**Effects of n-3 fatty acid supplements on glycemic traits in Chinese type 2 diabetic patients: a double-blind randomized controlled trial**

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| Keywords:                   | n-3 fatty acids, type 2 diabetes, randomized controlled trial, Chinese, glycemic traits |
Effects of n-3 fatty acid supplements on glycemic traits in Chinese type 2 diabetic patients: a double-blind randomized controlled trial

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Key terms: n-3 fatty acids, randomized controlled trial, type 2 diabetes

Abbreviations: ALA, alpha-linolenic acid; CO, corn oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; FSO, flaxseed oil; HbA1c, glycated hemoglobin A₁c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; PUFA, polyunsaturated fatty acids; T2D, type 2 diabetes; TG, triacylglycerol
Abstract

Scope: To investigate the effects of n-3 fatty acid supplements, both marine and plant-based, on glycemic traits in Chinese type 2 diabetes (T2D) patients.

Method and results: In a double-blind randomized controlled trial, 185 recruited Chinese T2D patients were randomized to either fish oil (FO, n=63), flaxseed oil (FSO, n=61) or corn oil group (CO, served as control group, n=61) for 180 days. The patients were asked to take corresponding oil capsules (4 capsules/day), which totally provided 2 g/day of eicosapentaenoic acid + docosahexaenoic acid in FO group and 2.5 g/day of alpha-linolenic acid in FSO group. No group×time interaction was observed for HOMA-insulin resistance, fasting insulin or glucose. Significant group×time interaction (P =0.035) was observed for glycated haemoglobin (HbA1c), with HbA1c decreased in FO group compared with CO group (P=0.037). We also found significant group×time interactions for lipid traits, including low-density lipoprotein cholesterol (P=0.043), total cholesterol (TC) (P=0.021), total cholesterol/ high-density lipoprotein cholesterol (TC/HDL-C) (P=0.009) and triacylglycerol (TG) (P=0.003), with the lipid profiles improved in FO group. No significant effects of FSO on glycemic traits or blood lipids were observed.

Conclusions: Marine n-3 PUFA supplements may improve glycemic control and lipid profiles among Chinese type 2 diabetic patients.

Clinical trial reg. no. NCT01857167, clinicaltrials.gov
Introduction

The epidemic of type 2 diabetes (T2D) continues to grow worldwide, especially in developing countries, such as China and India [1]. It is projected from a nationally representative samples that up to 113.9 million and 493.4 million Chinese adults have diabetes and prediabetes, respectively [2]. Epidemiologic studies consistently show that T2D is largely preventable through diet and lifestyle modification [1, 3]. One type of the candidate dietary factors is long-chain marine sources of n-3 polyunsaturated fatty acids (PUFA) (eicosapentaenoic acid [EPA, C20:5n3], docosapentaenoic acid [DPA, C22:5n3] and docosahexaenoic acid [DHA, C22:6n3]), as indicated by a number of rodent models, which consistently suggest improvement of insulin sensitivity by marine n-3 PUFA [4-6]. Of note, most rodent models use amounts of n-3 PUFA that are in considerable excess (on a pro-rata basis) to that allowed in human [7]; and therefore the results may not be applicable among humans. Yet, results from human observational and intervention studies remain inconclusive [8-10]. Meta-analyses suggest that marine n-3 PUFA are inversely associated with risk of T2D in Asian populations, including Chinese populations [8, 11, 12]. Furthermore, plasma marine n-3 PUFA are inversely associated with insulin resistance in Chinese T2D patients [13]. However, in a Cochrane systematic review and meta-analysis of trials involving n-3 PUFA treatment in T2D patients [14], marine n-3 PUFA supplements do not affect insulin sensitivity or glucose metabolism of these patients. Of note, no included or excluded trials in the meta-analysis are conducted among Chinese [14]. Another two meta-analyses of randomized controlled trials also suggests a lack of marine n-3 PUFA effect on insulin sensitivity in T2D patients, with only one small trial among Chinese [9, 15]. In addition to marine n-3 PUFA, alpha-linolenic acid (ALA, C18:3n3), a plant-based n-3 PUFA, has also shown inverse association with T2D in Chinese populations in observational studies [16, 17]. However, to the best of our knowledge, there is no published randomized
controlled trial among Chinese T2D patients for the ALA (mainly from flaxseed oil) intervention.

Therefore, to fill the gap and to confirm the results generated from observational studies, a large randomized controlled trial of n-3 PUFA supplements in Chinese T2D patients is highly necessary. In the present study, we conducted a randomized, multicenter, double-blind, placebo-controlled trial to investigate the effects of n-3 PUFA supplements, both marine and plant-based, on glycemic traits in Chinese T2D patients.

