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Dose-dependent effects of fish oil on cardio-metabolic biomarkers in healthy middle-aged and elderly Chinese people: a double-blind randomized controlled trial

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n-3PUFA consumption has been widely accepted as a nutritional strategy for the secondary prevention of cardiovascular events in patients at high risk of cardiovascular disease (CVD), but little is known about the dose–response relationship between dietary *n*-3PUFA and serum biomarkers associated with cardiovascular health in the general population. The present study involved a 12-week double-blind, randomized controlled trial to explore the effects of fish oil with different doses (0.31, 0.62 and 1.24 g d^{-1} of EPA and DHA) on serum fatty acids and cardio-metabolic biomarkers including adiponectin, inflammatory markers, lipid profiles and fasting glucose in healthy middle-aged and elderly Chinese people. 240 volunteers met our inclusion criteria. A total of 39 subjects dropped out and 201 finally completed the intervention. No significant differences in baseline characteristics and daily intakes of dietary nutrients were detected among all groups. After a 12-week intervention, fish oil dose-dependently enhanced serum EPA, DHA, *n*-3PUFA and adiponectin (except for 0.31 g d^{-1}), but decreased serum *n*-6/*n*-3PUFA, TG and fasting glucose. Changes in the above indicators from the baseline to week 12 in fish oil groups significantly differed from those in the control. Meanwhile, all the doses of EPA and DHA led to decreases in serum CRP; only 1.24 g d^{-1} led to an increase in HDL-C with a concurrent decrease in TC/HDL-C even though these changes were not significantly different among all groups. All the findings suggested that fish oil dose-dependently regulated serum PUFA and cardio-metabolic biomarkers including adiponectin, CRP, lipid profiles and fasting glucose in healthy middle-aged and elderly Chinese people who consumed insufficient dietary *n*-3PUFA, and the most desirable changes were observed for 1.24 g d^{-1}.

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1. Introduction

Cardiovascular disease (CVD) have emerged as the leading cause of death worldwide. Data updated by the World Health Organization demonstrated that in 2015, approximately 7.4 million people died from coronary heart disease (CHD) and 6.7 million died from stroke.[1] A reduction in morbidity and mortality of CVD is considered to be of utmost importance to improve public health.

In addition to medicine and surgery, nutritional intervention has been recognized as an effective strategy for the primary and secondary prevention of CVD. Several dietary nutrients were previously reported to reduce the risk of cardiovascular events like fibre[2] and vitamin E,[3] among which *n*-3 polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from fatty fish draw the most attention. On the basis of data obtained from large randomized controlled trials (RCTs) such as the Japan EPA Lipid Intervention Study (JELIS)[4] and the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial,[5] the American Heart Association (AHA) recently recommended *n*-3PUFA (EPA + DHA) supplementation to patients with prevalent CHD or heart failure with reduced left ventricular ejection fraction to reduce mortality.[6]

The underlying mechanisms for the cardio-protective actions of *n*-3PUFA or fish oil (a reliable source of EPA and DHA) remain to be elucidated. Adiponectin, an adipocyte-derived adipokine with anti-atherogenic, anti-diabetic and anti-inflammatory properties,[7] has been identified as a protective molecule in limiting the pathogenesis of CVD.[8]

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Individuals with high levels of circulating adiponectin had less chance than those with low levels to develop myocardial infarction[9] and CHD.[10] A meta-analysis performed by Wu J. H. *et al.*[11] showed that a fish oil supplement moderately increases the circulating adiponectin level (3.7 μg ml^{-1}, 95% confidence interval: 0.07, 0.67) even though unexplained heterogeneity and potential publication bias existed. Increasing adiponectin is therefore hypothesized as a possible mechanism by which *n*-3PUFA protects against CVD. Besides, fish oil is also reported to exert its cardio-protective activities by lowering serum triglyceride (TG) and inhibiting the inflammatory response.[12]

It is noteworthy that findings from previous research studies assessing the effects of dietary *n*-3PUFA on circulating adiponectin and other CVD-related parameters like lipid profiles and inflammatory markers have been controversial, and most available RCTs recruited individuals at high risk or patients with prevalent CVD rather than healthy subjects.[13–18] Furthermore, little is known about the proper dose of EPA and DHA in relation to cardiovascular health. Hence, we carried out a 12-week double-blind, randomized controlled trial to explore the effects of fish oil with different doses (0.31, 0.62 and 1.24 g d^{-1} of EPA and DHA) on serum PUFA and cardio-metabolic biomarkers including serum adiponectin, inflammatory markers, lipid profiles and fasting glucose in healthy middle-aged and elderly Chinese people. We hypothesized that fish oil consumption improved serum PUFA and cardio-metabolic biomarkers in a dose-dependent manner in healthy adults.

