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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1   **Effects of n-3 fatty acid supplements on glycemic traits in Chinese type 2 diabetic**

2   **patients: a double-blind randomized controlled trial**

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15 **Abbreviated Title**: n-3 fatty acids and glycemic control

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

21 **Abbreviations**: ALA, alpha-linolenic acid; CO, corn oil; DHA, docosahexaenoic acid; EPA,

22 eicosapentaenoic acid; FO, fish oil; FSO, flaxseed oil; HbA1c, glycated hemoglobin $A_{1c;}$

23 HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of

24 insulin resistance; LDL-C, low-density lipoprotein cholesterol; PUFA, polyunsaturated fatty

25 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

52   **Introduction**

53   The epidemic of type 2 diabetes (T2D) continues to grow worldwide, especially in

54   developing countries, such as China and India [1]. It is projected from a nationally

55   representative samples that up to 113.9 million and 493.4 million Chinese adults have

56   diabetes and prediabetes, respectively [2]. Epidemiologic studies consistently show that T2D

57   is largely preventable through diet and lifestyle modification [1, 3]. One type of the candidate

58   dietary factors is long-chain marine sources of n-3 polyunsaturated fatty acids (PUFA)

59   (eicosapentaenoic acid [EPA, C20:5n3], docosapentaenoic acid [DPA, C22:5n3] and

60   docosahexaenoic acid [DHA, C22:6n3]), as indicated by a number of rodent models, which

61   consistently suggest improvement of insulin sensitivity by marine n-3 PUFA [4-6]. Of note,

62   most rodent models use amounts of n-3 PUFA that are in considerable excess (on a pro-rata

63   basis) to that allowed in human [7]; and therefore the results may not be applicable among

64   humans. Yet, results from human observational and intervention studies remain inconclusive

65   [8-10]. Meta-analyses suggest that marine n-3 PUFA are inversely associated with risk of

66   T2D in Asian populations, including Chinese populations [8, 11, 12]. Furthermore, plasma

67   marine n-3 PUFA are inversely associated with insulin resistance in Chinese T2D patients

68   [13]. However, in a Cochrane systematic review and meta-analysis of trials involving n-3

69   PUFA treatment in T2D patients [14], marine n-3 PUFA supplements do not affect insulin

70   sensitivity or glucose metabolism of these patients. Of note, no included or excluded trials in

71   the meta-analysis are conducted among Chinese [14]. Another two meta-analyses of

72   randomized controlled trials also suggests a lack of marine n-3 PUFA effect on insulin

73   sensitivity in T2D patients, with only one small trial among Chinese [9, 15].

74   In addition to marine n-3 PUFA, alpha-linolenic acid (ALA, C18:3n3), a plant-based n-3

75   PUFA, has also shown inverse association with T2D in Chinese populations in observational

76   studies [16, 17]. However, to the best of our knowledge, there is no published randomized

4

77 controlled trial among Chinese T2D patients for the ALA (mainly from flaxseed oil)

78 intervention.

79

80 Therefore, to fill the gap and to confirm the results generated from observational studies, a

81 large randomized controlled trial of n-3 PUFA supplements in Chinese T2D patients is highly

82 necessary. In the present study, we conducted a randomized, multicenter, double-blind,

83 placebo-controlled trial to investigate the effects of n-3 PUFA supplements, both marine and

84 plant-based, on glycemic traits in Chinese T2D patients.

85

86 **Materials and Methods**

87 The trial was registered at ClinicalTrials.gov (No. NCT01857167). The trial was approved by

88 the Ethics Committee of College of Biosystem Engineering and Food Science at Zhejiang

89 University (No. 2013011). All the participants gave written informed consent.

90

91 *Experiment oil capsule preparation*

92 We standardized each of fish oil (FO), flaxseed oil (FSO) or corn oil (CO) capsules to one

93 gram with identical appearance. Each FO capsule provided 500mg of EPA + DHA (EPA:

94 DHA=3:2), and other major fatty acids in each FO capsule were C16:0 (71.4mg), C18:1n-9

95 (58.4mg), C16:1 (56mg), C20:0 (39.4mg) and C14:0 (34.6mg). Each FSO capsule contained

96 630mg of ALA, 155mg of C18:2n-6 and 137mg of C18:1n-9. Major fatty acids in each CO

97 capsule were C18:2n-6 (534mg), C18:1n-9 (299mg) and C16:0 (121mg). All the capsules

98 were made in the Neptunus Bioengineering Co., Ltd (Hangzhou, China). All the capsules

99 were kept in white bottles (90 capsules/bottle), which were labeled as Oil A, Oil B and Oil C

100 for the three types of capsules. None of the participants or the nurses/physicians in the study

101 centers knew the oil types during the intervention.