Materials and Methods

The trial was registered at ClinicalTrials.gov (No. NCT01857167). The trial was approved by the Ethics Committee of College of Biosystem Engineering and Food Science at Zhejiang University (No. 2013011). All the participants gave written informed consent.

Experiment oil capsule preparation

We standardized each of fish oil (FO), flaxseed oil (FSO) or corn oil (CO) capsules to one gram with identical appearance. Each FO capsule provided 500mg of EPA + DHA (EPA: DHA=3:2), and other major fatty acids in each FO capsule were C16:0 (71.4mg), C18:1n-9 (58.4mg), C16:1 (56mg), C20:0 (39.4mg) and C14:0 (34.6mg). Each FSO capsule contained 630mg of ALA, 155mg of C18:2n-6 and 137mg of C18:1n-9. Major fatty acids in each CO capsule were C18:2n-6 (534mg), C18:1n-9 (299mg) and C16:0 (121mg). All the capsules were made in the Neptunus Bioengineering Co., Ltd (Hangzhou, China). All the capsules were kept in white bottles (90 capsules/bottle), which were labeled as Oil A, Oil B and Oil C for the three types of capsules. None of the participants or the nurses/physicians in the study centers knew the oil types during the intervention.
Inclusion and exclusion criteria of the study participants

The inclusion criteria were (1) fasting blood glucose > 7.0 mmol/L or on use of diabetic medications; (2) between 35 and 80 years old for men and between post-menopausal and 80 years old for women. The exclusion criteria were (1) having familial hyperlipemia or with blood triacylglycerol (TG) concentrations >4.56 mmol/L; (2) a history of hepatic or kidney disease, or any type of cancer; (3) participation in another clinical trial within 30 days prior to screening.

Randomization of the participants and intervention

Two hundred and fifty-two potentially adults with known T2D status were screened in three study centers between June 2013 to June 2014. One hundred and eighty-five T2D patients were recruited based on the inclusion and exclusion criteria in the three study centers: Wuhan (Central China) (n=59), Changshan (Southeast China) (n=47) and Lanzhou (Western China) (n=79).

All the included participants were randomly allocated to one of the three treatments by computer-generated random numbers with a block size of six: FO group (n=63), FSO group (n=61) and CO group (n=61). Allocation sequence was generated by JSZ. Doctors/nurses at each study center enrolled and assigned participants to the intervention groups. The participants in each of the trial arms were required to take 4 capsules/day, which would totally provide 2 g/day of EPA+DHA in FO group or 2.5 g/day of ALA in FSO group. The dosage of n-3 PUFA supplements were consistent with those used in previous trials [10]. The duration of the intervention was 180 days. All the patients were given four bottles of capsules at baseline, and given another four bottles at 90 days of the intervention when they came back.
to the study centers for health examination. At visit of 180 days of intervention, patients came
back to the study centers for the final on-site examination. About 84.3% (n=157) of included
patients took diabetic medications, with 37 patients (13, 12 and 12 for FO, FSO and CO
group, respectively) using insulin only, 83 patients (27, 31 and 25 for FO, FSO and CO group,
respectively) using oral glucose-lowering drugs only, and 36 patients (11, 10 and 15 for FO,
FSO and CO group, respectively) using both insulin and glucose-lowering drug. All the
patients were asked to maintain their usual diet, lifestyle or use of prescribed medications,
and avoid use of n-3 fatty acid supplements during the intervention.

Measurements of biochemical parameters and erythrocyte fatty acids

We took fasting blood samples (10 mL) of all the participants at baseline, day 90 and day 180
of intervention. Serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein
cholesterol (LDL-C), total cholesterol, TG, glucose, uric acid, blood urea nitrogen (BUN),
creatinine, liver function markers (alanine transaminase [ALT], aspartate transaminase
[AST]), blood total protein, globulin (GLB), albumin (ALB), total bilirubin (TBIL), direct
bilirubin (DBIL), indirect bilirubin (IDBIL) were measured by commercially available kits on
HITACHI 7020 chemistry analyzer using enzyme-based colorimetric test supplied by Diasys
Diagnostic Systems (Shanghai) Co., Ltd. at each of the study centers. Serum insulin was
measured by ARCHITECT insulin reagent kit (Abbott Laboratories, Abbott Park, IL, USA).
Using fasting glucose and insulin, homeostatic model assessment of insulin resistance
(HOMA-IR) was calculated by using the formula: glucose (mmol/L) × insulin (mU/L)/22.5
[18]. HOMA-IR, but not HOMA2 model, was used to represent insulin resistance as it was
more widely used in Chinese populations, and therefore more comparable among related
studies in China. Blood glycated hemoglobin A1c (HbA1c) was measured by automated
Hemoglobin A1c Analyzer. Blood samples not immediately measured were stored under -
80°C for further analysis. Anthropometric parameters, including weight, height, waist and hip circumference, were measured at baseline and end-point of the intervention.