2. Materials and methods

2.1 Subjects

Healthy middle-aged and elderly people were recruited in a community health service centre in Liwan District, Guangzhou, Guangdong, China. The inclusion criteria were: age above 40 years; local residents for more than 3 years; and normal lipids or borderline hypertriglyceridemia (TG: 1.70–2.25 mmol L^{-1}). The exclusion criteria were: obesity [body mass index (BMI) ≥ 28 kg m^{-2} according to the Chinese criteria]; a history of hypertension, CVD, type 2 diabetes (T2D), digestive system diseases and cancers; use of fish oil or lipid-lowering supplements (or drugs) within the latest 3 months; fish allergy; and obstacles to communication. All participants signed their written informed consent before the randomization. This trial was approved by the ethics committee of the Medical Sciences Department of Wuhan University in accordance with the Helsinki Declaration, and is registered at http://www.chictr.org.cn (ChiCTR-IOR-17012053).

2.2 Study design

240 participants were enrolled in this double-blind randomized controlled trial and randomly assigned to 4 groups: control, fish oil group 1 (FO1), fish oil group 2 (FO2) and fish oil group 3 (FO3). Randomization was conducted by a statistician using computer-generated random numbers. Subjects in FO1, FO2 and FO3 groups were daily administered 1, 2 and 4 fish oil capsules (By-Health, Zhuhai, Guangdong, CHN) equivalent to 0.31, 0.62 and 1.24 g d^{-1} of EPA and DHA (EPA/DHA = 1.5 : 1) respectively for 12 weeks, while the control did not take any fish oil supplement. The doses of fish oil corresponded to the acceptable macronutrient distribution ranges of EPA plus DHA (0.25–2 g d^{-1}) recommended by the Chinese Nutrition Society and the amount of *n*-3 PUFA consumption (≈1 g d^{-1}) suggested by the AHA for patients with CHD.[6] Subjects were asked to maintain their routine dietary habits and record the actual intake of fish oil in self-made monitoring tables during the intervention. Telephone and face-to-face follow-up was performed in the 7th week. Compliance with the treatment was determined by counting the remaining fish oil capsules and serum levels of EPA and DHA at week 12.

2.3 Anthropometric measurements and dietary assessment

Anthropometric measurements were carried out at the baseline and at the end of the intervention. Doctors measured height, body weight, waist circumference, hip circumference and blood pressure with professional instruments which had been calibrated. BMI and waist–hip ratio (WHR) were calculated: BMI = body weight (kg)/height (m)/height (m); WHR = waist circumference (cm)/hip circumference (cm). A dietary survey was performed by trained investigators using 24-hour recall on 3 separate days: week 0, 7 and 12 for each. Food models and common food containers were used to estimate the accurate intake of foods. The intake of energy and dietary nutrients like protein, fat and carbohydrates was analyzed using professional software (Zhending, Shanghai, CHN).

2.4 Blood samples and serum fatty acids

At the baseline and the end of the intervention, subjects were asked to fast for 12 hours before collection of venous blood samples. The blood samples were centrifuged at 3000 rpm for 10 minutes to acquire serum samples, which were stored at −80 °C.

Extraction of serum fatty acids was performed as follows: 1 ml of 20% vitriol–methanol (1 : 4, V/V) was added into 200 μl of serum, and the samples were heated in a water bath at 80 °C for 90 minutes. After the samples were cooled to room temperature, 1 ml of saturated sodium chloride and l ml of *n*-hexane were added. After standing for 30 minutes, the mixed samples were centrifuged at 3000 rpm for 10 minutes. The supernatants were dried under nitrogen to prepare serum fatty acid samples. Before analyses, the serum fatty acid samples were dissolved by 60 μl of *n*-hexane. The fatty acid analysis was performed by gas chromatography (GC) using an Agilent 7890A GC chromatograph system, a flame ionization detector (FID) and J&W DB-23 columns of 60 m × 0.25 mm × 0.15 μm (Agilent, Santa Clara, CA, USA). Temperature program: 130 °C (held 10 minutes), then 10 °C min^{-1} to 180 °C (held 27 minutes) and 220 °C (held 8 minutes), and finally 30 °C min^{-1} to 232 °C (held 25 minutes). Fatty acids were identified by comparing the peaks with the external standards

(Nu-chek-Prep, Elysian, MN, USA) and quantified by the peak area normalization method.