102

Inclusion and exclusion criteria of the study participants

104   The inclusion criteria were (1) fasting blood glucose > 7.0 mmol/L or on use of diabetic

105   medications; (2) between 35 and 80 years old for men and between post-menopausal and 80

106   years old for women. The exclusion criteria were (1) having familial hyperlipemia or with

107   blood triacylglycerol (TG) concentrations >4.56 mmol/L; (2) a history of hepatic or kidney

108   disease, or any type of cancer; (3) participation in anther clinical trial within 30 days prior to

109   screening.

110

Randomization of the participants and intervention

112   Two hundred and fifty-two potentially adults with known T2D status were screened in three

113   study centers between June 2013 to June 2014. One hundred and eighty-five T2D patients

114   were recruited based on the inclusion and exclusion criteria in the three study centers: Wuhan

115   (Central China) (n=59), Changshan (Southeast China) (n=47) and Lanzhou (Western China)

116   (n=79).

117

118   All the included participants were randomly allocated to one of the three treatments by

119   computer-generated random numbers with a block size of six: FO group (n=63), FSO group

120   (n=61) and CO group (n=61). Allocation sequence was generated by JSZ. Doctors/nurses at

121   each study center enrolled and assigned participants to the intervention groups. The

122   participants in each of the trial arms were required to take 4 capsules/day, which would

123   totally provide 2 g/day of EPA+DHA in FO group or 2.5 g/day of ALA in FSO group. The

124   dosage of n-3 PUFA supplements were consistent with those used in previous trials [10]. The

125   duration of the intervention was 180 days. All the patients were given four bottles of capsules

126   at baseline, and given another four bottles at 90 days of the intervention when they came back

127 to the study centers for health examination. At visit of 180 days of intervention, patients came

128 back to the study centers for the final on-site examination. About 84.3% (n=157) of included

129 patients took diabetic medications, with 37 patients (13, 12 and 12 for FO, FSO and CO

130 group, respectively) using insulin only, 83 patients (27, 31 and 25 for FO, FSO and CO group,

131 respectively) using oral glucose-lowering drugs only, and 36 patients (11, 10 and 15 for FO,

132 FSO and CO group, respectively) using both insulin and glucose-lowering drug. All the

133 patients were asked to maintain their usual diet, lifestyle or use of prescribed medications,

134 and avoid use of n-3 fatty acid supplements during the intervention.

135

136 *Measurements of biochemical parameters and erythrocyte fatty acids*

137 We took fasting blood samples (10 mL) of all the participants at baseline, day 90 and day 180

138 of intervention. Serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein

139 cholesterol (LDL-C), total cholesterol, TG, glucose, uric acid, blood urea nitrogen (BUN),

140 creatinine, liver function markers (alanine transaminase [ALT], aspartate transaminase

141 [AST]), blood total protein, globulin (GLB), albumin (ALB), total bilirubin (TBIL), direct

142 bilirubin (DBIL), indirect bilirubin (IDBIL) were measured by commercially available kits on

143 HITACHI 7020 chemistry analyzer using enzyme-based colorimetric test supplied by Diasys

144 Diagnostic Systems (Shanghai) Co., Ltd. at each of the study centers. Serum insulin was

145 measured by ARCHITECT insulin reagent kit (Abbott Laboratories, Abbott Park, IL, USA).

146 Using fasting glucose and insulin, homeostatic model assessment of insulin resistance

147 (HOMA-IR) was calculated by using the formula: glucose (mmol/L) × insulin (mU/L)/22.5

148 [18]. HOMA-IR, but not HOMA2 model, was used to represent insulin resistance as it was

149 more widely used in Chinese populations, and therefore more comparable among related

150 studies in China. Blood glycated hemoglobin A_{1c} (HbA1c) was measured by automated

151 Hemoglobin A1c Analyzer. Blood samples not immediately measured were stored under -

152   80°C for further analysis. Anthropometric parameters, including weight, height, waist and hip

153   circumference, were measured at baseline and end-point of the intervention.