Compliance of the patients to the intervention was evaluated by measurement of erythrocyte phospholipid fatty acid compositions at baseline and end-point of the intervention, and by counting the empty bottles they returned to the study centers at day 90 and 180. Additionally, trained nurses contacted patients via phone once per month to record their compliance of the previous month and to remind them to take the capsules. Erythrocyte phospholipid fatty acids were measured by gas chromatography as previous described [19]. Briefly, we extracted total lipids from erythrocytes with chloroform/methanol (1:1), and separated phospholipid fraction from the total lipids by thin-layer chromatography. Then, we converted the phospholipid fatty acids to methylester and extracted them into n-hexane and dried on anhydrous Na$_2$SO$_4$. Finally, we filtered fatty acid methylesters by Sep-Pak Silica column before gas chromatography separation and analysis.

**Statistical analysis**

We conducted all the statistical analyses in Stata (version 13; StataCorp, College Station, TX, USA). The total sample size was calculated based on 80% power ($\alpha_{\text{two-tailed}}=0.05$) to detect difference in HOMA-IR by 20% or 0.63 (SD=1.1) between groups (n=150, 50/group), considering 20% drop out rate (n=187, 62/group), based on our previous work [20]. This sample size (n=50/group) will also enable us to have 84% power ($\alpha_{\text{two-tailed}}=0.05$) to detect difference in HbA1c by 20% or 12 mmol/mol (SD=20) between groups.

All the outcomes variables were checked for the normal distribution, and were log transformed if they were not normal distributed (HOMA-IR, glucose, insulin, HbA1c and TG
were natural log transformed). One-way ANOVA (for continuous variables) or Chi-square

test (for categorical variables) was performed to test the group difference at baseline.

Difference in change of the three fatty acid compositions for FO or FSO compared with CO
group was examined by linear regression, adjusting for age, sex, study center and baseline
BMI and baseline corresponding fatty acid composition.

Following an intention-to-treat principal, linear mixed models were used to compare
differences between intervention groups in four glycemic traits (HOMA-IR, insulin, glucose,
HbA1c) overtime (day 0, 90 and 180). The mixed model analysis without any ad hoc
imputation for missing data would provide equal or more power than dose analysis with data
imputation [21]. Time since baseline randomization was included in the model as a
categorical variable (day 0, 90 and 180), and the group×time interaction was treated as the
fixed effect in the model and was the primary effect of interest. Other potential confounders
included in the model as fixed effects were age, sex, study center and baseline BMI.

Sensitivity analysis was conducted (1) by including only two time points (day 0 and 180) in
the linear mixed models; (2) by including baseline value of corresponding outcome as a
covariate in the linear mixed models.

In addition to the primary analyses on glycemic traits, we performed secondary analyses on
lipid traits, including HDL-C, LDL-C, TC, TC/HDL-C and TG using the linear mixed models.

If significant time × group interaction was observed for primary or secondary analyses, we
conducted post-hoc analyses to examine the group × time interaction between FO and CO,
between FO and FSO, and between FSO and CO, respectively, based on the linear mixed
models. P-value (two-tailed) <0.05 was considered significant.
**Results**

At baseline, we did not observe any significant difference in blood lipids or glycemic traits between participants of the three intervention groups (Table 1). During the intervention, five participants in the FO group, eight participants in the FSO group and six participants in the CO group dropped out of the trials, leaving 58, 53 and 55 participants in the FO, FSO and CO group, respectively (Figure 1).

Erythrocyte EPA and DHA was significantly increased in the FO group compared with the CO group ($P<0.001$ for both fatty acids) (Figure 2). FSO group, compared with CO group, had significant increased composition of ALA ($P=0.043$), but non-significant increase of EPA ($P=0.084$) and DHA ($P=0.056$). EPA was significantly increased in FO compared with FSO group ($P=0.001$).

No significant difference among the three groups was observed on fasting serum glucose, insulin or HOMA-IR (Table 2). However, we found significant interaction between study groups (3 groups) and time (3 time points) for HbA1c ($P=0.035$). Post-hoc analysis showed that HbA1c was significantly decreased in FO group ($P=0.037$) but not FSO group ($P=0.30$), compared with CO group. Sensitivity analysis by including 2 time points (day 0 and 180) found similar tendency that HbA1c decreased in FO group ($P=0.088$) and in FSO group ($P=0.152$), compared with CO group. Further sensitivity analysis by including baseline outcome value as a covariate found that FO compared with CO group, showed a stronger statistical significance in the HbA1c ($P=0.009$ from the full analysis including 3 time points; $P=0.029$ from the restricted analysis including 2 time points [day 0 and day 180]), while no significant difference in the HbA1c ($P=0.23$ and 0.06 from the full analysis and restricted analysis respectively) observed for FSO compared with CO group. No significant
results/material changes were found for other glycemic traits in FO or FSO group in the above two steps of sensitivity analyses.

