



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Synergistic Effects of Omega-3 Fatty Acids and Coenzyme Q10 on Gut Microbiota-Mediated Lipid Metabolism in Borderline Dyslipidemia: A Single-center Randomized Open Labeled Controlled Clinical Trial

Xinsheng Zhang^{a,1}, Tingting Miao^{b,c,1}, Yong zhang^a, Shuting Tan^c, Qing Xu^a, Li Yang^c, Jingting Chen^c, Chenyang Ai^c, Feng Su^d, Lu Liu^a, Zhimeng Yu^a, Liya Li^a, Xueyong Wang^{c,*}, Yinghua Liu^{a,*}

^a The First Medical Center of PLA General Hospital of China, Beijing 100853, China

^b Health Management Institute, The Second Medical Center & National Clinical Research Center for Geriatric Diseases, Chinese PLA General Hospital, Beijing 100853, China

^c School of Chinese Meteria Medica, Beijing University of Chinese Medicine, Beijing 102488, China

^d School of Public Health, Zhejiang Chinese Medicine University, Hangzhou 310053, China

ABSTRACT: Background: Borderline dyslipidemia is a prevalent cardiovascular risk factor for which pharmacological intervention is not always indicated. Omega-3 polyunsaturated fatty acids and coenzyme Q10 (CoQ10) have been reported to influence lipid metabolism and oxidative status; however, their combined effects in individuals with borderline dyslipidemia remain unclear. **Objective:** This study aimed to explore the associations of combined supplementation with α -linolenic acid (ALA) and CoQ10 with lipid metabolism, gut microbiota, and serum metabolites in patients with borderline dyslipidemia. **Methods:** In a 12-week, single-center randomized, open-label controlled trial, 90 participants with borderline dyslipidemia were assigned to receive either ALA (1.6 g/d) plus CoQ10 (136 mg/d) or standard dietary and exercise guidance alone. Primary outcomes included changes in serum lipids, lipoprotein subfractions (by NMR spectroscopy), gut microbiota (16S rRNA sequencing), and serum metabolomic profiles. **Results:** Eighty-two participants completed the trial. Compared with the control group, the Omega-3_Q10 group showed significantly different change-from-baseline values in multiple lipid parameters, with mixed directions of change. Exploratory analyses identified concurrent changes in selected lipoprotein subfractions, gut microbiota composition, bile acid-related metabolites, and antioxidant markers. **Conclusions:** Combined supplementation with ALA and CoQ10 was associated with short-term changes in lipid-related and metabolic markers in individuals with borderline dyslipidemia. Given the exploratory nature of the analyses, short intervention duration, and absence of clinical cardiovascular endpoints, the long-term clinical implications of these findings remain uncertain and warrant confirmation in larger, prospective studies with defined clinical outcomes.

Keywords: Borderline dyslipidemia; α -linolenic acid; Coenzyme Q10; Gut microbiota; Lipoprotein subfractions

1. Introduction

Dyslipidemia, characterized by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or reduced high-density lipoprotein cholesterol (HDL-C), represents a major

¹ These authors contributed equally to this work.

*Corresponding author

wxyph.d@163.com (X.Y.Wang); liuyinghua77@163.com (Y.H.Liu)

Received 24 January 2025

Received in revised form 17 November 2025

Accepted 16 January 2026

modifiable risk factor for atherosclerotic cardiovascular disease (ASCVD)[1]. In China, the prevalence of dyslipidemia among adults exceeds 40%, with borderline dyslipidemia (defined as serum LDL-C 3.4-4.1 mmol/L and/or TG 1.7-2.3 mmol/L) accounting for over 60% of cases, indicating its substantial public health impact[2]. While borderline dyslipidemia frequently precedes overt cardiovascular events, pharmacological intervention is not routinely recommended at this stage due to cost-benefit considerations and potential adverse effects[3]. Current guidelines emphasize lifestyle modifications, including dietary changes, exercise, and weight management, as first-line treatment approaches[4]. However, adherence to these lifestyle interventions remains suboptimal, necessitating complementary nutritional supplementation strategies.

Beyond conventional lipid profiles, advanced lipid subfraction analysis reveals nuanced cardiovascular risks. Very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) particles demonstrate positive associations with cardiovascular diseases (CVD), while high-density lipoprotein (HDL) particles show inverse associations with CVD, except for small HDL[5]. Additionally, elevated VLDL and IDL subfractions correlate strongly with ASCVD progression [6,7]. Nuclear magnetic resonance (NMR) determines the mass, particle number and particle size of lipoprotein subcomponents by detecting the number of four types of terminal methyl protons. This technique quantitatively detects average LDL particle size, subtypes of VLDL, IDL, LDL, HDL, and lipid distribution within each subtype, and has been implemented in several population-based epidemiological and genetic studies[8].

Nutrient interventions may selectively modulate these high-risk subcomponents. Omega-3 polyunsaturated fatty acids (ω -3 PUFAs), particularly EPA and DHA, have been reported to reduce TG and small dense low-density lipoprotein (sdLDL) density in borderline dyslipidemia, potentially through effects on hepatic lipid metabolism[9]. Additionally, Omega-3 facilitates the transformation of LDL particles from small and dense to large and loose configurations, which exhibit reduced atherogenic potential[10,11]. α -Linolenic acid (ALA), a plant-derived ω -3 PUFA, demonstrates TG-lowering and anti-inflammatory effects, and despite limited evidence for LDL subfraction modulation, increased intake correlates with reduced cardiovascular risk[12]. Elevated n-3 PUFA levels in erythrocyte membranes correlate with altered LDL particle characteristics, including increased diameter, decreased sdLDL-c, and reduced electronegative LDL.

Coenzyme Q10 (CoQ10) has been reported to reduce all-cause mortality and cardiovascular event risk in heart failure patients[13]. As an endogenous antioxidant, CoQ10 may ameliorate lipid metabolism disorders by diminishing oxidative stress and inflammatory responses[14]. Meta-analysis reveals that CoQ10 significantly reduces TC and LDL-C compared to controls, with enhanced effects observed at doses ≤ 100 mg/day and treatment courses under 12 weeks[15], though effects on lipoprotein subfractions remain underexplored.