154

155   Compliance of the patients to the intervention was evaluated by measurement of erythrocyte

156   phospholipid fatty acid compositions at baseline and end-point of the intervention, and by

157   counting the empty bottles they returned to the study centers at day 90 and 180. Additionally,

158   trained nurses contacted patients via phone once per month to record their compliance of the

159   previous month and to remind them to take the capsules. Erythrocyte phospholipid fatty acids

160   were measured by gas chromatography as previous described [19]. Briefly, we extracted total

161   lipids from erythrocytes with chloroform/methanol (1:1), and separated phospholipid fraction

162   from the total lipids by thin-layer chromatography. Then, we converted the phospholipid fatty

163   acids to methylester and extracted them into n-hexane and dried on anhydrous Na_2SO_4.

164   Finally, we filtered fatty acid methylesters by Sep-Pak Silica column before gas

165   chromatography separation and analysis.

166

167   *Statistical analysis*

168   We conducted all the statistical analyses in Stata (version 13; StataCorp, College Station, TX,

169   USA). The total sample size was calculated based on 80% power ($\alpha_{two-tailed}$=0.05) to detect

170   difference in HOMA-IR by 20% or 0.63 (SD=1.1) between groups (n=150, 50/group),

171   considering 20% drop out rate (n=187, 62/group), based on our previous work [20]. This

172   sample size (n=50 /group) will also enable us to have 84% power ($\alpha_{two-tailed}$=0.05) to detect

173   difference in HbA1c by 20% or 12 mmol/mol (SD=20) between groups.

174

175   All the outcomes variables were checked for the normal distribution, and were log

176   transformed if they were not normal distributed (HOMA-IR, glucose, insulin, HbA1c and TG

177   were natural log transformed). One-way ANOVA (for continuous variables) or Chi-square

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

179   Difference in change of the three fatty acid compositions for FO or FSO compared with CO

180   group was examined by linear regression, adjusting for age, sex, study center and baseline

181   BMI and baseline corresponding fatty acid composition.

182

183   Following an intention-to-treat principal, linear mixed models were used to compare

184   differences between intervention groups in four glycemic traits (HOMA-IR, insulin, glucose,

185   HbA1c) overtime (day 0, 90 and 180). The mixed model analysis without any ad hoc

186   imputation for missing data would provide equal or more power than dose analysis with data

187   imputation [21]. Time since baseline randomization was included in the model as a

188   categorical variable (day 0, 90 and 180), and the group×time interaction was treated as the

189   fixed effect in the model and was the primary effect of interest. Other potential confounders

190   included in the model as fixed effects were age, sex, study center and baseline BMI.

191   Sensitivity analysis was conducted (1) by including only two time points (day 0 and 180) in

192   the linear mixed models; (2) by including baseline value of corresponding outcome as a

193   covariate in the linear mixed models.

194

195   In addition to the primary analyses on glycemic traits, we performed secondary analyses on

196   lipid traits, including HDL-C, LDL-C, TC, TC/HDL-C and TG using the linear mixed models.

197   If significant time × group interaction was observed for primary or secondary analyses, we

198   conducted post-hoc analyses to examine the group × time interaction between FO and CO,

199   between FO and FSO, and between FSO and CO, respectively, based on the linear mixed

200   models. *P*-value (two-tailed) <0.05 was considered significant.

201

9

202 **Results**

203 At baseline, we did not observe any significant difference in blood lipids or glycemic traits

204 between participants of the three intervention groups (**Table 1**). During the intervention, five

205 participants in the FO group, eight participants in the FSO group and six participants in the

206 CO group dropped out of the trials, leaving 58, 53 and 55 participants in the FO, FSO and CO

207 group, respectively (**Figure 1**).

208

209 Erythrocyte EPA and DHA was significantly increased in the FO group compared with the

210 CO group (P<0.001 for both fatty acids) (**Figure 2**). FSO group, compared with CO group,

211 had significant increased composition of ALA (P=0.043), but non-significant increase of EPA

212 (P=0.084) and DHA (P=0.056). EPA was significantly increased in FO compared with FSO

213 group (P=0.001).