We found significant time by group interaction for each of serum LDL-C ($P=0.043$), TC ($P=0.021$), TC/HDL-C ($P=0.009$) and TG ($P=0.003$), but not for HDL-C (Table 3). In the post-hoc analyses, serum concentrations of TC ($P=0.029$), TC/HDL-C ($P=0.038$) and TG ($P=0.001$) were significantly decreased in FO group compared with that in CO group, but with no statistical significance on LDL-C ($P=0.081$). FO group compared with FSO group, had significant decrease of LDL-C ($P=0.025$), TC ($P=0.007$), TC/HDL-C ($P<0.001$) and TG ($P=0.043$) after the intervention. No significant difference between FSO and CO groups was found for any blood lipid trait. No significantly different change between treatments during the intervention was observed for other biochemical parameters, except for IDBIL ($P=0.047$) (Table 4).

**Discussion**

To the best of our knowledge, the present randomized controlled trial was among the first to examine the effect of both marine and plant-based n-3 PUFA supplements on Chinese T2D patients. Our trial also had larger sample size than most of previous trial in other population settings. We found that FO supplements may potentially improve glycemic control in the T2D patients in terms of decreasing HbA1c, a marker of blood glucose status over the past few months. Moreover, FO consistently lowered blood lipids in these patients, including TC, LDL-C and TC/HDL-C. No significant effects on glycemic traits or blood lipids were found in patients taking FSO supplements.

Fish oil supplements were found to increase blood glucose levels with borderline significance.
among T2D patients in an early meta-analysis [22]. In addition, every one g/day increase of EPA and DHA was significantly associated with 0.38% and 0.6% increase of HbA1c, respectively, in T2D patients [22]. However, an updated Cochrane review of n-3 PUFA intervention among T2D patients based on 23 randomized controlled trial (1075 participants) showed that n-3 PUFA supplements did not have any significant effect on glycemic traits, including fasting insulin, glucose and HbA1c [14]. In several other reviews of n-3 PUFA supplements, the authors suggested no benefit of n-3 PUFA on glycemic traits or insulin sensitivity among T2D patients [10]. Of note, all of the trials included in the above meta-analyses or review [9, 10, 14] came from Western populations, with no trials from Asian populations (except for one from India). These results were consistent with that from meta-analyses of prospective cohort studies, which suggested that marine n-3 PUFA/fish intake showed null or even positive association with T2D risk in Western populations [8, 11]. In contrast, marine n-3 PUFA intake was associated with lower risk of T2D in Asian populations [8, 11]. Furthermore, the only randomized controlled trial in the Asian Indian population [23] in the aforementioned meta-analysis [14] suggested that n-3 PUFA could improve glycemic status. Recently, several randomized controlled trials of n-3 PUFA supplements among T2D patients have been conducted in Asian countries (from Iran and Japan) [24-26]. For example, in a randomized double-blind placebo-controlled trial among 81 T2D patients in Iran, n-3 PUFA supplementation for 2 months significantly decreased HbA1c compared with control group [26]. Among another trial of 67 Iranian T2D patients over a period of 3-month intervention [24], n-3 PUFA (EPA) supplementation improved glycemic control by decreasing fasting serum insulin, glucose, HbA1c and HOMA-IR. Furthermore, in a randomized controlled trial among 30 elderly Japanese T2D patients, EPA/DHA-rich liquid diet, compared with liquid diet lacking EPA/DHA, significantly decreased fasting plasma glucose and HbA1c over a period of 3 months [25]. These new evidence suggested that n-3
PUFA supplements could potentially improve glycemic control in Asian populations, while no evidence was available among Chinese populations prior to our present trial.

Our current trial supported that marine n-3 PUFA supplements may improve glycemic control by decreasing HbA1c level. The reason for the non-significant change for HOMA-IR or fasting glucose was not clear, which may be due to the influence of regular oral drug use or insulin injection days before the blood draw. We also observed that HOMA-IR, insulin and glucose level were lowest at 90 days. This may be due to the decrease of compliance in the late stage of the trial or some unknown confounding factors. In addition, we found that HbA1c in FSO group continuously decreased from baseline to day 90 and subsequently to day 180; while in FO group it decreased from baseline to day 90 and then increased at day 180. This observation may be because of the poorer compliance after day 90 in FO group, or because of the fact that the degree of response to either treatment has reached a plateau at the HbA1c value of around 54 mmol/mol. Nevertheless, HbA1c is a stable marker of glucose status over several months, and the decreased level of HbA1c indicates improved glycemic control after the marine n-3 PUFA supplements. The disparate findings for marine n-3 PUFA with glycemic control and T2D in Asian and Western populations may be due to the different genetic backgrounds. For example, in a recent study [27], researchers find that an adaptive genetic polymorphism within *FADS2* gene conferred an adaptive advantage in Asians because of the traditional plant-based diet practice, which suggests that Asians are more likely to synthesize long-chain PUFA from plant PUFA precursors. Based on the aforementioned evidence, we therefore hypothesized that Asians may be more sensitive to marine n-3 PUFA in terms of glycemic control compared with Western populations, which warrants further investigation.
In contrast to glycemic traits, previous meta-analyses consistently supported that marine n-3 PUFA improved lipid profiles by reducing blood TG level \[14, 22\] among T2D patients. However, both of these meta-analyses suggested that n-3 PUFA may increase serum LDL-C concentration among T2D patients; while no significant change in TC or HDL-C was observed after n-3 PUFA supplements \[14, 22\]. In the secondary analyses of the present study, TG, TC and TC/HDL-C were significantly decreased in FO group compared with CO group. In addition, FO had the tendency to decrease LDL-C compared with the CO. The present results suggested that, in Chinese T2D patients, beneficial effect of n-3 PUFA on blood lipids was not limited to TG, but also to other lipid profiles, such as TC, TC/HDL-C and potentially LDL-C. The reason for the difference in TC and LDL-C between the present results and that of previous meta-analyses was not clear. It may be that the present study had longer intervention period than most of previous randomized trials included in the meta-analyses \[14, 22\]. Furthermore, we did not collect information on statin use for the patients, which may potentially bias the lipid response to n-3 PUFA intervention. Nevertheless, these results indicated that fish oil might play an important role in the prevention of cardiovascular events, a most important complication of T2D. We noticed a slightly different response of lipids to n-3 PUFA at day 90 and day 180, which suggested that the effects of n-3 PUFA on blood lipids may have some optimal threshold in terms of intervention period (reaching optimal blood n-3 PUFA levels), which warrants further investigation.

Different from FO, the present study did not observed any significant effect of FSO supplements, source of a plant-based n-3 PUFA: ALA, on glycemic traits or lipid profiles. Our results were consistent with several previous trials. In two randomized controlled trials of FSO supplements among T2D individuals \[28, 29\], 7.4g/d and 5g/d of ALA was used in the trial arm of each study. However, there was no significant change of glycemic traits in
response to FSO supplements in the two studies. Given that dose of ALA used in the present
study (2.5g/d) was much lower than that of previous trials [28, 29], it could be postulated that
in the present study we would observed no significant effect of ALA supplements on
glycemic traits as consistent with the previous trials [28, 29]. Yet, further randomized trials of
higher flaxseed oil/ALA dose are needed to investigate the effect of FSO/ALA on Chinese
T2D patients.

The beneficial effects of the marine n-3 PUFA on glycemic control and glucose homeostasis
are biological plausible and may involve various mechanisms. For example, with the
increasing incorporation of marine n-3 PUFA into cell membranes via supplementation, the
membrane fluidity will be increased, leading to increased insulin sensitivity [30]. Marine n-3
PUFA may also improve glucose homeostasis through regulating inflammatory status [31, 32].
In addition, a variety of animal models have suggested that marine n-3 PUFA may improve
insulin sensitivity and glucose homeostasis by influencing insulin signaling pathway [33-36].

The present study has several strengths. First, the sample size of the present trial provided
sufficient power to examine the effect of n-3 PUFA supplements on glycemic traits. Second,
study duration was longer than most of previous trials in the T2D patients. Third, this was a
multicenter trial, which represented participants from Western, Central and Southeast China.
The limitation of the present trial was that 180-day of intervention was still too short for us to
examine the effect of n-3 PUFA supplements on T2D complications, such as cardiovascular
events among the patients. In addition, we suggested that the patients maintain their usual diet
and physical activity, but did not monitor their lifestyles during the intervention, although we
obtained oral agreements from the patients that they would not change their diet or lifestyles
during the intervention. Third, 84.3% of study participants took diabetic medications, which
may be a potential source of confounding to the present trial. Yet, the distribution of
medications used was similar among groups, and unlikely to affect the study results. Fourth,
the repeated measure approach may not capture the potentially specific response at day 90
and at day 180 to the treatments, which may influence the overall results. This speculation
was supported by our sensitivity analysis of including only 2 time points (day 0 and day 180)
in the models that the effect of FO was attenuated, while effect of FSO strengthened. Last, we
did not randomize the participants based on HbA1c at baseline, which may potentially affect
the effect of n-3 supplements on HbA1c, a main outcome of this trial. Future researchers in
this area need to balance trial arms for the main outcomes at baseline.

In conclusion, the present randomized controlled trial suggested that marine n-3 PUFA
supplements for 180 days potentially improved glycemic control in Chinese T2D patients.
This study provides new evidence of using marine n-3 PUFA for the glycemic control of
Chinese T2D patients; however, there is no convincing evidence showing that marine n-3
PUFA are superior to plant-based n-3 PUFA. More studies with longer follow-up duration
and larger sample size in Chinese populations are warranted to replicate and confirm the
results of the present study.
Acknowledgement

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Author contributions: The author’s responsibilities were as follows: DL (Principal Investigator) and JSZ: designed the study; ML, FL, YY, LY, JF, WC, DDL, YJ, LW, HY: conducted the clinical trials in study centers; JT, WC, MS, ZL and FW: contributed to data collection and sample measurements; JSZ analyzed data and performed statistical analysis; JSZ, DL: wrote paper; DL had primary responsibility for final content. All authors contributed to the manuscript review and approved the final version.

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Conflict of Interest: Nothing to disclose.
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Table 1 Baseline characteristics of study participants involved in the randomized controlled trial

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<td>138.3±18.6</td>
<td>133.7±21.4</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78.2±10.5</td>
<td>78.6±8.1</td>
<td>79.5±14.9</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.14±0.3</td>
<td>1.16±0.29</td>
<td>1.19±0.20</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.97±0.80</td>
<td>2.89±0.85</td>
<td>3.05±0.85</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.66±0.96</td>
<td>4.68±0.96</td>
<td>4.88±1.01</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.28±1.05</td>
<td>4.23±1.13</td>
<td>4.17±0.92</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.68±0.74</td>
<td>1.93±1.30</td>
<td>1.85±0.97</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, except for women (n). *P<0.05 indicated significantly different between the three trial arms. One-way ANOVA (for continuous variables) or Chi-square test (for categorical variables) was performed to test the group difference at baseline.

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol
Table 2 Effect of n-3 PUFA supplements on glycemic traits in Chinese patients with type 2 diabetes