Omega-3 PUFA and coenzyme Q10 represent commonly utilized nutritional supplements. ALA has advantages such as a wide range of sources, low cost, and low allergy risk, and has therefore been considered

as a plant-derived omega-3 fatty acid in nutritional research contexts.. There is insufficient evidence that plant-derived n-3 fatty acids improve lipid profiles. Coenzyme Q10 functions as an antioxidant and is involved in cellular energy metabolic homeostasis. However, research evidence regarding their combined effects remains limited. Potential pathways related to lipid metabolism, bile acid metabolism, inflammation, oxidative stress, and intestinal flora have been proposed in previous studies, although direct evidence remains insufficient. Additional high-quality studies are required to elucidate their precise impact on lipid subcomponents. This open-label randomized controlled trial explores the combined effects of ω -3 PUFAs (ALA) and CoQ10 on gut microbiota composition, serum metabolomics, and advanced lipid subfractions in individuals with borderline dyslipidemia.

2. Materials and methods

2.1 Study Design

This 12-week single-center randomized open-label controlled trial was registered at Current Controlled Trials (<http://www.chictr.org>, ChiCTR2200063253). Participants with established hyperlipidemia (HLP) were recruited from the Chinese People's Liberation Army (PLA) General Hospital in Beijing between September 2022 and February 2023, and randomly allocated to either the control group (receiving dietary and exercise guidance from nutritionists) or the Omega3_Q10 supplementation group (administered α -linolenic acid 1.6 g/d and coenzyme Q10 136 mg/d) according to a computer-generated randomization schedule by statisticians. All participants were registered by the researchers and received standardized dietary and physical activity guidance from a hospital nutritionist. Compliance was monitored through scheduled telephone interviews and biweekly intake records. Masking was not implemented in this trial. Participants, investigators and staff were aware of the treatment allocation; however, the statisticians remained blinded. The study was conducted in accordance with the Declaration of Helsinki, with written informed consent obtained from all participants. The protocol and amendments were approved by the Medical Ethics Committee of Chinese PLA General Hospital (No. S2022-252-02).

2.2 Participants

Inclusion criteria: Aged 18–65 years; Multi-ethnic, gender-inclusive; Adequate cognitive function and medication compliance; Voluntary informed consent; No use of antibiotics, probiotics, or prebiotics within 3 months prior to enrollment; Documented borderline dyslipidemia within 6 months, defined as: borderline hypercholesterolemia (serum LDL-C 3.40–4.10 mmol/L or TC 5.2–6.2 mmol/L), borderline hypertriglyceridemia (serum TG 1.70–2.3 mmol/L), or mixed borderline dyslipidemia (serum LDL-C 3.40–4.10 mmol/L or TC 5.2–6.2 mmol/L and TG 1.70–2.3 mmol/L).

Exclusion criteria: Use of traditional Chinese medicine or lipid-lowering drugs within 1 month; Secondary borderline dyslipidemia (e.g., nephrotic syndrome, hypothyroidism, hepatorenal dysfunction); Severe comorbidities (poorly controlled diabetes/complications, recent acute cardio-cerebrovascular events,

malignancies, autoimmune diseases, hematological disorders, mental illness); Pregnancy/lactation; Drug allergies; Poor compliance.

2.3 Intervention Measures

Ninety eligible participants were randomized to the Omega3_Q10 group or control group (n=45 each) using a random number table. Both groups received low-salt, low-fat dietary advice and exercise guidance based on the 2022 Chinese Dietary Guidelines for Residents. Daily energy requirements were calculated based on the subject's standard body weight, ranging from 25–30 kcal/kg, it is recommended that the intake of omega-3 be approximately 2.00g/1000kcal. And provide recommendations for aerobic exercise and resistance exercise. A nutritionist provides daily reminders, and subjects are required to record and monitor their dietary intake and exercise performance using nutrition software. The Omega3_Q10 group received omega-3 fatty acid capsules (2 capsules thrice daily, 280 mg α -linolenic acid and 0.05 mg vitamin E per capsule) and coenzyme Q10 capsules (2 capsules twice daily, 34 mg coenzyme Q10 per capsule) for 12 weeks, resulting in a daily intake of 1.6 g α -linolenic acid and 136 mg coenzyme Q10. The control group received standard dietary and exercise guidance alone. Intervention materials were obtained from Guangdong Renyuan Health Management Co., LTD.

2.4 Demographic Characteristics

Baseline demographic data were collected through standardized questionnaires, including age, sex, ethnicity, occupation, HLP duration, comorbidities, blood pressure, physical activity, smoking, and drinking habits. Dietary intake was assessed using a validated food-frequency questionnaire at baseline and week 12.

2.5 Anthropometric and Blood Chemistry Measurements

Anthropometric indices were measured with participants in light clothing and barefoot, including body weight (± 0.1 kg) and height (± 0.1 cm). Body composition was analyzed using the KY-5200 Individual Nutrition Detector (Kangyu Medical Devices, Beijing). Fasting venous blood samples (10-h fast) were collected at baseline and week 12. Complete blood counts were determined using a Mindray BC-6800Plus Automatic Hematology Analyzer (Mindray, Shenzhen). Serum biochemical parameters were measured via a Beckman Coulter AU5800 Automatic Biochemical Analyzer (Beckman Coulter, Shanghai). Serum inflammatory factors were detected using an Orienter LA2000 Automatic Chemiluminescence Immunoanalyzer (Orienter Bioengineering, Sichuan). Serum antioxidant indices were assayed with a microplate reader using commercial ELISA kits (Nanjing, Jiangsu).

2.6 Serum Lipid Profiles and Lipid Distribution

Lipid profiles were analyzed using the AVANCE IVDr Magnetic Resonance Spectrometer System (Bruker Biospin, USA) following standard operating procedures, with raw data processed via Topspin software[16]. Chemical shifts were calibrated using trimethylsilylpropanoic acid and the alanine doublet at 1.48 ppm. Lipoprotein subclasses were quantified via integrals of CH₃ (0.8 ppm) and CH₂ (1.25 ppm) signals.

Key parameters: Total TG particles (TPTG), total TC particles (TPCH), LDL-C (LDCH), HDL-C (HDCH), total Apo-A1 particles (TPA1), total Apo-A2 particles (TPA2), total Apo-B100 particles (TPAB); calculated ratios: LDL-C/HDL-C (LDHD), Apo-B100/Apo-A1 (ABA1); total ApoB-carrying lipoprotein particles (TBPB) derived from VLDL (VLPB), IDL (IDPB), and LDL (LDPB) particle counts. LDL was subclassified into LDL-1~LDL-6 (L1PB~L6PB).