214

215 No significant difference among the three groups was observed on fasting serum glucose,

216 insulin or HOMA-IR (**Table 2**). However, we found significant interaction between study

217 groups (3 groups) and time (3 time points) for HbA1c (P=0.035). Post-hoc analysis showed

218 that HbA1c was significantly decreased in FO group (P=0.037) but not FSO group (P=0.30),

219 compared with CO group. Sensitivity analysis by including 2 time points (day 0 and 180)

220 found similar tendency that HbA1c decreased in FO group (P=0.088) and in FSO group

221 (P=0.152), compared with CO group. Further sensitivity analysis by including baseline

222 outcome value as a covariate found that FO compared with CO group, showed a stronger

223 statistical significance in the HbA1c (P=0.009 from the full analysis including 3 time points;

224 P=0.029 from the restricted analysis including 2 time points [day 0 and day 180]), while no

225 significant difference in the HbA1c (P=0.23 and 0.06 from the full analysis and restricted

226 analysis respectively) observed for FSO compared with CO group. No significant

227   results/material changes were found for other glycemic traits in FO or FSO group in the

228   above two steps of sensitivity analyses.

229

230   We found significant time by group interaction for each of serum LDL-C (P=0.043), TC

231   (P=0.021), TC/HDL-C (P=0.009) and TG (P=0.003), but not for HDL-C (**Table 3**). In the

232   post-hoc analyses, serum concentrations of TC (P=0.029), TC/HDL-C (P=0.038) and TG

233   (P=0.001) were significantly decreased in FO group compared with that in CO group, but

234   with no statistical significance on LDL-C (P=0.081). FO group compared with FSO group,

235   had significant decrease of LDL-C (P=0.025), TC (P=0.007), TC/HDL-C (P<0.001) and TG

236   (P=0.043) after the intervention. No significant difference between FSO and CO groups was

237   found for any blood lipid trait. No significantly different change between treatments during

238   the intervention was observed for other biochemical parameters, except for IDBIL (P=0.047)

239   (**Table 4**).

240

241   **Discussion**

242   To the best of our knowledge, the present randomized controlled trial was among the first to

243   examine the effect of both marine and plant-based n-3 PUFA supplements on Chinese T2D

244   patents. Our trial also had larger sample size than most of previous trial in other population

245   settings. We found that FO supplements may potentially improve glycemic control in the T2D

246   patients in terms of decreasing HbA1c, a marker of blood glucose status over the past few

247   months. Moreover, FO consistently lowered blood lipids in these patients, including TC,

248   LDL-C and TC/HDL-C. No significant effects on glycemic traits or blood lipids were found

249   in patients taking FSO supplements.

250

251   Fish oil supplements were found to increase blood glucose levels with borderline significance

11

252   among T2D patients in an early meta-analysis [22]. In addition, every one g/day increase of

253   EPA and DHA was significantly associated with 0.38% and 0.6% increase of HbA1c,

254   respectively, in T2D patients [22]. However, an updated Cochrane review of n-3 PUFA

255   intervention among T2D patients based on 23 randomized controlled trial (1075 participants)

256   showed that n-3 PUFA supplements did not have any significant effect on glycemic traits,

257   including fasting insulin, glucose and HbA1c [14]. In several other reviews of n-3 PUFA

258   supplements, the authors suggested no benefit of n-3 PUFA on glycemic traits or insulin

259   sensitivity among T2D patients [10]. Of note, all of the trials included in the above meta-

260   analyses or review [9, 10, 14] came from Western populations, with no trials from Asian

261   populations (except for one from India). These results were consistent with that from meta-

262   analyses of prospective cohort studies, which suggested that marine n-3 PUFA/fish intake

263   showed null or even positive association with T2D risk in Western populations [8, 11]. In

264   contrast, marine n-3 PUFA intake was associated with lower risk of T2D in Asian populations

265   [8, 11]. Furthermore, the only randomized controlled trial in the Asian Indian population [23]

266   in the aforementioned meta-analysis [14] suggested that n-3 PUFA could improve glycemic

267   status. Recently, several randomized controlled trials of n-3 PUFA supplements among T2D

268   patients have been conducted in Asian countries (from Iran and Japan) [24-26]. For example,

269   in a randomized double-blind placebo-controlled trial among 81 T2D patients in Iran, n-3

270   PUFA supplementation for 2 months significantly decreased HbA1c compared with control

271   group [26]. Among another trial of 67 Iranian T2D patients over a period of 3-month

272   intervention [24], n-3 PUFA (EPA) supplementation improved glycemic control by

273   decreasing fasting serum insulin, glucose, HbA1c and HOMA-IR. Furthermore, in a

274   randomized controlled trial among 30 elderly Japanese T2D patients, EPA/DHA-rich liquid

275   diet, compared with liquid diet lacking EPA/DHA, significantly decreased fasting plasma

276   glucose and HbA1c over a period of 3 months [25]. These new evidence suggested that n-3

277 PUFA supplements could potentially improve glycemic control in Asian populations, while

278 no evidence was available among Chinese populations prior to our present trial.