<table>
<thead>
<tr>
<th>Time</th>
<th>Fish oil</th>
<th>Flaxseed oil</th>
<th>Corn oil</th>
<th>P-time</th>
<th>P-group</th>
<th>P-time×group interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>day 0</td>
<td>8.18±3.03</td>
<td>8.61±3.64</td>
<td>8.31±3.63</td>
<td>0.221</td>
<td>0.811</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>7.60±1.93</td>
<td>8.23±2.83</td>
<td>7.71±2.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>8.46±3.02</td>
<td>8.16±2.70</td>
<td>8.22±2.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>day 0</td>
<td>14.7±8.7</td>
<td>20.3±18</td>
<td>16.5±16.1</td>
<td>&lt;0.001</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>11.4±10.6</td>
<td>13.1±10.8</td>
<td>11.5±9.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>11.6±6.76</td>
<td>15.6±11.3</td>
<td>18.1±19.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>day 0</td>
<td>5.48±3.97</td>
<td>7.57±6.39</td>
<td>6.21±5.89</td>
<td>&lt;0.001</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>4.19±4.76</td>
<td>5.10±4.62</td>
<td>4.00±4.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>4.68±4.12</td>
<td>5.74±4.67</td>
<td>6.46±6.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>day 0</td>
<td>63.9±22.9</td>
<td>62.3±20.8</td>
<td>57.6±16.4</td>
<td>&lt;0.001</td>
<td>0.571</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>52.7±13.8</td>
<td>59.2±17.1</td>
<td>56.2±17.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>54.4±13.4a</td>
<td>54.2±14ab</td>
<td>55.0±16.4b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Groups sharing the same superscript (a or b) have no significant difference from each other in the post-hoc analysis ($P\geq0.05$). HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin A$_1c$. 
Table 3 Effect of n-3 PUFA on blood lipids in Chinese patients with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Fish oil</th>
<th>Flaxseed oil</th>
<th>Corn oil</th>
<th>P-time</th>
<th>P-group</th>
<th>P-time×group interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>1.14±0.3</td>
<td>1.16±0.29</td>
<td>1.19±0.20</td>
<td>&lt;0.001</td>
<td>0.112</td>
<td>0.436</td>
</tr>
<tr>
<td>day 90</td>
<td>1.25±0.33</td>
<td>1.20±0.33</td>
<td>1.27±0.25</td>
<td>0.112</td>
<td>0.055</td>
<td>0.043</td>
</tr>
<tr>
<td>day 180</td>
<td>1.23±0.30</td>
<td>1.22±0.29</td>
<td>1.25±0.25</td>
<td>0.241</td>
<td>0.016</td>
<td>0.021</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>2.97±0.80</td>
<td>2.89±0.85</td>
<td>3.05±0.85</td>
<td>0.017</td>
<td>0.055</td>
<td>0.043</td>
</tr>
<tr>
<td>day 90</td>
<td>2.92±0.78</td>
<td>2.85±0.80</td>
<td>3.14±0.96</td>
<td>0.25</td>
<td>0.016</td>
<td>0.021</td>
</tr>
<tr>
<td>day 180</td>
<td>2.62±0.81a</td>
<td>2.80±0.72b</td>
<td>2.99±0.90ab</td>
<td>0.112</td>
<td>0.241</td>
<td>0.009</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>4.66±0.96</td>
<td>4.68±0.96</td>
<td>4.88±1.01</td>
<td>0.25</td>
<td>0.016</td>
<td>0.021</td>
</tr>
<tr>
<td>day 90</td>
<td>4.56±0.99</td>
<td>4.83±0.82</td>
<td>5.04±1.00</td>
<td>4.03</td>
<td>0.057</td>
<td>0.003</td>
</tr>
<tr>
<td>day 180</td>
<td>4.50±1.00a</td>
<td>4.90±0.85b</td>
<td>5.04±1.10b</td>
<td>4.19</td>
<td>0.112</td>
<td>0.016</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>4.28±1.05</td>
<td>4.23±1.13</td>
<td>4.17±0.92</td>
<td>4.03</td>
<td>0.057</td>
<td>0.003</td>
</tr>
<tr>
<td>day 90</td>
<td>3.81±0.98</td>
<td>4.30±1.40</td>
<td>4.03±0.84</td>
<td>4.19</td>
<td>0.112</td>
<td>0.016</td>
</tr>
<tr>
<td>day 180</td>
<td>3.82±1.02a</td>
<td>4.22±1.12b</td>
<td>4.19±1.46b</td>
<td>4.19</td>
<td>0.112</td>
<td>0.016</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>1.68±0.74</td>
<td>1.93±1.30</td>
<td>1.85±0.97</td>
<td>0.006</td>
<td>0.057</td>
<td>0.003</td>
</tr>
<tr>
<td>day 90</td>
<td>1.34±0.56</td>
<td>1.96±1.46</td>
<td>1.78±1.03</td>
<td>4.19</td>
<td>0.112</td>
<td>0.016</td>
</tr>
<tr>
<td>day 180</td>
<td>1.45±0.75a</td>
<td>1.94±1.15b</td>
<td>1.75±0.87b</td>
<td>4.19</td>
<td>0.112</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Groups sharing the same superscript (a or b) have no significant difference from each other in the post-hoc analysis (P≥0.05). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol.
Table 4 Effect of n-3 PUFA supplements on liver and kidney function markers and other blood parameters.