2.5 Serum indicators

Serum adiponectin and inflammatory markers including tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and C-reactive protein (CRP) were determined by using ELISA kits (adiponectin from R&D, Minneapolis, MN, USA; inflammatory markers from ExCell, Shanghai, CHN). Fasting glucose, serum TG, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric analysis using commercial kits (all from BioSino, Beijing, CHN). As a biomarker of atherosclerosis,[19] the ratio of TC to HDL-C was calculated.

2.6 Statistical analysis

All the measurements were repeated three times. Analyses included only participants who completed the study. Data were presented as means ± standard deviations (SD) or medians with interquartile, and analyzed using SPSS version 20 software (Chicago, IL, USA). Before any statistical analysis, the variables were checked for normality by the Kolmogorov–Smirnov test. Log transformation was applied to correct partial abnormal variables. Differences among groups were analyzed using One-way ANOVA followed by a Least Significant Difference (LSD)-test or a nonparametric test. Paired-*t* test or Wilcoxon signed-rank test was performed to identify within-group differences. A value of P less than 0.05 was considered statistically significant.

3. Results

3.1 Study participants

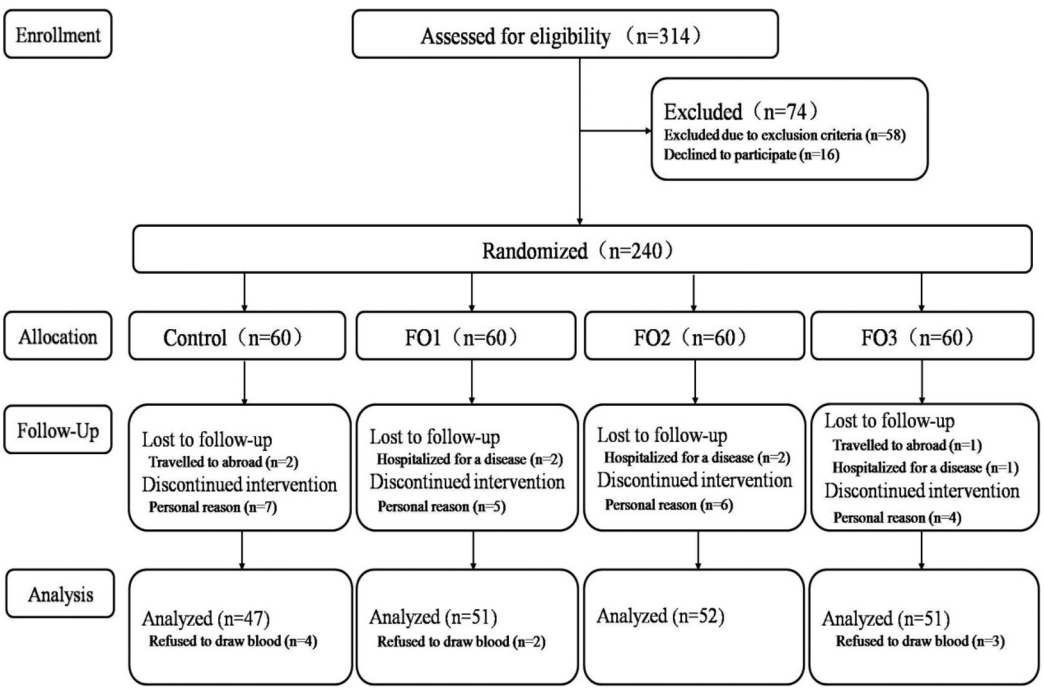
We recruited 314 volunteers, and 240 of them met our inclusion criteria. During the intervention, 22 subjects withdrew voluntarily, 8 were lost to follow-up for reasons such as being hospitalized for a disease or travelling abroad, and 9 refused their blood to be drawn at week 12 (the end of the study). In sum, a total of 39 subjects dropped out and 201 finally completed the intervention (Fig. 1).

3.2 Baseline characteristics

At the baseline, no significant differences among groups were detected with respect to age, gender and anthropometric parameters including height, weight, BMI, WHR and blood pressure (Table 1). At week 12, all the anthropometric parameters did not change markedly in all groups.

3.3 Intake of dietary nutrients

Results of dietary assessment indicated that there were no clear differences in the daily intake of energy, protein, total fat, carbohydrates and fatty acids among all groups (Table 2). The average intake of EPA in each group ranged from 0.06 to 0.08 g d^{-1}, that of DHA ranged from 0.06 to 0.12 g d^{-1}, and the



FO1, FO2 and FO3 denoted daily supplementation with 1, 2 and 4 fish oil capsules, which contained 0.31, 0.62 and 1.24g/d of EPA and DHA, respectively

Fig. 1 Flow diagram of the study participants.