279

280 Our current trial supported that marine n-3 PUFA supplements may improve glycemic control

281 by decreasing HbA1c level. The reason for the non-significant change for HOMA-IR or

282 fasting glucose was not clear, which may be due to the influence of regular oral drug use or

283 insulin injection days before the blood draw. We also observed that HOMA-IR, insulin and

284 glucose level were lowest at 90 days. This may be due to the decrease of compliance in the

285 late stage of the trial or some unknown confounding factors. In addition, we found that

286 HbA1c in FSO group continuously decreased from baseline to day 90 and subsequently to

287 day 180; while in FO group it decreased from baseline to day 90 and then increased at day

288 180. This observation may be because of the poorer compliance after day 90 in FO group, or

289 because of the fact that the degree of response to either treatment has reached a plateau at the

290 HbA1c value of around 54 mmol/mol. Nevertheless, HbA1c is a stable marker of glucose

291 status over several months, and the decreased level of HbA1c indicates improved glycemic

292 control after the marine n-3 PUFA supplements. The disparate findings for marine n-3 PUFA

293 with glycemic control and T2D in Asian and Western populations may be due to the different

294 genetic backgrounds. For example, in a recent study [27], researchers find that an adaptive

295 genetic polymorphism within *FADS2* gene conferred an adaptive advantage in Asians because

296 of the traditional plant-based diet practice, which suggests that Asians are more likely to

297 synthesize long-chain PUFA from plant PUFA precursors. Based on the aforementioned

298 evidence, we therefore hypothesized that Asians may be more sensitive to marine n-3 PUFA

299 in terms of glycemic control compared with Western populations, which warrants further

300 investigation.

301

302 In contrast to glycemic traits, previous meta-analyses consistently supported that marine n-3

303 PUFA improved lipid profiles by reducing blood TG level [14, 22] among T2D patients.

304 However, both of these meta-analyses suggested that n-3 PUFA may increase serum LDL-C

305 concentration among T2D patients; while no significant change in TC or HDL-C was

306 observed after n-3 PUFA supplements [14, 22]. In the secondary analyses of the present study,

307 TG, TC and TC/HDL-C were significantly decreased in FO group compared with CO group.

308 In addition, FO had the tendency to decrease LDL-C compared with the CO. The present

309 results suggested that, in Chinese T2D patients, beneficial effect of n-3 PUFA on blood lipids

310 was not limited to TG, but also to other lipid profiles, such as TC, TC/HDL-C and potentially

311 LDL-C. The reason for the difference in TC and LDL-C between the present results and that

312 of previous meta-analyses was not clear. It may be that the present study had longer

313 intervention period than most of previous randomized trials included in the meta-analyses [14,

314 22]. Furthermore, we did not collect information on statin use for the patients, which may

315 potentially bias the lipid response to n-3 PUFA intervention. Nevertheless, these results

316 indicated that fish oil might play an important role in the prevention of cardiovascular events,

317 a most important complication of T2D. We noticed a slightly different response of lipids to n-

318 3 PUFA at day 90 and day 180, which suggested that the effects of n-3 PUFA on blood lipids

319 may have some optimal threshold in terms of intervention period (reaching optimal blood n-3

320 PUFA levels), which warrants further investigation.

321

322 Different from FO, the present study did not observed any significant effect of FSO

323 supplements, source of a plant-based n-3 PUFA: ALA, on glycemic traits or lipid profiles.

