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Meta-analyses

Fatty acid and non-alcoholic fatty liver disease: Meta-analyses of case-control and randomized controlled trials

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SUMMARY

Background and aims: Blood and/or liver fatty acid contents of healthy subjects and non-alcoholic fatty liver disease (NAFLD) patients have shown inconsistent associations. In addition, the results of randomized controlled trials (RCTs) in relation to the effects of n-3 polyunsaturated fatty acid (PUFA) supplementation on alanine aminotransferase (ALT), aspartate aminotransferase (AST), liver fat, triglyceride (TAG) and fasting glucose levels are inconsistent. The present study aimed to investigate the differences of fatty acid content in the blood and/or liver tissue between healthy subjects and NAFLD patients, and to quantify the benefits of n-3 PUFA therapy in NAFLD patients.

Methods: A systematic literature search was performed up to November 2016 using PubMed and Scopus databases. The differences of fatty acid content between cases and controls were calculated as weighted mean differences (WMD) by using a random-effects model. The intervention effects of RCTs were calculated as WMD for net changes in ALT, AST, liver fat, TAG and fasting glucose levels, respectively. Meta-regression with restricted maximum likelihood estimation was used to evaluate a potential linear relationship between confounding factors and effect sizes. Generalized least square was performed for dose-response analysis.

Results: Ten eligible case-control studies and 11 RCTs were included. The pooled estimates of case-control studies showed that blood and/or liver docosahexaenoic acid (DHA) content was significantly higher in the controls compared with cases. The pooled estimates of RCTs showed that n-3 PUFA supplementation significantly reduced the ALT (−7.53 U/L; 95% CI: −9.98, −5.08 U/L), ASL (−7.10 U/L, 95% CI: −11.67, −2.52 U/L) and TAG (−36.16 mg/dL, 95% CI: −49.15, −23.18 mg/dL) concentrations, and marginally reduced the liver fat content (−5.11%, 95% CI: −10.24, 0.02%, P = 0.051), but not fasting glucose. Dose-response analysis of RCTs showed that 1 g per day increment of eicosapentaenoic acid (EPA)+DHA was associated with a 3.14 U/L, 2.43 U/L, 2.74% and 9.97 mg/dL reduction in ALT (95% CI: −5.25, −1.02 U/L), AST (95% CI: −3.90, −0.90 U/L), liver fat (95% CI: −4.32, −1.16%) and TAG (95% CI: −14.47, −5.48 mg/dL) levels, respectively.

Conclusions: The present meta-analysis provides substantial evidence that n-3 PUFA supplementation, especially DHA, has a favorable effect in treatment of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), which is a leading cause of chronic liver disease, consists of a wide spectrum of conditions, ranging from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis and hepatocellular carcinoma [1]. It has been demonstrated that the prevalence of NAFLD is 10% in children and affects up to as high as 30% adults in Western countries [2–4]. The NAFLD patients are asymptomatic at the beginning, but increasing findings have shown that NAFLD is significantly associated with increased risk of type 2 diabetes mellitus (T2DM) [5], and cardiovascular diseases (CVD) [6,7]. Thus, management and treatment of NAFLD are a challenge for public health.

Changes in lifestyles are a feasible and practical way to improve histological features of NAFLD [8,9]. Meanwhile, lifestyle interventions have shown to be associated with decreased concentrations of triglyceride (TAG), alanine aminotransferase (ALT) and

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aspartate aminotransferase (AST) [10]. Besides, drug treatment with thiazolidinediones has shown to improve physical condition of NAFLD patients [11]. Given the poor compliance of lifestyle interventions and side-effect of pharmacotherapy, an effective and safe therapeutic approach is urgently needed for treatment of NAFLD. Up to now, mounting attention has focused on dietary and nutritional interventions. Of these, n-3 polyunsaturated fatty acids (PUFA) of marine origin have been paid increasing attention, due to their ability to improve glucose and lipid metabolism and inflammation [12,13].