Lipid distribution: Triglycerides (VLDL-TG [VLTG], IDL-TG [IDTG], LDL-TG [LDTG], HDL-TG [HDTG]); cholesterol (VLDL-C [VLCH], IDL-C [IDCH], LDL-C [LDCH], HDL-C [HDCH]); free cholesterol (VLDL-FC [VLFC], IDL-FC [IDFC], LDL-FC [LDFC], HDL-FC [HDFC]); phospholipids (VLDL-PL [VLPL], IDL-PL [IDPL], LDL-PL [LDPL], HDL-PL [HDPL]); Apo-A (HDL-Apo-A1 [HDA1], HDL-Apo-A2 [HDA2]); Apo-B (VLDL-Apo-B [VLAB], IDL-Apo-B [IDAB], LDL-Apo-B [LDAB]).

Subclass distributions: VLDL subclasses (V1TG~V5TG, V1CH~V5CH, V1FC~V5FC, V1PL~V5PL); LDL subclasses (L1TG~L6TG, L1CH~L6CH, L1FC~L6FC, L1PL~L6PL, L1AB~L6AB); HDL subclasses (H1TG~H4TG, H1CH~H4CH, H1PL~H4PL, H1A1~H4A1, H1A2~H4A2).

2.7 16S rRNA Gene Sequencing

Genomic DNA was extracted from fecal samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA). DNA quality was evaluated through 1% agarose gel electrophoresis, while concentration and purity were determined using a NanoDrop2000 Spectrophotometer (Thermo Scientific, USA). The V3-V4 region of the 16S rRNA gene underwent PCR amplification using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Species richness was assessed through alpha-diversity analysis using operational taxonomic units (OTUs). Beta-diversity visualization was accomplished through principal coordinate analysis based on Bray-Curtis distances. The identification of intestinal microbial biomarkers utilized linear discriminant analysis effect size (LEfSe).

2.8 Metabolomics analysis

Targeted metabolite standards were acquired from Sigma-Aldrich (St. Louis, MO), Steraloids Inc. (Newport, RI), and TRC Chemicals (Toronto, ON). Stock solutions (5.0 mg/mL) were formulated in water, methanol, NaOH, or HCl, and combined for calibration purposes. The analysis employed Optima-grade formic acid (Sigma-Aldrich), methanol, acetonitrile, and isopropanol (Thermo-Fisher Scientific, Fairlawn, NJ), along with ultrapure water (Mill-Q Reference System, Millipore, Billerica, MA).

Sample preparation involved thawing specimens on ice to minimize degradation. Twenty microliters of plasma were dispensed into 96-well plates, followed by automated addition of 120 μ L ice-cold methanol containing internal standards using an Eppendorf epMotion Workstation (Eppendorf, Hamburg). The mixture underwent vortexing for 5 min and centrifugation at 4000 g for 30 min (Allegra X-15R, Beckman Coulter, Indianapolis). Thirty microliters of supernatant were transferred to new plates, combined with 20 μ L fresh derivatization reagent, sealed, and incubated at 30°C for 60 min. The samples were then diluted with 338 μ L ice-cold 50% methanol, maintained at -20°C for 20 min, and centrifuged at 4000 g (4°C) for 30 min.

Subsequently, 135 microliters of supernatant were transferred to plates containing 10 μ L internal standards, and derivatized standards underwent serial dilution in left wells prior to LC-MS analysis.

2.9 Statistical Analysis

The sample size calculation utilized G*Power software V3.1.9.3 (Heinrich-Heine University, Germany), based on previous studies[17], with parameters set at $\alpha = 0.05$, effect size = 0.75, and power = 0.9, two-sided, requiring 78 cases. Accounting for a 10% dropout rate, 90 subjects were planned for inclusion. Subjects were randomly allocated in a 1:1 ratio between two groups of 45 participants each. Randomization proceeded through a central randomization system managed by independent data centers, using subjects' unique outpatient numbers.

Data are presented as mean $\pm$ standard deviation. Baseline participant characteristics were compared using independent t-tests or χ^2 tests. Independent t-tests analyzed serum lipids, antioxidant indexes, serum lipid distribution and their changes at baseline and 12 weeks. Gut microbiome comparisons between groups employed Wilcoxon rank-sum tests. Serum metabolomics differences were assessed via independent t-tests or Wilcoxon tests and Pearson correlation. Spearman correlation analyzed overall relationships among gut microbiome, serum metabolites, and clinical indexes. KEGG pathway analysis utilized hypergeometric tests. Statistical analyses were conducted using SPSS 23.0 (Beijing Stats Data Mining Co., LTD), with statistical significance set at two-tailed $P < 0.05$.

Since the gut microbiota and metabolomics analyses were conducted with 10 participants per group, these results are exploratory and hypothesis-generating, rather than confirmatory mechanistic evidence. For serum metabolite and lipidomic analyses involving multiple comparisons, false discovery rate (FDR) correction was applied using the Benjamini-Hochberg (BH) method, with adjusted P -values (Q -values) reported. Statistical significance was established at $Q < 0.05$. Results with uncorrected P -values < 0.05 but lacking significance after FDR correction were classified as preliminary exploratory analyses.

3. Results

3.1 Participants Characteristics

A total of 120 individuals were enrolled, and 90 eligible participants were randomized to the Omega3_Q10 group or control group after screening, with both groups receiving standardized dietary and exercise guidance. Seven participants discontinued in the control group (4 due to non-adherence to diet/exercise advice, 2 gave up the intervention and 1 due to business trips), while 1 participant in the Omega3_Q10 group withdrew due to non-compliance with supplementation. Ultimately, 82 participants completed the trial (Fig. 1 flow diagram). At baseline, no significant differences were observed between groups in age, gender, ethnicity, occupation, BMI, borderline dyslipidemia duration, comorbidities, blood pressure, smoking/drinking status, or physical activity level (Table 1). There were also no differences in borderline dyslipidemia or lipid-lowering statin use duration. Dietary nutrient intake (energy, protein, fat,

carbohydrate, minerals, vitamins, and dietary fiber) was comparable between groups at baseline and post-intervention (Supplementary material: Table S1).