324 Our results were consistent with several previous trials. In two randomized controlled trials of

325 FSO supplements among T2D individuals [28, 29], 7.4g/d and 5g/d of ALA was used in the

326 trial arm of each study. However, there was no significant change of glycemic traits in

327   response to FSO supplements in the two studies. Given that dose of ALA used in the present

328   study (2.5g/d) was much lower than that of previous trials [28, 29], it could be postulated that

329   in the present study we would observed no significant effect of ALA supplements on

330   glycemic traits as consistent with the previous trials [28, 29]. Yet, further randomized trials of

331   higher flaxseed oil/ALA dose are needed to investigate the effect of FSO/ALA on Chinese

332   T2D patients.

333

334   The beneficial effects of the marine n-3 PUFA on glycemic control and glucose homeostasis

335   are biological plausible and may involve various mechanisms. For example, with the

336   increasing incorporation of marine n-3 PUFA into cell membranes via supplementation, the

337   membrane fluidity will be increased, leading to increased insulin sensitivity [30]. Marine n-3

338   PUFA may also improve glucose homeostasis through regulating inflammatory status [31, 32].

339   In addition, a variety of animal models have suggested that marine n-3 PUFA may improve

340   insulin sensitivity and glucose homeostasis by influencing insulin signaling pathway [33-36].

341

342   The present study has several strengths. First, the sample size of the present trial provided

343   sufficient power to examine the effect of n-3 PUFA supplements on glycemic traits. Second,

344   study duration was longer than most of previous trials in the T2D patients. Third, this was a

345   multicenter trial, which represented participants from Western, Central and Southeast China.

346   The limitation of the present trial was that 180-day of intervention was still too short for us to

347   examine the effect of n-3 PUFA supplements on T2D complications, such as cardiovascular

348   events among the patients. In addition, we suggested that the patients maintain their usual diet

349   and physical activity, but did not monitor their lifestyles during the intervention, although we

350   obtained oral agreements from the patients that they would not change their diet or lifestyles

351   during the intervention. Third, 84.3% of study participants took diabetic medications, which

15

352   may be a potential source of confounding to the present trial. Yet, the distribution of

353   medications used was similar among groups, and unlikely to affect the study results. Fourth,

354   the repeated measure approach may not capture the potentially specific response at day 90

355   and at day 180 to the treatments, which may influence the overall results. This speculation

356   was supported by our sensitivity analysis of including only 2 time points (day 0 and day180)

357   in the models that the effect of FO was attenuated, while effect of FSO strengthened. Last, we

358   did not randomize the participants based on HbA1c at baseline, which may potentially affect

359   the effect of n-3 supplements on HbA1c, a main outcome of this trial. Future researchers in

360   this area need to balance trial arms for the main outcomes at baseline.

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362   In conclusion, the present randomized controlled trial suggested that marine n-3 PUFA

363   supplements for 180 days potentially improved glycemic control in Chinese T2D patients.

364   This study provides new evidence of using marine n-3 PUFA for the glycemic control of

365   Chinese T2D patients; however, there is no convincing evidence showing that marine n-3

366   PUFA are superior to plant-based n-3 PUFA. More studies with longer follow-up duration

367   and larger sample size in Chinese populations are warranted to replicate and confirm the

368   results of the present study.

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Table 1 Baseline characteristics of study participants involved in the randomized

controlled trial

	Fish oil (n=63)	Flaxseed oil (n=61)	Corn oil (n=61)
Age, y	59.7±8.8	59.7±11.1	59.1±10
Women, n (%)	25 (39.7)	41 (67.2)	32 (52.4)*
Weight, kg	68.4±11.5	63.8±11.5	68.9±15.2
BMI, kg/m^2	24.7±2.8	24.7±3.9	25.5±4.1
SBP, mmHg	137.2±20.2	138.3±18.6	133.7±21.4
DBP, mmHg	78.2±10.5	78.6±8.1	79.5±14.9
HDL-C, mmol/L	1.14±0.3	1.16±0.29	1.19±0.20
LDL-C, mmol/L	2.97±0.80	2.89±0.85	3.05±0.85
TC, mmol/L	4.66±0.96	4.68±0.96	4.88±1.01
TC/HDL-C	4.28±1.05	4.23±1.13	4.17±0.92
TG, mmol/L	1.68±0.74	1.93±1.30	1.85±0.97

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

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Table 2 Effect of n-3 PUFA supplements on glycemic traits in Chinese patients with type 2 diabetes

