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Research Paper

Replication of a Gene-Diet Interaction at *CD36*, *NOS3* and *PPARG* in Response to Omega-3 Fatty Acid Supplements on Blood Lipids: A Double-Blind Randomized Controlled Trial☆

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ABSTRACT

Background: Modulation of genetic variants on the effect of omega-3 fatty acid supplements on blood lipids is still unclear.
Methods: In a double-blind randomized controlled trial, 150 patients with type 2 diabetes (T2D) were randomized into omega-3 fatty acid group (n = 56 for fish oil and 44 for flaxseed oil) and control group (n = 50) for 180 days. All patients were genotyped for genetic variants at *CD36* (rs1527483), *NOS3* (rs1799983) and *PPARG* (rs1801282). Linear regression was used to examine the interaction between omega-3 fatty acid intervention and *CD36*, *NOS3* or *PPARG* variants for blood lipids.
Findings: Significant interaction with omega-3 fatty acid supplements was observed for *CD36* on triglycerides (p-interaction = 0.042) and *PPAGR* on low-density lipoprotein-cholesterol (p-interaction = 0.02). We also found a significant interaction between change in erythrocyte phospholipid omega-3 fatty acid composition and *NOS3* genotype on triglycerides (p-interaction = 0.042), total cholesterol (p-interaction = 0.013) and ratio of total cholesterol to high-density lipoprotein cholesterol (p-interaction = 0.015). The T2D patients of *CD36*-G allele, *PPARG*-G allele and *NOS3*-A allele tended to respond better to omega-3 fatty acids in improving lipid profiles. The interaction results of the omega-3 fatty acid group were mainly attributed to the fish oil supplements.
Interpretation: This study suggests that T2D patients with different genotypes at *CD36*, *NOS3* and *PPARG* respond differentially to intervention of omega-3 supplements in blood lipid profiles.

1. Introduction

There has been a pronounced progress in the field of nutrigenetics or gene-diet interaction in the past decade, thanks to the great achievement in the identification of novel genetic variants related to diseases in large-scale epidemiological studies and international consortium [1–4]. The goal of gene-diet interaction is to tailor one's diet based on his genetic background in contrast to the traditional "one-size-fits-all" dietary recommendation. Although the concept of gene-diet interaction is appealing, and progress within recent years is encouraging, lack of

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2D, type 2 diabetes; SNP, single-nucleotide polymorphisms; TG, triacylglycerol; TC, total cholesterol..

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replication has become a major barrier affecting the acceleration of the field and its translation into practice [1,4,5].

Omega-3 (or n-3) fatty acids, both marine (C20:5n-3, C22:5n-3, C22:6n-3) and plant based (C18:3n-3), could improve blood lipid profiles and decrease risk of cardiovascular diseases [6–9]. Some intervention studies suggest that effects of omega-3 fatty acids on blood lipids could be modified by genetic variants and supports the existence of gene-diet interaction for omega-3 fatty acids with regard to the lipid outcomes [10–12]. In a systematic review, Corella et al. [13] suggested that only three genes (*CD36*, *NOS3* and *PPARG*) showed interactions with omega-3 fatty acids to affect the levels of blood lipids in the intervention studies, while no replication among trials has been reported so far.

Therefore, the aim of the present study was, to use a well-conducted randomized controlled trial to replicate the previous findings from intervention studies about the interaction of genetic variants (single-nucleotide polymorphisms, SNP) at *CD36*, *NOS3* and *PPARG* with omega-3 fatty acid intervention for the blood lipids.

2. Materials and Methods

2.1. Study Population and Design

This study was based on a double-blind randomized controlled trial. The trial was registered at ClinicalTrials.gov (No. NCT01857167), and approved by the Ethics Committee of Colledge of Biosystem Engineering and Food Science at Zhejiang University. All participants gave written informed consent.

The inclusion and exclusion criteria, and the detailed procedures of the trial has previous been reported [14]. Briefly, the inclusion criteria were fasting blood glucose>7.0 mmol/L or on use of diabetic medications, participants between 35 and 80 years for men or between postmenopausal and 80 years for women; the exclusion criteria were having familial hyperlipidemia or with blood triglycerides >4.56 mmol/L, having a history of hepatic or kidney disease or any type of cancer, or participation in another clinical trial within 30 days prior to screening. The total sample size was calculated based on a 80% power with $\alpha = 0.05$ to detect a difference in HOMA-IR by 0.63 (SD 1.1) between groups, considering a 20% drop out rate [14]. A total of 185 patients with type 2 diabetes (T2D) were recruited in three research centers at Wuhan, Changshan and Lanzhou, and randomized into three groups: fish oil ($n = 63$), flaxseed oil ($n = 61$) and corn oil group ($n = 61$). This was a double-blind randomized controlled trial, and all the participants were randomly allocated to one of the three treatments by computer-generated random sequence. Neither the researchers randomizing the patients nor the patients knew the treatments they were allocated before the randomization or during the trial. Participants at each of the group took 4 capsules/day, corresponding to 2 g/day of C20:5n-3 and C22:6n-3 in fish oil group, and 2.5 g/day of C18:3n-3 in flaxseed oil group, with corn oil used as a control oil. The trial lasted for 180 days. All patients were asked to maintain their usual diet, lifestyle and medication, and avoid use of omega-3 fatty acid supplements. Compliance of the participants to the intervention was objectively assessed by the measurement of erythrocyte fatty acids that C20:5n-3 and C22:6n-3 was significantly increased in fish oil group compared with corn oil group, and C18:3n-3 was significantly increased in flaxseed oil group compared with corn oil group [14]. Among the trial participants, 150 patients provided DNA samples, of which 100 in omega-3 supplement group (56 in fish oil group, 44 in flaxseed oil group) and 50 in corn oil control group.

2.2. SNP Selection and Genotyping

SNPs rs1527483 at *CD36*, rs1799983 at *NOS3*, and rs1801282 at *PPARG* were selected for genotyping based on the prior evidence [13]. Blood DNA was isolated by using the QIAamp DNA Blood Mini Kits (Qiagen, Valenica, CA, USA). The selected SNPs were genotyped using the standard protocol recommended by the MassARRAY RS1000 (Sequenom, San Diego, CA, USA) manufacturer, and the data were analyzed by Typer 4.0 Software (Sequenom) [15], with an average genotyping success rate of 98%.

2.3. Measurement of Blood Lipids, Erythrocyte Phospholipid Fatty Acids, and Other Parameters

Fasting blood samples were collected at baseline and the end of the intervention. Serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) were measured by commercially available kits with HITACHI 7020 chemistry analyser using enzyme-based colorimetric test supplied by Diasys Diagnostic Systems (Shanghai) Co., Ltd. Erythrocyte phospholipid fatty acid composition was measured by gas chromatography, as has been described previously [14]. Body weight and height were measured by trained nurses at baseline and the end of the intervention. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

2.4. Statistical Analyses

All the statistical analyses were conducted using Stata (version 14; StataCorp, College Station, TX, USA). All the lipid variables (HDL-C, LDL-C, TC, TC/HDL-C, and TG) were checked for normal distribution and were natural log-transformed if not normally distributed (for TG only). Dominant models were used to assess the genetic effects and the gene-diet interactions in the present study, as to maximise the sample size in each genetic group. At baseline, the association between blood lipids and genetic variants at *CD36*, *NOS3* and *PPARG* was examined by linear regression models, adjusted for age, sex, study center and BMI.

As the primary analysis, we examined the interaction of genetic variants at *CD36*, *NOS3* and *PPARG* with omega-3 fatty acid supplements on the change in blood lipids during the intervention based on the complete case analysis. We used general linear model to test the genotype-by-intervention interaction as independent predictors of change in blood lipids, adjusting for age, sex, study center, BMI and baseline value of the corresponding outcome trait. To increase the sample size and the power to detect the interaction, we combined fish oil and flaxseed oi group into one omega-3 fatty acid supplement group, as erythrocyte phospholipid C20:5n-3 and C22:6n-3 were increased in both fish oil and flaxseed oil groups as reported previously [14]. Quanto 1.2.4 (University of Southern California) was used to estimate the detectable effect size of genotype-by-diet interactions. For example, this study had 80% power to detect significant gene-diet interaction effect sizes (for rs1527483) of 0.27 mmol/L, 0.85 mmol/L, 0.97 mmol/L, 1.05, and 0.9 mmol/L for change in HDL-C, LDL-C, TC, TC/HDL-C ratio and TG under a dominant model, respectively.

In a secondary analysis, we examined the interaction between change in erythrocyte phospholipid omega-3 fatty acids (sum of C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3, as a continuous variable) and NOS3 genotypes for the change in lipid outcomes using the general linear model, adjusting for age, sex, study center, BMI and baseline value of the corresponding outcome trait. We conducted this secondary analysis because original paper reporting this gene-fatty acid interaction was based on the interaction between change in plasma total omega-3 fatty acids and NOS3 variant on change in TG in an intervention study [10].

If a significant interaction ($p < 0.05$) was detected, we conducted a stratified analysis by the genotype groups and by intervention groups using the general linear model. In addition, we also separately examined the effects of different omega-3 fatty acid group (i.e. fish oil and flaxseed oil group) on blood lipids by the tested genotypes.

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