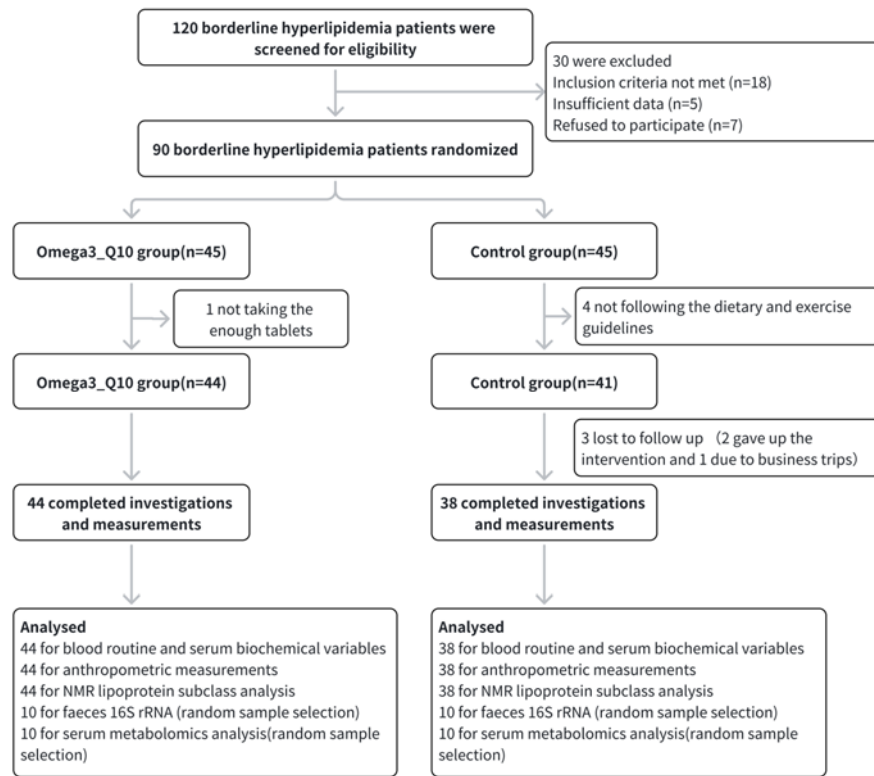


Figure 1: flow diagram

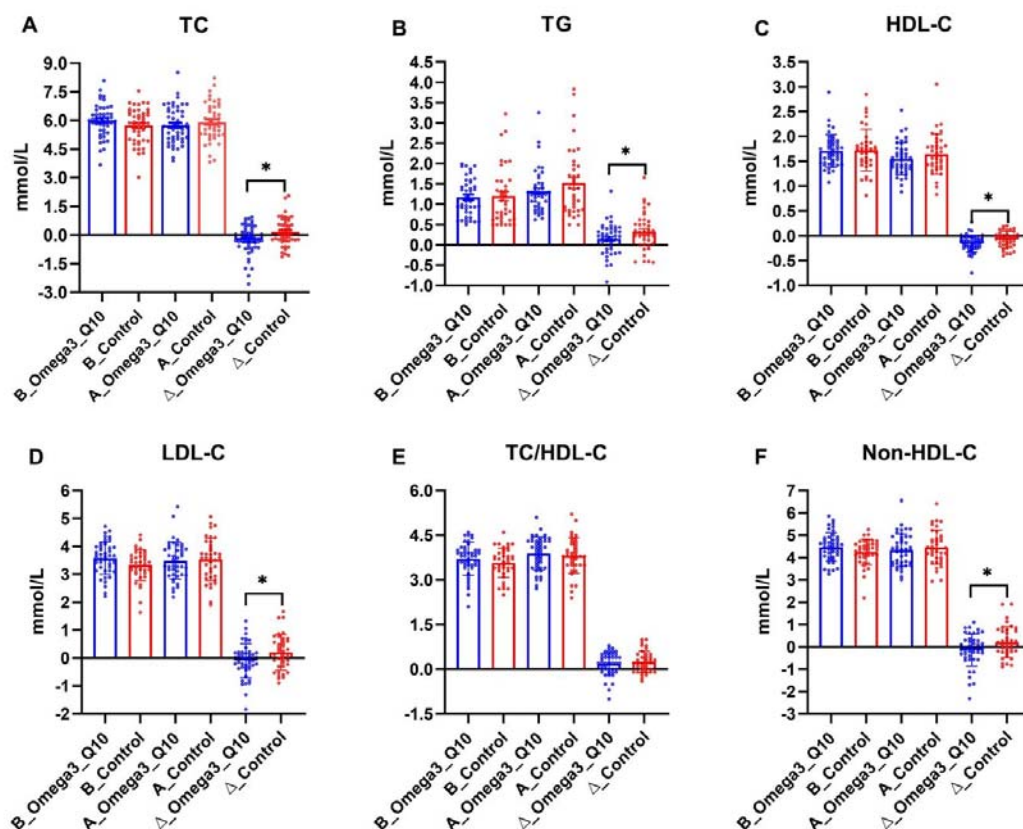
Table 1. Characteristics of participants at baseline.

Variable	Omega3_Q10 group (n=44)	Control group (n=38)	P-value
Age (years, Mean±SD)	50.46±9.31	52.25±9.39	0.391 ^a
Gender (n, %)			
Male	15 (34.09%)	11 (28.95%)	0.618 ^b
Female	29 (65.91%)	27 (71.05%)	
ethnic			
han ethnicity	40(90.91%)	36 (94.74%)	0.617 ^b
other ethnic groups	4 (9.09%)	2 (5.26%)	
occupational			
professional and technical personnel	19 (43.18%)	16 (42.11%)	0.437 ^b
business practitioners	10 (22.73%)	4 (10.53%)	
freelancer	1 (2.27%)	3(7.89%)	
retired personnel	11 (25.00%)	13 (34.21%)	
other personnel	3 (6.82%)	2 (5.26%)	
BMI (kg/m ² , mean±SD)	24.43±3.68	25.40±3.13	0.208 ^a
borderline dyslipidemia duration (years, mean±SD)	4.21±3.14	3.25±2.39	0.198 ^a
chronic disease situation			
hypertension (n, %)	6(13.64%)	6 (15.79%)	0.783 ^b
coronary heart disease (n, %)	1(2.27%)	0 (0.00%)	1.000 ^b
type 2 diabetes (n, %)	0(0.00%)	3 (7.89%)	0.191 ^b
gout (n, %)	0(0.00%)	2 (5.26%)	0.212 ^b
Systolic BP (mm Hg, mean±SD)	121.18±14.05	124.66±13.69	0.262 ^a
Diastolic BP (mm Hg, mean±SD)	78.25±10.25	80.00±0.03	0.418 ^a
Current smokers (n, %)	2 (4.55%)	6 (15.79%)	0.181 ^b
Current drinkers (n, %)	4 (9.09%)	6 (15.79%)	0.585 ^b
Physical activity level (Score:1~3, mean±SD)	2.39±0.89	2.47±0.86	0.655 ^a

Note: P-values were calculated for the characteristics differences between the two groups at baseline. ^a Independent t-test; ^b The χ^2 test.