	Time	Fish oil	Flaxseed oil	Corn oil	P-time	P-group	P-time×group interaction
Fasting glucose, mmol/L	day 0	8.18±3.03	8.61±3.64	8.31±3.63	0.221	0.811	0.703
	day 90	7.60±1.93	8.23±2.83	7.71±2.37			
	day 180	8.46±3.02	8.16±2.70	8.22±2.51			
Fasting insulin, mU/L	day 0	14.7±8.7	20.3±18	16.5±16.1	<0.001	0.069	0.281
	day 90	11.4±10.6	13.1±10.8	11.5±9.67			
	day 180	11.6±6.76	15.6±11.3	18.1±19.8			
HOMA-IR	day 0	5.48±3.97	7.57±6.39	6.21±5.89	<0.001	0.107	0.404
	day 90	4.19±4.76	5.10±4.62	4.00±4.57			
	day 180	4.68±4.12	5.74±4.67	6.46±6.46			
HbA1c, mmol/mol	day 0	63.9±22.9	62.3±20.8	57.6±16.4	<0.001	0.571	0.035
	day 90	52.7±13.8	59.2±17.1	56.2±17.2			
	day 180	54.4±13.4a	54.2±14ab	55.0±16.4b			

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 ($P \geq 0.05$). HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin A_{1c}.

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Table 3 Effect of n-3 PUFA on blood lipids in Chinese patients with type 2 diabetes

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

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.

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Table 4 Effect of n-3 PUFA supplements on liver and kidney function markers and other

blood parameters.

	Time	Fish oil	Flaxseed oil	Corn oil	*P*-time	*P*-group	*P*-time×group interaction
BUN	day 0	6.04±2.15	6.02±1.88	5.54±1.36	0.017	0.375	0.677
	day 180	6.10±2.24	6.31±1.62	5.88±1.60			
Creatinine	day 0	75.0±28.0	64.6±22.0	66.1±23.5	0.905	0.301	0.387
	day 180	73.8±36.2	65.9±22.8	68.1±34.1			
Uric acid	day 0	322.4±86.9	322.0±78.9	320.3±98.4	0.485	0.95	0.207
	day 180	332.4±81.1	313.6±88.7	307.8±73.6			
ALT	day 0	28.0±17.8	31.1±22.6	28.2±14.8	0.074	0.36	0.839
	day 180	29.5±16.8	30.6±25.9	31.6±18.8			
AST	day 0	26.6±10.8	29.1±16.2	25.7±8.00	0.091	0.535	0.774
	day 180	25.1±11.0	26.2±11.7	25.4±10.3			
TBIL	day 0	12.7±5.72	12.2±6.32	13.4±6.3	0.148	0.077	0.151
	day 180	11.2±4.39	12.4±6.94	15.5±20.0			
DBIL	day 0	5.28±2.54	5.02±2.04	5.36±2.24	<0.001	0.632	0.344
	day 180	4.47±1.47	4.76±2.09	4.49±1.54			
IBIL	day 0	7.92±4.18	8.35±4.92	8.72±4.91	0.155	0.181	0.047
	day 180	6.69±3.48a	7.90±4.44ab	8.71±4.68b			
TP	day 0	70.0±5.60	71.4±8.16	71.1±6.84	0.055	0.43	0.90
	day 180	71.7±4.47	72.2±5.92	73.3±5.76			
GLB	day 0	27.6±2.97	28.9±5.11	28.6±3.43	0.461	0.978	0.234
	day 180	28.1±3.09	28.4±3.96	28.5±3.93			
ALB	day 0	43.1±2.92	43.2±3.15	44.0±3.01	0.881	0.032	0.308
	day 180	42.7±2.9	43.1±3.04	44.7±5.2			

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).
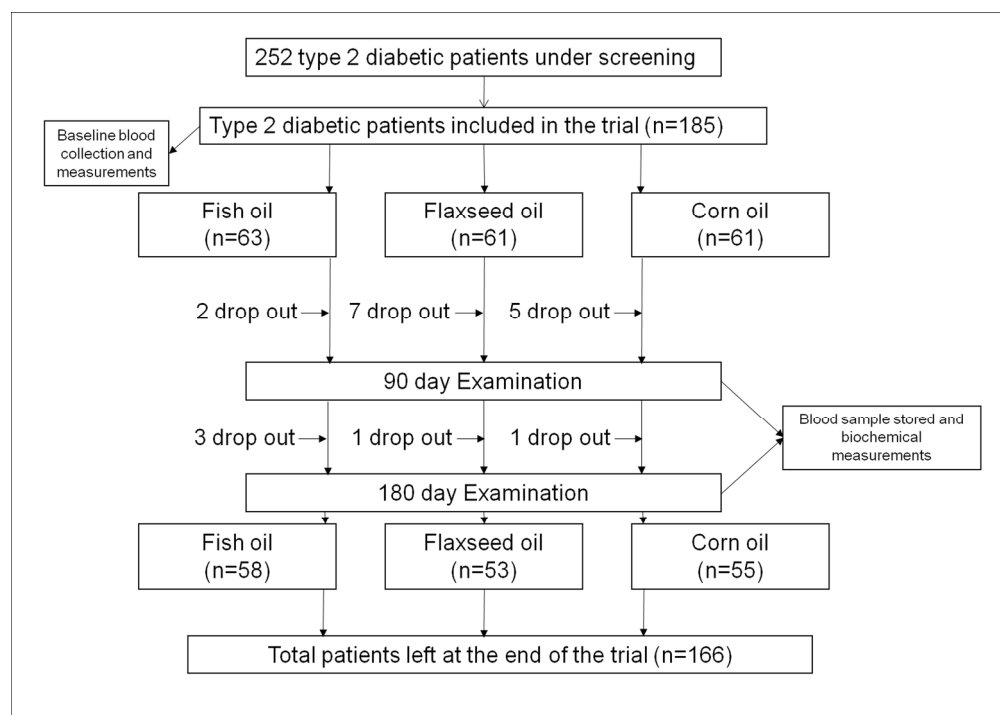
Figure 1 Flow chart of present randomized controlled trial.
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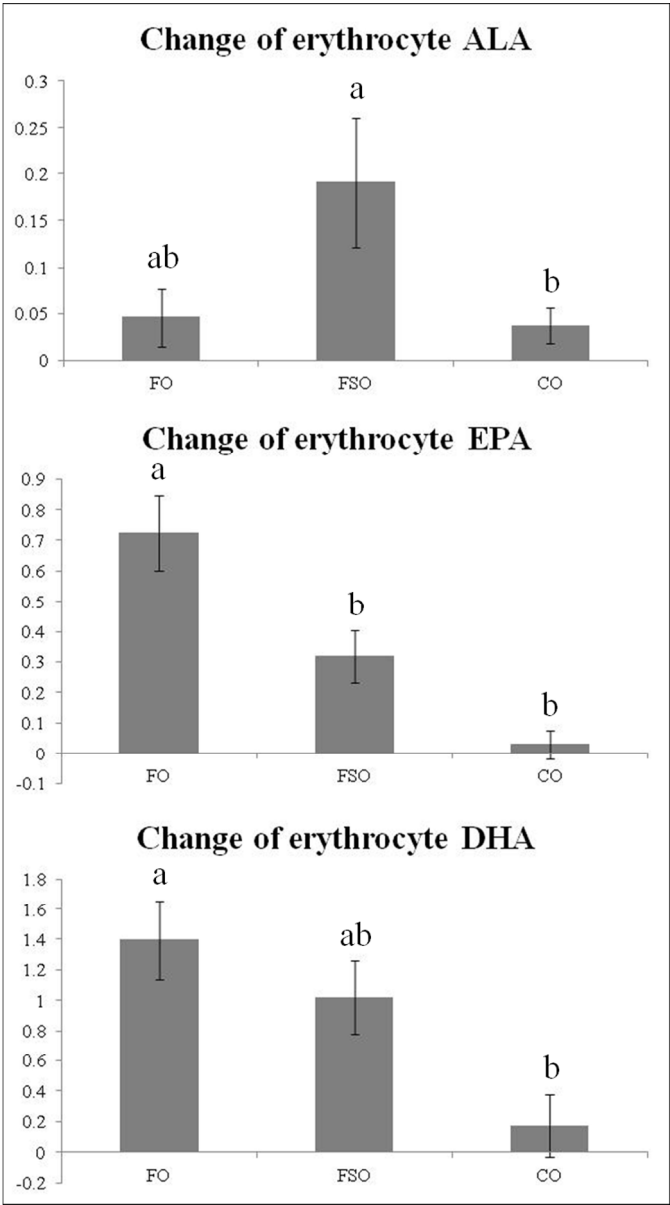51
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Figure 2 Effect of n-3 PUFA supplements on erythrocyte phospholipid n-3 fatty acid compositions
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