Table 1 Baseline characteristics of subjects

	Control (n = 47)	FO1 (n = 51)	FO2 (n = 52)	FO3 (n = 51)	P value
Gender (male/female)	10/37	14/37	13/39	20/31	0.219
Age (years)	61 ± 7	62 ± 8	60 ± 8	61 ± 8	0.652
Height (m)	1.58 ± 0.07	1.57 ± 0.08	1.57 ± 0.08	1.60 ± 0.07	0.093
Weight (kg)	57.1 ± 8.5	56.9 ± 9.7	56.9 ± 9.0	60.3 ± 8.7	0.151
BMI (kg m^{-2})[a]	22.8 ± 2.8	22.9 ± 2.9	23.2 ± 3.5	23.5 ± 2.9	0.668
WHR[b]	0.86 ± 0.06	0.87 ± 0.10	0.86 ± 0.07	0.89 ± 0.08	0.156
SBP (mmHg)	127 ± 15	127 ± 19	123 ± 23	128 ± 16	0.675
DBP (mmHg)	78 ± 12	78 ± 12	76 ± 12	77 ± 10	0.790

Data are presented as means ± SD. [a] BMI = weight (kg)/height (m)/height (m). [b] WHR = waist (cm)/hip (cm). BMI: body mass index; WHR: waist–hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2 Daily intake of dietary energy and nutrients during the intervention

	Control (n = 47)	FO1 (n = 51)	FO2 (n = 52)	FO3 (n = 51)	P value
Energy (kcal)	1437 ± 277	1486 ± 395	1497 ± 331	1541 ± 362	0.454
Protein (g)	64 ± 16	65 ± 20	69 ± 18	70 ± 19	0.174
Total fat (g)	45 ± 16	47 ± 17	47 ± 17	49 ± 22	0.716
Carbohydrates (g)	208 ± 43	213 ± 59	211 ± 58	218 ± 50	0.802
SFA (g)	6.4 ± 4.0	6.0 ± 3.1	6.4 ± 3.2	6.3 ± 4.3	0.807
MUFA (g)	9.1 ± 5.8	8.8 ± 4.9	9.5 ± 5.2	9.6 ± 7.9	0.628
PUFA (g)	4.9 ± 2.7	5.6 ± 3.1	5.7 ± 4.1	5.4 ± 3.2	0.646
n-6PUFA (g)[a]	4.4 ± 2.5	5.1 ± 3.0	5.1 ± 3.6	4.9 ± 3.1	0.623
n-3PUFA (g)[b]	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.7	0.5 ± 0.3	0.488
n-6/n-3PUFA[c]	12.4	16.2	11.9	13.6	0.289
EPA (g)	0.07 ± 0.09	0.08 ± 0.10	0.07 ± 0.09	0.06 ± 0.07	0.968
DHA (g)	0.06 ± 0.07	0.12 ± 0.15	0.09 ± 0.14	0.08 ± 0.09	0.555

Data are presented as means ± SD. [a] n-6PUFA: C18:2, C18:3-γ, C20:3, C20:4. [b] n-3PUFA:C20:5, C22:5, C22:6. [c] n-6/n-3PUFA = n-6PUFA (g)/n-3PUFA (g). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

ratio of dietary n-6 to n-3 PUFA (n-6/n-3PUFA) ranged from 11.9 to 16.2.

3.4 Serum n-3 and n-6 PUFAs

At the baseline, the relative levels of serum EPA, DHA, n-3PUFA, n-6PUFA and n-6/n-3PUFA were not statistically different among all groups (Table 3). At week 12, fish oil supplementation led to dose-dependent increases in serum EPA, DHA and n-3PUFA, and a concurrent decrease in n-6/n-3PUFA compared to those of the control ($P < 0.05$ or $P < 0.01$). n-6PUFA was only markedly decreased in the FO3 group ($P < 0.01$). Changes in serum EPA, DHA, n-3PUFA and n-6/n-3PUFA from the baseline to week 12 in FO1, FO2 and FO3 groups significantly differed from those in the control ($P < 0.05$ or $P < 0.01$). The most obvious changes of serum PUFA were observed in the FO3 group. These findings also indicated a good compliance of subjects in the present study.