3.2 Clinical Variables, Serum Lipids, Antioxidant Indexes and serum omega-3 fatty acids

Δ indicates the change from baseline to week 12. No significant differences in complete blood counts or clinical variables were noted between groups at baseline or week 12 (Supplementary material: Table S2). Body composition indexes (Supplementary material: Table S3) and inflammatory factors (Supplementary material: Table S4) also showed no inter-group differences. Following 12 weeks of intervention, the serum ALA content in the Omega3_Q10 group was significantly elevated compared to the control group, with a concurrent significant increase in total omega-3 fatty acid levels. No significant changes were noted in EPA and DHA (Supplementary material: Table S5). TG increased in both groups over the 12-week intervention; however, the magnitude of increase differed significantly between the Omega3_Q10 group and the control group (Δ TG: 0.15 ± 0.38 vs. 0.33 ± 0.40 , $P = 0.040$). In contrast, TC, LDL-C, and non-HDL-C showed significantly different change-from-baseline values between the Omega3_Q10 group and the control group. Specifically, the change-from-baseline values were -0.25 ± 0.77 vs. 0.16 ± 0.73 for TC ($P = 0.008$), -0.09 ± 0.61 vs. 0.20 ± 0.64 for LDL-C ($P = 0.035$), and -0.14 ± 0.73 vs. 0.21 ± 0.69 for non-HDL-C ($P = 0.029$). HDL-C decreased in both groups, with a larger decline observed in the Omega3_Q10 group (Δ HDL-C: -0.16 ± 0.17 vs. -0.08 ± 0.16 , $P = 0.026$). At baseline or week 12, there were no significant differences in lipid variables between the two groups (Supplementary material: Table S6). Additionally, significant differences in serum Δ SOD (-0.58 ± 28.08 vs. -26.08 ± 33.48 , $P < 0.001$) and Δ GSH-PX (5.84 ± 39.21 vs. -26.81 ± 34.35 , $P < 0.001$) were observed between the Omega3_Q10 group and the control group. After 12 weeks of intervention, the MDA level in the Omega3_Q10 group was significantly lower than that in the control group, while the GSH-PX level was significantly higher than that in the control group ($P < 0.05$) (Fig.2).



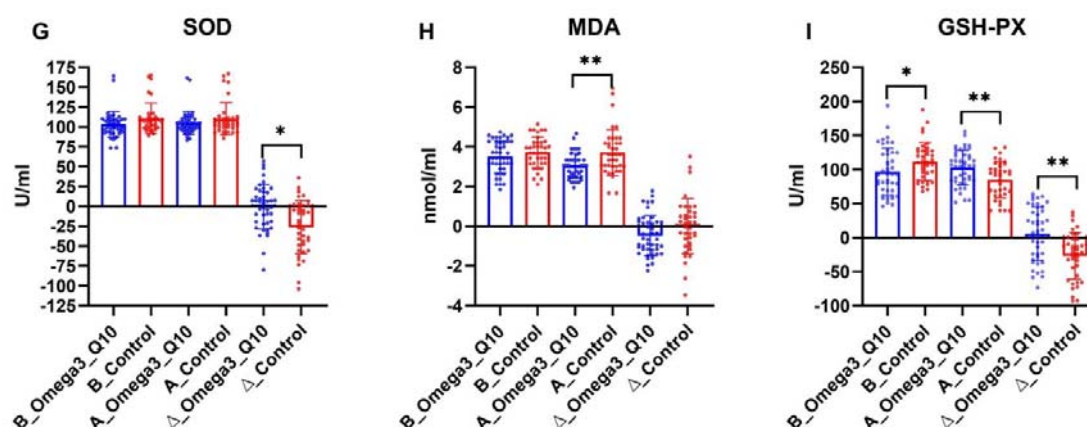


Figure 2: Serum lipids and antioxidant indexes at baseline and 12 weeks in the two groups. The columns of the serum lipids and antioxidant indexes were based on 82 patients (Omega3_Q10 group, $n = 44$; Control group, $n = 38$). (A) TC: total cholesterol; (B) TG: triglyceride; (C) HDL-C: high density lipoprotein cholesterol; (D) LDL-C: low density lipoprotein cholesterol; (E) TC/HDL-C: total cholesterol/high density lipoprotein cholesterol; (F) N-HDL-C: non-high density lipoprotein cholesterol; (G) SOD: superoxide dismutase; (H) MDA: malondialdehyde; (I) GSH-PX: glutathione peroxidase. The columns represent the mean, while the top symbols represent the standard deviation. These variables were analyzed by ANCOVA. B_Omega3_Q10: Omega3_Q10 group at baseline; A_Omega3_Q10: Omega3_Q10 group after 12 weeks intervention; Δ_Omega3_Q10: Omega3_Q10 group after 12 weeks intervention minus at baseline; B_Control: Control group at baseline; A_Control: Control after 12 weeks intervention; Δ_Control: Control group after 12 weeks intervention minus at baseline; P -values were calculated for the differences before and after the intervention between the two groups at 12 weeks, * $P < 0.05$, ** $P < 0.01$.

3.3 Serum Lipid Profiles and Distribution by NMR

Main parameters: Significant differences in change-from-baseline values of ΔTPTG, ΔTPCH, ΔLDCH, ΔTPA2, and ΔTPAB were observed between the Omega3_Q10 group and the control group after 12 weeks ($P < 0.05$) (Fig.3A-G). Total ApoB-carrying lipoprotein particles: Significant differences in change-from-baseline values of serum ΔTBPN were observed between the Omega3_Q10 group and the control group ($P < 0.05$) (Fig.3J). Lipoprotein main fractions: Significant differences in change-from-baseline values of ΔLDPN, ΔLDPL, ΔVLTG, and ΔIDTG were observed between the Omega3_Q10 group and the control group ($P < 0.05$) (Fig.3K-N). LDL subfractions: Significant differences in change-from-baseline values of ΔL5PN were observed between the Omega3_Q10 group and the control group ($P < 0.05$), along with differences in ΔL5CH, ΔL1TG, ΔL3TG, ΔL5PL, and ΔL5AB ($P < 0.05$) (Fig.3O-T). Other lipoprotein subfractions: Significant differences in change-from-baseline values of ΔLDPL, ΔHDA2, ΔLDAB were observed between the Omega3_Q10 group and the control group ($P < 0.05$). For VLDL subfractions, significant differences in ΔV1TG were observed between groups ($P < 0.05$). For HDL subfractions, significant difference in ΔH4A2 were observed between the Omega3_Q10 group and the control group ($P < 0.05$), (Fig.3U-Y).