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Fish oil</th>
<th>Flaxseed oil</th>
<th>Corn oil</th>
<th>P-time</th>
<th>P-group</th>
<th>P-time×group interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>day 0</td>
<td>6.04±2.15</td>
<td>6.02±1.88</td>
<td>5.54±1.36</td>
<td>0.017</td>
<td>0.375</td>
<td>0.677</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>6.10±2.24</td>
<td>6.31±1.62</td>
<td>5.88±1.60</td>
<td>0.007</td>
<td>0.633</td>
<td>0.829</td>
</tr>
<tr>
<td>Creatinine</td>
<td>day 0</td>
<td>75.0±28.0</td>
<td>64.6±22.0</td>
<td>66.1±23.5</td>
<td>0.905</td>
<td>0.301</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>73.8±36.2</td>
<td>65.9±22.8</td>
<td>68.1±34.1</td>
<td>0.374</td>
<td>0.774</td>
<td>0.774</td>
</tr>
<tr>
<td>Uric acid</td>
<td>day 0</td>
<td>322.4±86.9</td>
<td>322.0±78.9</td>
<td>320.3±98.4</td>
<td>0.485</td>
<td>0.95</td>
<td>0.207</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>332.4±81.1</td>
<td>313.6±88.7</td>
<td>307.8±73.6</td>
<td>0.477</td>
<td>0.807</td>
<td>0.225</td>
</tr>
<tr>
<td>ALT</td>
<td>day 0</td>
<td>28.0±17.8</td>
<td>31.1±22.6</td>
<td>28.2±14.8</td>
<td>0.074</td>
<td>0.36</td>
<td>0.839</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>29.5±16.8</td>
<td>30.6±25.9</td>
<td>31.6±18.8</td>
<td>0.001</td>
<td>0.535</td>
<td>0.774</td>
</tr>
<tr>
<td>AST</td>
<td>day 0</td>
<td>26.6±10.8</td>
<td>29.1±16.2</td>
<td>25.7±8.00</td>
<td>0.091</td>
<td>0.535</td>
<td>0.774</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>25.1±11.0</td>
<td>26.2±11.7</td>
<td>25.4±10.3</td>
<td>0.001</td>
<td>0.535</td>
<td>0.774</td>
</tr>
<tr>
<td>TBIL</td>
<td>day 0</td>
<td>12.7±5.72</td>
<td>12.2±6.32</td>
<td>13.4±6.3</td>
<td>0.148</td>
<td>0.077</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>11.2±4.39</td>
<td>12.4±6.94</td>
<td>15.5±20.0</td>
<td>0.001</td>
<td>0.077</td>
<td>0.151</td>
</tr>
<tr>
<td>DBIL</td>
<td>day 0</td>
<td>5.28±2.54</td>
<td>5.02±2.04</td>
<td>5.36±2.24</td>
<td>&lt;0.001</td>
<td>0.632</td>
<td>0.344</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>4.47±1.47</td>
<td>4.76±2.09</td>
<td>4.49±1.54</td>
<td>0.001</td>
<td>0.077</td>
<td>0.151</td>
</tr>
<tr>
<td>IBIL</td>
<td>day 0</td>
<td>7.92±4.18</td>
<td>8.35±4.92</td>
<td>8.72±4.91</td>
<td>0.155</td>
<td>0.181</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>6.69±3.48a</td>
<td>7.90±4.44ab</td>
<td>8.71±4.68b</td>
<td>0.085</td>
<td>0.774</td>
<td>0.225</td>
</tr>
<tr>
<td>TP</td>
<td>day 0</td>
<td>70.0±5.60</td>
<td>71.4±8.16</td>
<td>71.1±6.84</td>
<td>0.055</td>
<td>0.43</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>71.7±4.47</td>
<td>72.5±5.92</td>
<td>73.3±5.76</td>
<td>0.001</td>
<td>0.077</td>
<td>0.151</td>
</tr>
<tr>
<td>GLB</td>
<td>day 0</td>
<td>27.6±2.97</td>
<td>28.9±5.11</td>
<td>28.6±3.43</td>
<td>0.461</td>
<td>0.978</td>
<td>0.234</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>28.1±3.09</td>
<td>28.4±3.96</td>
<td>28.5±3.93</td>
<td>0.001</td>
<td>0.077</td>
<td>0.151</td>
</tr>
<tr>
<td>ALB</td>
<td>day 0</td>
<td>43.1±2.92</td>
<td>43.2±3.15</td>
<td>44.0±3.01</td>
<td>0.881</td>
<td>0.032</td>
<td>0.308</td>
</tr>
<tr>
<td></td>
<td>day 180</td>
<td>42.7±2.9</td>
<td>43.1±3.04</td>
<td>44.7±5.2</td>
<td>0.001</td>
<td>0.077</td>
<td>0.151</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Groups sharing the same superscript (a or b) have no significant difference from each other in the post-hoc analysis (P≥0.05). BUN, blood urea nitrogen; ALT, alanine transaminase; AST, aspartate transaminase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TP, total protein; GLB, globin-like protein; ALB, albumin.
Figure legends

Figure 1 Flow chart of present randomized controlled trial.

Figure 2 Effect of n-3 PUFA supplements on erythrocyte phospholipid n-3 fatty acid compositions. Groups sharing the same superscript (a or b) have no significant difference from each other in the post-hoc analysis ($P \geq 0.05$), after adjustment for age, sex, study center and baseline BMI and baseline corresponding fatty acid composition. ALA, alpha-linolenic acid (C18:3n3); EPA, eicosapentaenoic acid (C20:5n3); DHA, docosahexaenoic acid (C22:6n3).
Figure 1 Flow chart of present randomized controlled trial.

252 type 2 diabetic patients under screening

Baseline blood collection and measurements

Type 2 diabetic patients included in the trial (n=185)

Fish oil (n=63)

Flaxseed oil (n=61)

Corn oil (n=61)

2 drop out → 7 drop out → 6 drop out

90 day Examination

3 drop out → 1 drop out → 1 drop out

180 day Examination

Blood samples stored and biochemical measurements

Fish oil (n=58)

Flaxseed oil (n=53)

Corn oil (n=55)

Total patients left at the end of the trial (n=166)
Figure 2 Effect of n-3 PUFA supplements on erythrocyte phospholipid n-3 fatty acid compositions

229x412mm (300 x 300 DPI)