3.5 Serum adiponectin

All groups demonstrated a similar level of serum adiponectin at the baseline (Table 4). After the 12-week intervention, the adiponectin levels in FO2 and FO3 groups respectively increased by 15.9% ($P < 0.01$) and 28.2% ($P < 0.01$). Adiponectin in the FO3 group was higher than that in the control ($P < 0.05$). Changes in adiponectin from the baseline to week 12 were significantly different among all groups ($P = 0.02$), and the most obvious change was found in the FO3 group.

3.6 Serum inflammatory markers

At the baseline, no significant differences among all groups with regard to serum TNF-α, IL-6 and CRP were found (Table 4). At the end of the intervention, serum CRP levels rather than TNF-α and IL-6 levels in FO1, FO2 and FO3 groups were markedly lower compared to their corresponding baseline levels ($P < 0.05$ or $P < 0.01$). Nevertheless, changes of serum inflammatory markers among all groups did not reach statistical significance.

3.7 Serum lipid and glucose metabolic indicators

Differences in serum lipid profiles and fasting glucose among all groups were not marked before the intervention (Table 4). At week 12, serum TG decreased significantly with increased levels of fish oil supplementation, and changes of TG from the baseline to week 12 in FO2 and FO3 groups were more obvious than that in the control ($P < 0.05$ or $P < 0.01$). Meanwhile, an increase in HDL-C and a decrease in TC/HDL-C were observed in the FO3 group compared with the baseline levels ($P < 0.01$),

Table 3 Serum PUFA relative levels at the baseline and week 12

Relative level (%)	Control (n = 47)	FO1 (n = 51)	FO2 (n = 52)	FO3 (n = 51)	P value
EPA					
Baseline	0.90 ± 0.55	0.77 ± 0.48	0.91 ± 0.61	0.86 ± 0.58	0.651
Week 12	0.78 ± 0.55	1.05 ± 0.64[e,g]	1.33 ± 0.77[e,h]	1.63 ± 0.97[e,h]	<0.01
Change (%)[a]	−5.84(−37.62, 37.80)	38.00(−23.02, 114.63)[g]	55.28(−18.20, 152.96)[h]	108.65(19.69, 239.43)[h]	<0.01
DHA					
Baseline	3.89 ± 1.00	3.74 ± 1.01	4.07 ± 1.02	3.78 ± 0.96	0.276
Week 12	3.88 ± 1.07	4.31 ± 1.19[e,g]	4.46 ± 1.35[f,h]	4.90 ± 1.05[e,h]	<0.01
Change (%)	−0.05(−13.83, 14.93)	16.81(−7.53, 40.76)[g]	13.21(−9.27, 35.93)[g]	29.94(13.87, 51.51)[h]	<0.01
***n*-6PUFA**[b]					
Baseline	31.13 ± 2.34	30.89 ± 2.17	30.59 ± 2.43	30.47 ± 2.05	0.597
Week 12	31.14 ± 1.82	30.48 ± 2.34	30.52 ± 3.02	29.23 ± 2.26[e,h]	0.001
Change (%)	−0.45(−4.92, 5.51)	−2.44(−6.49, 5.63)	−1.28(−6.86, 7.91)	−3.29(−9.94, 2.14)[g]	0.099
***n*-3PUFA**[c]					
Baseline	5.33 ± 1.45	5.06 ± 1.47	5.50 ± 1.59	5.12 ± 1.47	0.360
Week 12	4.96 ± 1.39	5.71 ± 1.72[f,g]	6.12 ± 2.06[f,h]	6.86 ± 1.86[e,h]	<0.01
Change (%)	−6.64(−20.11, 18.93)	7.83(−9.95, 47.14)[g]	14.03(−11.25, 37.45)[g]	34.65(13.82, 69.73)[h]	<0.01
***n*-6/*n*-3PUFA**[d]					
Baseline	6.38 ± 2.26	6.66 ± 2.10	6.06 ± 1.89	6.44 ± 1.85	0.478
Week 12	6.94 ± 2.69	6.10 ± 2.98[f,g]	6.03 ± 3.64[h]	4.66 ± 1.61[e,h]	<0.01
Change (%)	9.54(−13.24, 22.12)	−11.41(−31.15, 9.92)[g]	−13.28(−28.79, 16.18)[g]	−29.73(−47.08, −11.20)[h]	<0.01

Data are presented as means ± SD and change values (%) are presented as medians (P_{25}, P_{75}). [a] Change (%) = (week 12-baseline)/baseline × 100%. [b] *n*-6PUFA: C18:2, C18:3-γ, C20:3, C20:4. [c] *n*-3PUFA: C18:3-α, C20:5, C22:6. [d] *n*-6/*n*-3PUFA = *n*-6PUFA (%)/*n*-3PUFA (%). [e] $P < 0.01$ *versus* baseline. [f] $P < 0.05$ *versus* baseline. [g] $P < 0.05$ *versus* control. [h] $P < 0.01$ *versus* control. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PUFA: polyunsaturated fatty acid.

but the changes of HDL-C and TC/HDL-C in the FO3 group did not statistically differ from those in the other three groups. The levels of serum TC in all groups were unchanged. Unexpected decreases in serum LDL-C were found in all groups ($P < 0.05$ or $P < 0.01$) even though differences among groups were not significant.