There were no significant differences in other lipid profiles and distribution indicators between the two groups. After FDR correction at a threshold of $q < 0.05$, no single metabolite remained statistically significant (Supplementary material: Table S6). Therefore, serum lipid profiles and their nuclear magnetic resonance distributions are only used as exploratory analyses.

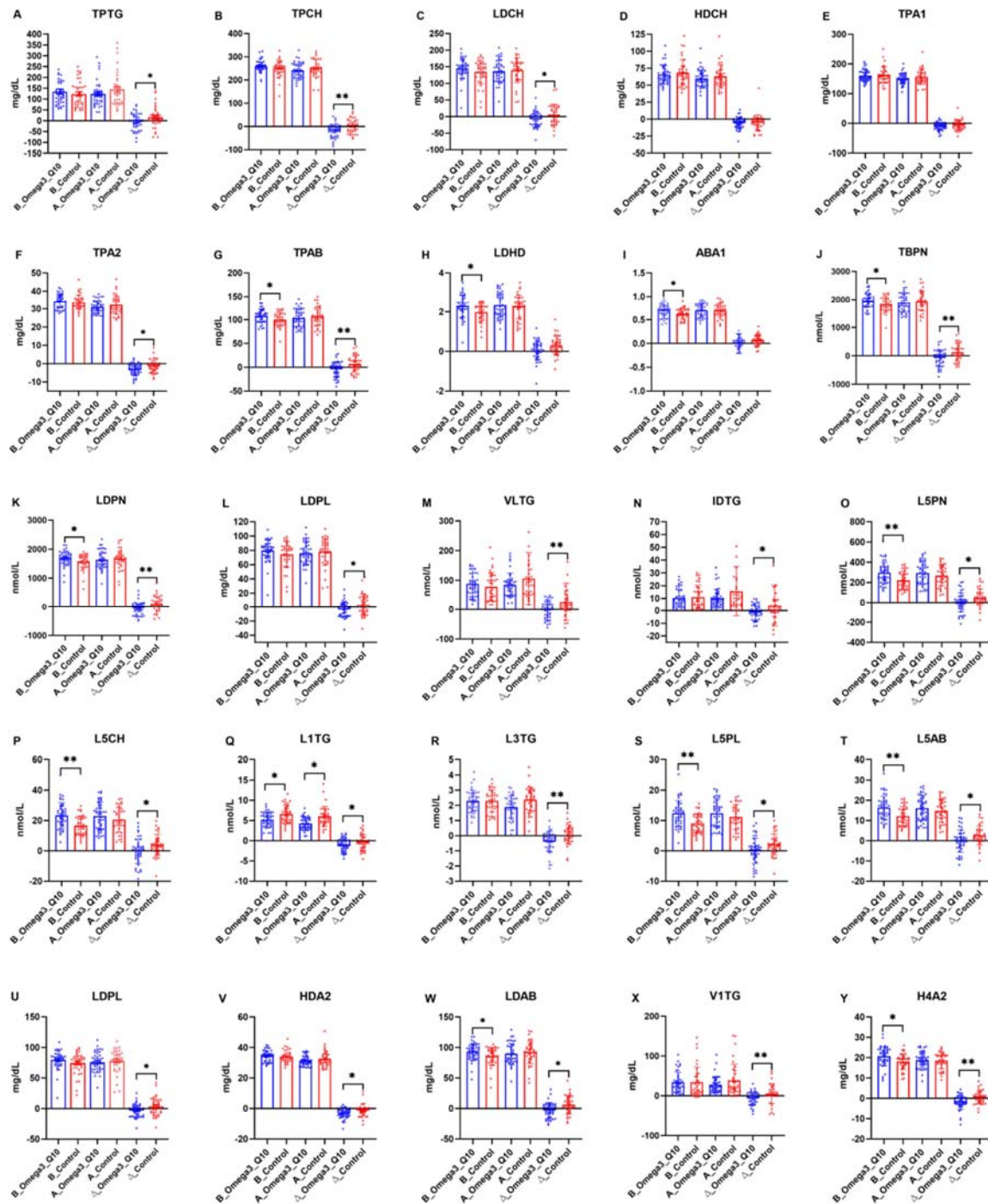
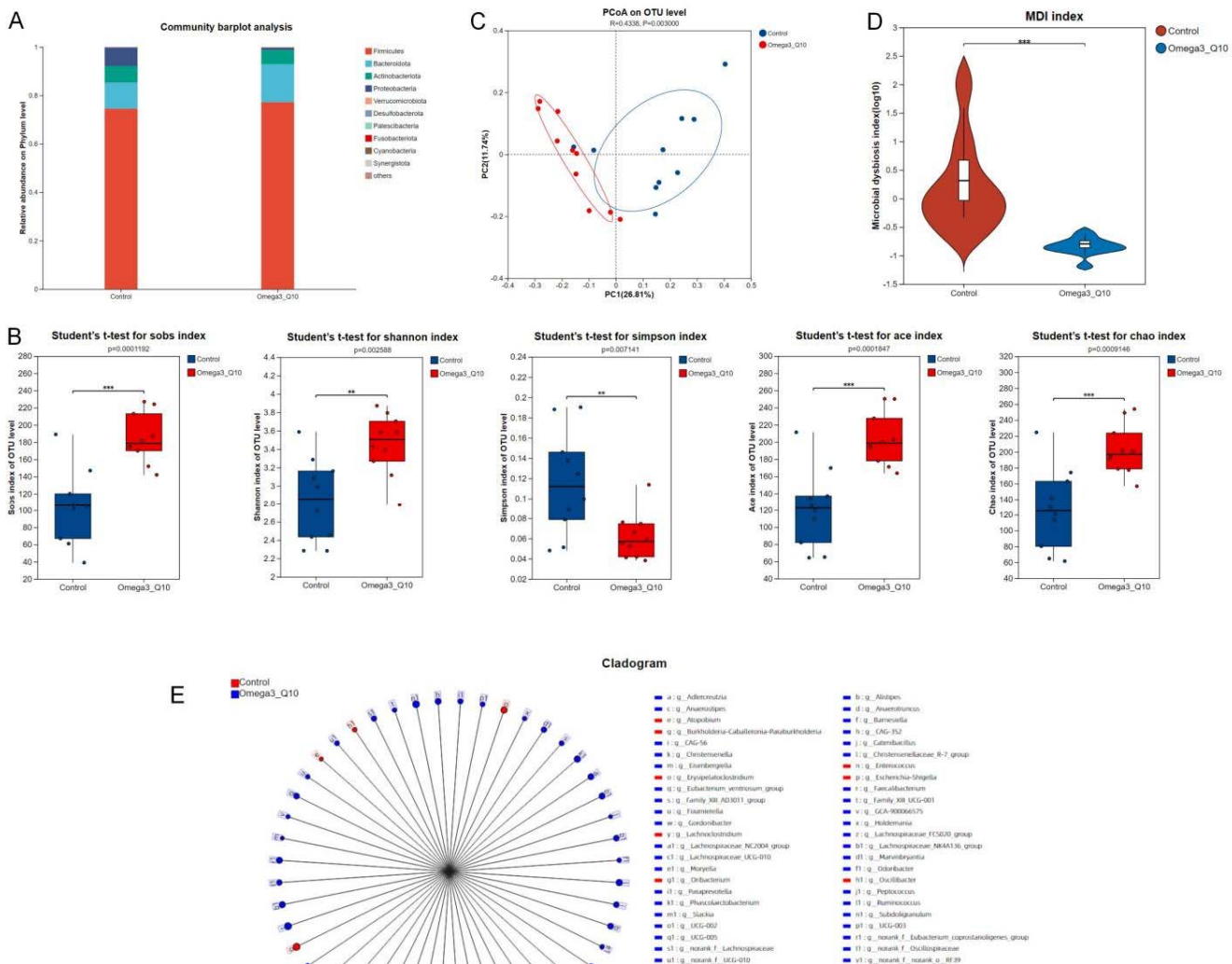