To our best knowledge, no prospective cohort study has been built to investigate the association of blood and/or liver fatty acid content with NAFLD. Several case-control studies have shown a significantly higher concentration of n-3 PUFA (especially docosahexaenoic acid (DHA)) in healthy subjects compared with NAFLD patients [14–20], whereas other studies have suggested opposite or null associations [21–23]. Similarly, randomized controlled trials (RCTs) with n-3 PUFA intervention have shown inconsistent results in relation to ALT, AST, liver fat, TAG and fasting glucose levels in patients with NAFLD [24–34]. A meta-analysis of 3 RCTs has reported that n-3 PUFA supplementation exerted a significant reduction in concentration of ALT [11]. Another meta-analysis has showed that the n-3 PUFA supplementation significantly reduced the levels of AST and liver fat, but not ALT, however, the trials included in the meta-analysis were not restricted to RCTs [35]. Moreover, the effects of n-3 PUFA supplementation on concentrations of TAG and fasting glucose are unclear in NAFLD patients. Recently, several RCTs with n-3 PUFA supplementation on NAFLD patients have been published, while the trials have not drawn a definitive conclusion on biomarkers of liver damage [27,28,30,31]. Therefore, the present study aimed to investigate the differences of fatty acid content in the blood and/or liver tissue between cases and controls, with case-control studies. In addition, another meta-analysis of RCTs was performed to clarify whether n-3 PUFA supplementation could exert significant reductions in ALT, AST, liver fat, TAG, and fasting glucose levels in NAFLD patients.

2. Methods

The present systematic review and meta-analysis was based on the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [36].

2.1. Study selection

A systematic literature search was performed up to November 2016, using the databases of PubMed and Scopus. Fish oil, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), DHA, omega-3 and n-3 were paired with NAFLD, fatty liver, hepatic steatosis and non-alcoholic steatohepatitis (NASH) as search terms. Using Google scholar, manual search of meta-analyses, recent reviews, and original research articles were also scrutinized.

2.2. Eligible criteria

The original RCTs that were included in the present meta-analysis met the following criteria: (1) RCT; (2) using n-3 PUFA as the only intervention; and (3) available data were provided to calculate the mean differences between baseline and endpoint for ALT, ASL, liver fat, TAG, and fasting glucose. Meanwhile, case-control studies, which have reported the fatty acid content in the blood and/or liver tissue, were also included.

2.3. Data extraction and quality assessment

Data extraction was independently carried out by two investigators (XG and BY), and any discrepancy was resolved by discussion reaching a consensus. Data that were extracted, included first author, nation/country, published year, age, gender, duration of intervention, sample-size and types of intervention. In each RCT, the means and standard deviations (SDs) at baseline and endpoint in both the control and intervention groups were extracted. If the SDs were not reported in the study directly, we calculated them from standard error of mean (SEM) or 95% confidence interval (CI) with the equations listed in the Cochrane handbook [37]:

$$SD = SEM \times \sqrt{n} \quad (1)$$

and

$$SD = \sqrt{n} \times (upperlimit - lowerlimit) \div 3.92 \quad (2)$$

Change-from-baseline SD was calculated with the following equation:

$$SD_{change} = \sqrt{SD^2_{baseline} + SD^2_{final} - 2 \times R \times SD^2_{baseline} \times SD^2_{final}} \quad (3)$$

The R is the correlation coefficient. A minimum correlation coefficient of 0.5 was used to be conservative.

Quality assessment was conducted with the Jadad scoring criteria, including randomization, random sequence generation, blinding, reporting the reasons for withdraws and dropouts, and allocation concealment. A trial was scored 1 point for each aspect reported [38]. The trial with Jadad score $\geq$4 was regarded as a high quality study, whereas the score <4 was indicative of a low quality study.

2.4. Statistical analysis

Two types of meta-analysis were performed. First, we carried out a meta-analysis of case-control studies to estimate blood and/or liver fatty acid content between cases and controls. Using a randomized-effects model, the pooled effects were analyzed as weighted mean difference (WMD). The second meta-analysis of RCTs was to investigate the effects of n-3 PUFA supplementation on ALT, AST, liver fat, TAG, and fasting glucose levels. The pooled effects were calculated as WMD for net changes in these biomarkers by using a random-effects model [39].

Heterogeneity between studies was evaluated with I^2 statistic. The I^2 value of 25%, 50% and 75% as cut-off points represented low, moderate and high degrees of heterogeneity, respectively, and the I^2 value greater than 50% was regarded as high degree of heterogeneity [37]. To identify the possible sources of heterogeneity, subgroup and meta-regression analyses were performed to focus on the information of NAFLD patients: study duration ($\leq$6 and >6 months), dose of n-3 PUFA supplementation ($\leq$3 and >3 g), and region (Western countries and Asian). Moreover, meta-regression was used to evaluate whether there was a significant linear relationship between effect sizes and confounding factors (dose and duration of n-3 PUFA supplementation) [40]. Generalized least square was performed for dose-response analysis to examine the relationship between 1 g per day increment of EPA + DHA and decline in effect size [41]. To explore whether a trial imposed undue effect on the overall results, sensitivity analysis was conducted by omitting 1 study at a time, and the effect size was re-calculated. Publication bias was assessed with Begg's test (significant level at $P < 0.1$) [42]. If Begg's test was significant, the trim-and-fill method was used to correct the potential

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