After the intervention, fasting glucose in fish oil groups decreased dose-dependently ($P < 0.01$) with the most obvious decrease (approximately 16.5%) occurring in the FO3 group. Fasting glucose levels in the FO2 and FO3 groups were lower than that in the control ($P < 0.05$ or $P < 0.01$). Changes in fasting glucose in the FO3 group significantly differed from those in the control ($P < 0.01$).

4. Discussion

n-3PUFA consumption has been widely accepted as a nutritional strategy for the secondary prevention of cardiovascular events in patients at high risk of CVD,[6,12] but little is known about the dose–response relationship between dietary *n*-3PUFA and serum biomarkers associated with cardiovascular health in the general population. This present study showed that a 12-week EPA and DHA supplementation at the doses of 0.31 (FO1 group), 0.62 (FO2 group) and 1.24 (FO3 group) g d^{-1} dose-dependently enhanced serum EPA, DHA, *n*-3PUFA and adiponectin levels (except for 0.31 g d^{-1}), but decreased serum *n*-6/*n*-3PUFA, TG and fasting glucose levels in healthy middle-aged and elderly people. Besides, all the doses of EPA and DHA led to decreases in serum CRP, but only 1.24 g d^{-1} led to an increase in HDL-C with a concurrent decrease in TC/HDL-C even though changes of these indicators were not significantly different among groups.

High dietary *n*-6/*n*-3PUFA caused by the intake of excessive *n*-6PUFA and insufficient *n*-3PUFA has been proved to promote the pathogenesis of CVD, and this process can be suppressed by increasing consumption of fatty fish or *n*-3PUFA supplements.[20] The results of dietary assessment in the present study demonstrated that the daily intake of dietary EPA and DHA in all groups were considerably lower than the recommended amount (approximately 1 g d^{-1}) proposed by the AHA for the prevention of CVD.[6] After a 12-week intervention, fish oil led to obvious increases in serum EPA, DHA and *n*-3PUFA with a corresponding decrease in serum *n*-6/*n*-3PUFA. These findings were in line with a previous study[21] indicating that the cardio-protective activities of fish oil may be partially mediated by an improvement of the serum PUFA composition.

Earlier prospective cohort studies designed to observe the relationship between adiponectin and CVD showed that increased circulating adiponectin is independently associated with decreased risks of CHD[10,22] and myocardial infarction.[9] These documented benefits of adiponectin are likely attributable to its regulation of blood pressure,[23] plasma HDL-C,[22] inflammatory response[24] and endothelial nitric oxide production.[25] Unfortunately, the effects of *n*-3PUFA on adiponectin are controversial. The present study found that 0.62 and 1.24 g d^{-1} of EPA and DHA significantly enhanced serum adiponectin at week 12, which was consistent with a previous study,[26] but in contrast to the result obtained by Tsitouras P. D. *et al.*[27] demonstrating that the dietary intake of *n*-3PUFA did not affect serum adiponectin in healthy elderly people. The discrepancy depended, to some extent, on differences in the sample size, 12