Figure 3: Serum lipid profiles, lipid distribution and their changes at baseline and 12 weeks by NMR. The columns of those indexes were based on 82 patients (Omega3_Q10 group, $n = 44$; Control group, $n = 38$). (A) TPTG: total triglyceride particles; (B) TPCH: total cholesterol particles; (C) LDCH: low density lipoprotein cholesterol; (D) HDCH: high density lipoprotein cholesterol; (E) TPA1: total Apo-A1 particles; (F) TPA2: total Apo-A2 particles; (G) TPAB: total Apo-B100 particles; (H) LDHD: LDL-C/HDL-C; (I) ABA1: Apo-B100/Apo-A1; (J) TBPB: total ApoB-carrying lipoprotein particles; (K) LDPN: LDL particle counts; (L) LDPL: LDL-phospholipids; (M) VLTG: VLDL-triglycerides; (N) IDTG: IDL-triglycerides; (O) L5PN: LDL-5; (P) L5CH: LDL cholesterol-5; (Q) L1TG: LDL triglycerides-1; (R) L3TG: LDL triglycerides-3; (S) L5PL: LDL phospholipids-5; (T) L5AB: LDL Apo-B-5; (U) LDPL: LDL-Phospholipids; (V) HDA2: HDL-Apo-A2; (W) LDAB: LDL-Apo-B; (X) V1TG: VLDL triglycerides-1; (Y) H4A2: LDL Apo-A2-4. The columns represent the mean, while the top symbols represent the standard deviation. These variables were analyzed by ANCOVA. B_Omega3_Q10: Omega3_Q10 group at baseline; A_Omega3_Q10: Omega3_Q10 group after 12 weeks intervention; Δ_Omega3_Q10: Omega3_Q10 group after 12 weeks intervention minus at baseline; B_Control: Control group at baseline; A_Control: Control after 12 weeks intervention; Δ_Control: Control group after 12 weeks intervention minus at baseline; P -values were calculated for the differences before and after the intervention between the two groups at 12 weeks, $*P < 0.05$, $**P < 0.01$. Adjusted for multiple comparisons using FDR control (BH method), Q -values < 0.05 are considered statistically significant, not significant after FDR correction.



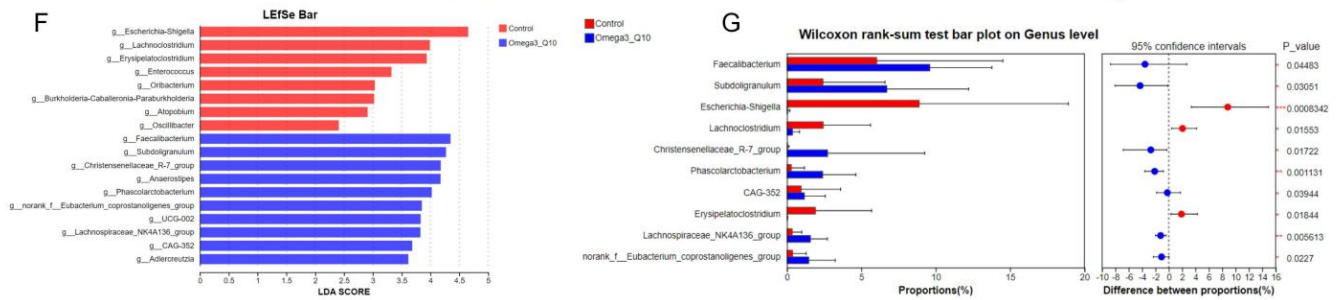
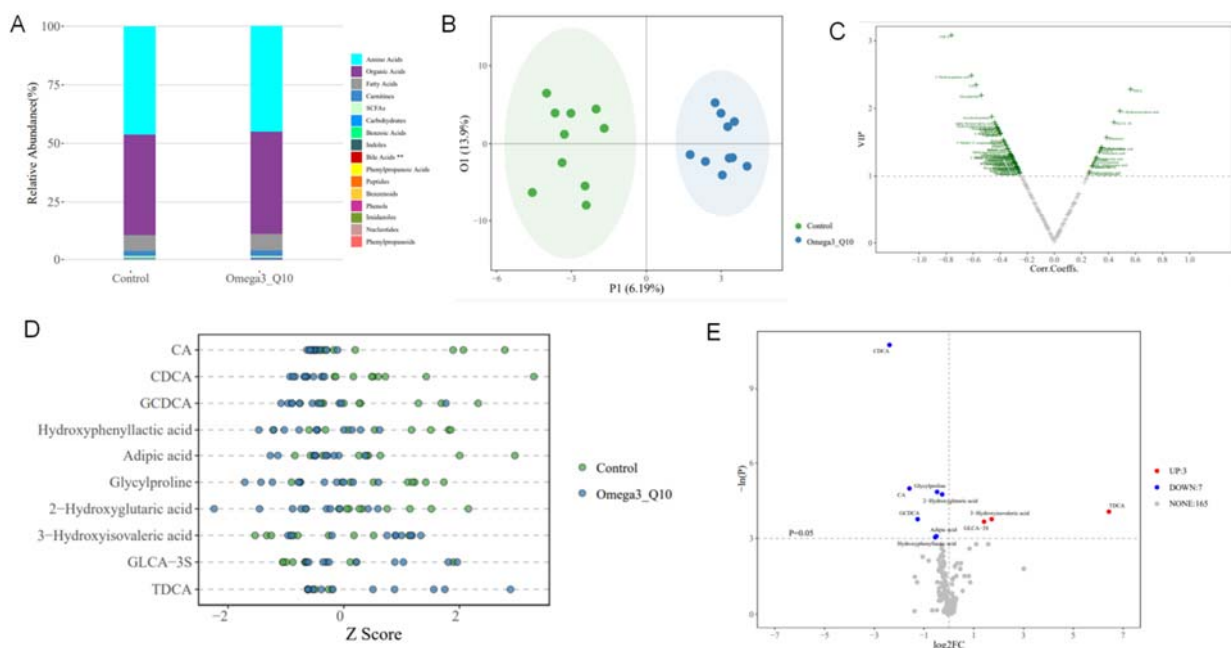


Figure 4: The differences of the gut microbiome between the two groups (n=10). (A) The Community barplot analysis at 12 weeks in the two groups; (B) The Sobs, Ace, Chao index of community richness; the Shannon, Simpson index of community diversity. Comparisons were analyzed by Student's t-test; (C) The PCoA analysis on OTU level of the gut microbiome between the two groups. Comparisons were analyzed by Anosim; (D) The microbial dysbiosis index (MDI) of community dysbiosis. Comparisons were analyzed by Wilcox test; (E) The cladogram of the LefSe results. Comparisons were analyzed by t-test or Wilcox test. Only the genus with a significant logarithmic LDA threshold score of > 2.5 and $P < 0.05$ were shown; (F) and (G) The difference test of genus between the two groups. Comparisons were analyzed by Wilcox rank-sum test. Omega3_Q10, Omega3 fatty acids combined with coenzyme Q10.

3.5 Serum Metabolomics Analysis

Targeted quantitative metabolomics identified 175 serum metabolites across 16 categories in 20 matched fecal samples at week 12 (Fig. 3A). OPLS-DA showed clear separation of metabolites between groups (Fig. 5B). Volcano plot analysis (based on VIP and correlation coefficients) and Z-score analysis identified 10 differential metabolites (Figs. 5C, D). Among these, 3-Hydroxyisovaleric acid, Glycodeoxycholic acid_3s (GLCA-3S), and Taurodeoxycholic acid (TDCA) were significantly upregulated in the Omega3_Q10 group ($P < 0.05$), while Cholic acid (CA), Glycylproline, 2-Hydroxyglutaric acid, Glycochenodeoxycholic acid (GCDCA), Chenodeoxycholic acid (CDCA), Adipic acid, and Hydroxyphenyllactic acid were downregulated ($P < 0.05$) (Fig. 5E–G). A heat map of Pearson correlations visualized relative intensities of these metabolites (Fig. 5H). We corrected all variable combinations. Following FDR correction with a threshold of $q < 0.05$, only CDCA among the metabolomics indicators retained statistical significance; the remaining indicators did not meet this criterion ($q > 0.05$).



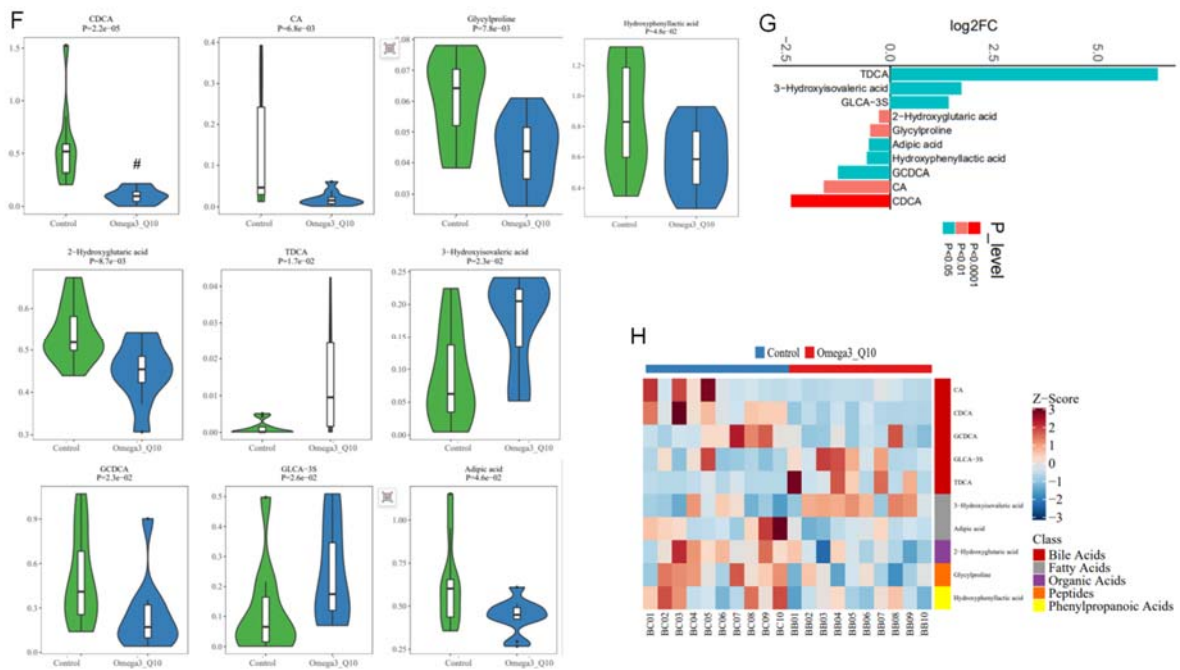


Figure 5: The differences of the serum metabolomics between two groups (n=10). (A) The classifications of metabolites in two groups. Comparisons were analyzed by Independent t-test or Wilcox test. * $P < 0.05$; (B) The OPLS-DA analysis plots. Comparisons were analyzed by Independent t-test or Wilcox test; (C) The volcano plots of differential metabolites based on the OPLS-DA. Comparisons were analyzed by Independent t-test or Wilcox test; (D) The Z-Score plot of 10 differential metabolites, (E) single-dimensional volcano plots of the metabolites metabolite. Comparisons were analyzed by Independent t-test or Wilcox test; (F) Ten differential metabolites between the two groups after intervention. Comparisons were analyzed by Independent t-test or Wilcox test; (G) The P values of these potential biomarkers between the two groups. Comparisons were analyzed by Independent t-test or Wilcox test; (G) The potential biomarker in each individual. