risk stratification by the "EPA+DHA level". *Herz.*, **31**, 30-49.
Rupp, H., Wagner, D., Rupp, T., Schulte, L.M. & Maisch, B. (2004) Risk stratification by the "EPA+DHA level" and the "EPA/AA ratio" focus on anti-inflammatory and antiarrhythmogenic effects of long-chain omega-3 fatty acids. *Herz.*, **29**, 673-685.
Sako, T., Mori, A., Lee, P., Goto, H., Fukuta, H., Oda, H., Saeki, K., Miki, Y., Makino, Y., Ishioka, K., Mizutani, H., Kojima, Y., Koikeda, S. & Arai, T. (2010) Supplementing transglucosidase with a high-fiber diet for prevention of postprandial hyperglycemia in streptozotocin-induced diabetic dogs. *Vet. Res. Commun.*, **34**, 161-172.
Sasongko, M.B., Wong, T.Y., Nguyen, T.T., Kawasaki, R., Jenkins, A., Shaw, J. & Wang, J.J. (2011) Serum apolipoprotein AI and B are stronger biomarkers of diabetic retinopathy than traditional lipids. *Diabetes Care*, **34**, 474-479.
Senmaru, T., Fukui, M., Kobayashi, K., Iwase, H., Inada, S., Okada, H., Asano, M., Yamazaki, M., Hasegawa, G., Nakamura, N., Iwasaki, M., Yabe, D., Kurose, T. & Seino, Y. (2012) Dipeptidyl-peptidase IV inhibitor is effective in patients with type 2 diabetes with high serum eicosapentaenoic acid concentrations. *J. Diabetes Invest.*, **3**, 498-502.
Sierra-Johnson, J., Romero-Corral, A., Somers, V.K., Lopez-Jimenez, F., Walldius, G., Hamsten, A., Hellénius, M.L. & Fisher, R.M. (2007) ApoB/apoA-I ratio: an independent predictor of insulin resistance in US non-diabetic subjects. *Eur. Heart J.*, **28**, 2637-2643.
Takaki, A., Umemoto, S., Ono, K., Seki, K., Ryoke, T., Fujii, A., Itagaki, T., Harada, M., Tanaka, M., Yonezawa, T., Ogawa, H. & Matsuzaki, M.; ELIA study group. (2011) Add-on therapy of EPA reduces oxidative stress and inhibits the progression of aortic stiffness in patients with coronary artery disease and statin therapy: a randomized controlled study. *J. Atheroscler. Thromb.*, **18**, 857-866.
Thompson, A. & Danesh, J. (2006) Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. *J. Intern. Med.*, **259**, 481-492.
Wallström, P., Sonestedt, E., Hlebowicz, J., Ericson, U., Drake, I., Persson, M., Gullberg, B., Hedblad, B. & Wirfält, E. (2012) Dietary fiber and saturated fat intake associations with cardiovascular disease differ by sex in the Malmö Diet and Cancer Cohort: a prospective study. *PLoS One*, **7**, e31637.
Wang, H., Khor, T.O., Saw, C.L., Lin, W., Wu, T., Huang, Y. & Kong, A.N. (2010) Role of Nrf2 in suppressing LPS-induced inflammation in mouse peritoneal macrophages by polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid. *Mol. Pharm.*, **7**, 2185-2193.
Weldon, S.M., Mullen, A.C., Loscher, C.E., Hurley, L.A. & Roche, H.M. (2007) Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J. Nutr. Biochem.*, **18**, 250-258.