Table 4 Serum indicator levels at the baseline and week 12

	Control (n = 47)	FO1 (n = 51)	FO2 (n = 52)	FO3 (n = 51)	P value
Adiponectin (μg ml^{-1})					
Baseline	6.24 ± 2.29	5.79 ± 2.68	5.72 ± 2.07	5.81 ± 2.13	0.504
Week 12	6.43 ± 2.91	6.36 ± 2.64	6.87 ± 2.58[d]	7.43 ± 2.63[d,f]	0.039
Change (%)[a]	−2.98(−15.42, 21.87)	14.19(−0.57, 33.54)[f]	15.91(−1.34, 37.92)[g]	28.23(−2.34, 75.28)[g]	0.002
TNF-α[b]					
Baseline	2.98 ± 0.28	3.01 ± 0.28	3.02 ± 0.26	2.94 ± 0.27	0.391
Week 12	3.02 ± 0.28	3.04 ± 0.33	3.06 ± 0.25	2.97 ± 0.27	0.415
Change (%)	0.00(−2.03, 4.82)	0.33(−2.56, 4.62)	0.99(−1.18, 5.36)	1.70(−3.69, 4.27)	0.824
IL-6[b]					
Baseline	1.13 ± 0.50	1.34 ± 0.63	1.32 ± 0.60	1.30 ± 0.61	0.275
Week 12	1.26 ± 0.86	1.57 ± 0.87	1.41 ± 0.94	1.42 ± 0.90	0.386
Change (%)	−6.90(−23.73, 32.91)	10.14(−22.70, 63.10)	3.35(−33.53, 41.44)	0.00(−33.76, 72.37)	0.790
CRP (mg L^{-1})					
Baseline	1.98 ± 2.00	2.15 ± 2.01	2.25 ± 2.36	1.57 ± 1.35	0.496
Week 12	1.70 ± 1.72	1.48 ± 1.49[e]	1.37 ± 1.6[d]	1.30 ± 1.70[d]	0.239
Change (%) TG (mmol L^{-1})	−19.12(−55.93, 48.73)	−37.14(−74.09, 10.85)	−42.10(−72.90, 26.97)	−33.81(−71.44, −1.84)	0.189
TG (mmol L^{-1})					
Baseline	1.31 ± 0.69	1.33 ± 0.49	1.45 ± 0.81	1.61 ± 1.14	0.244
Week 12	1.29 ± 0.63	1.17 ± 0.35[d]	1.18 ± 0.47[d]	1.23 ± 0.66[d]	0.641
Change (%)	−5.95(−21.25, 21.05)	−7.50(−20.84, 6.68)	−12.24(−29.04, 3.88)[f]	−18.00(−35.69, −4.86)[g]	0.019
TC (mmol L^{-1})					
Baseline	4.41 ± 1.43	4.30 ± 1.17	4.49 ± 1.63	4.43 ± 1.34	0.922
Week 12	4.34 ± 1.50	4.29 ± 0.88	4.20 ± 1.04	4.28 ± 1.17	0.951
Change (%)	1.32(−22.07, 23.19)	5.85(−10.67, 21.85)	0.00(−23.38, 25.28)	0.27(−19.51, 22.44)	0.920
LDL-C (mmol L^{-1})					
Baseline	2.62 ± 0.53	2.77 ± 0.73	2.81 ± 0.62	2.85 ± 0.62	0.285
Week 12	2.25 ± 0.63[d]	2.48 ± 0.77[e]	2.47 ± 0.79[e]	2.53 ± 0.63[d]	0.223
Change (%)	−16.19(−32.65, 11.62)	−5.06(−28.24, 12.69)	−14.70(−30.61, 7.39)	−11.51(−22.73, 4.00)	0.722
HDL-C (mmol L^{-1})					
Baseline	1.44 ± 0.65	1.37 ± 0.62	1.45 ± 0.55	1.31 ± 0.63	0.595
Week 12	1.61 ± 0.76	1.48 ± 0.59	1.48 ± 0.55	1.59 ± 0.75[d]	0.657
Change (%)	0.00(−16.03, 47.58)	12.84(−11.51, 47.48)	−0.64(−11.74, 25.84)	25.84(−7.60, 52.49)	0.245
TC/HDL-C[c]					
Baseline	3.76 ± 2.25	3.60 ± 1.41	3.50 ± 1.61	4.06 ± 2.84	0.682
Week 12	3.26 ± 2.00	3.23 ± 1.16	3.20 ± 1.41	3.14 ± 1.39[d]	0.757
Change (%)	−4.56(−41.63, 25.42)	−9.80(−28.49, 21.87)	−0.18(−30.98, 19.87)	−24.48(−35.81, 8.62)	0.321
Glucose (mmol L^{-1})					
Baseline	4.96 ± 1.26	4.99 ± 0.95	4.87 ± 0.85	5.15 ± 0.94	0.546
Week 12	4.70 ± 1.05	4.47 ± 0.82[d]	4.37 ± 0.58[d,f]	4.29 ± 0.49[d,g]	0.078
Change (%)	−7.18(−15.58, 11.03)	−8.06(−21.47, 3.78)	−9.18(−21.40, 2.83)	−16.52(−27.41, −2.20)[g]	0.023

Data are presented as means ± SD and change values (%) are presented as medians (P_{25}, P_{75}). [a] Change (%) = (week 12-baseline)/baseline × 100%. [b] Abnormal distribution data were log-transformed before analysis. [c] TC/HDL-C = TC(mmol L^{-1})/HDL-C(mmol L^{-1}). [d] P < 0.01 *versus* baseline. [e] P < 0.05 *versus* baseline. [f] P < 0.05 *versus* control. [g] P < 0.01 *versus* control. TNF-α: tumor necrosis factor α; IL-6: interleukin 6; CRP: C-reactive protein; TG: triglyceride; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol.

in Tsitouras P. D. *et al. versus* 201 in the present study. Besides, it should be noticed that the dose of *n*-3PUFA (720 g of fatty fish weekly plus 15 ml of sardine oil daily) used in Tsitouras P. D. *et al.* was much higher than that used in our study (≤1.24 g d^{-1}). High consumption of *n*-3PUFA was suggested to increase the risk of lipid peroxidation in healthy men,[28] which may eventually counteract some beneficial impact of *n*-3PUFA.

A growing body of evidence suggests that the cardio-protective function of *n*-3PUFA is due, in part, to the inhibition of vascular inflammation.[12] As a biomarker of plaque inflammation, serum CRP has been identified as a predictor of an adverse cardiovascular outcome owing to its pro-atherosclerotic activity.[29–31] In accordance with a previous study,[32] a reduction of serum CRP caused by fish oil supplementation was detected in the present study even though changes of CRP from the baseline to week 12 in all fish oil groups did not significantly differ from that in the control. Meanwhile, we observed no marked decreases in serum TNF-α and IL-6 after the intervention. An interesting corollary of earlier research indicated that only a high dose (>2 g d^{-1}) combined with a long duration (>16 weeks) of fish oil supplementation improved the inflammatory response,[26] and hence relatively low concentrations of EPA and DHA and a short duration, as in this study, were supposed to be responsible for the lack of lowering effects of fish oil on serum TNF-α and IL-6.

Lipid and glucose metabolism disorder has always been involved in the early pathogenesis of CVD. A well documented hypolipidemic function of *n*-3PUFA[15,16] was also found in the present study: levels of serum TG in fish oil groups decreased in a dose-dependent manner after the intervention. This effect

was previously reported to be mediated by inhibiting hepatic very low-density lipoprotein (VLDL),[33] and accelerating VLDL and chylomicron clearance rates.[34] In good agreement with the research conducted by Svensson M. *et al.*,[35] only 1.24 g d^{-1} of EPA and DHA significantly enhanced serum HDL-C with a concurrent reduction in TC/HDL-C (a biomarker of atherosclerosis). Meanwhile, unexpected decreases in serum LDL-C were observed in both the control and the fish oil groups. Reasons for this finding could not be readily determined because there were no significant differences in the intake of dietary nutrients among all groups, and no participant used LDL-lowering supplements or drugs except for fish oil according to the results of our questionnaire.

Insulin resistance related glucose abnormality constitutes a risk factor for CVD.[36] Findings from epidemiological[37] and animal studies[38,39] have been consistent, indicating that *n*-3 PUFA has the capacity to negatively regulate fasting glucose. Improvements of insulin signaling pathway and glucose transporter type 4 (GLUT4) content in insulin targeted tissues such as muscle and adipose tissue are supposed to be the mechanisms that link *n*-3PUFA to glycaemic control.[38–40] However, results of the hypoglycemic function of fish oil in available RCTs remain conflicting.[41,42] In the present study, fasting glucose exhibited a dose–response decrease in fish oil groups and the most obvious change was observed in the FO3 group (1.24 g d^{-1} of EPA and DHA). These findings were in agreement with a previous study,[43] but contradicted the result reported by Clark L. F. *et al.*[42] showing that fish oil had no impact on glycaemic control in persons with impaired glucose metabolism. The discrepancy may be possibly explained by distinct selection of participants.

This trial was not without any limitation. After a comprehensive consideration, a blank control rather than a placebo control was chosen in the present study even though it may theoretically weaken the strength of our evidence.

5. Conclusions

Fish oil dose-dependently regulated serum PUFA and cardiometabolic biomarkers containing adiponectin, CRP, lipid profiles and fasting glucose. The most desirable changes were observed in 1.24 g d^{-1} EPA plus DHA. A moderate supplementation of fish oil was considered as an effective approach for healthy middle-aged and elderly Chinese people who consumed insufficient dietary *n*-3PUFA to reduce risks of CVD.

Conflicts of interest

This research was funded by the nutrition scientific research foundation of By-Health Inc., which is a noncommercial foundation that aims to support research on the efficacy of dietary supplements, community-based popularization of science, health management and personalized nutrition intervention. By-Health Inc. also donated the fish-oil capsules that were used in this study. The ownership of these research results remains with the authors and not with By-Health Inc.

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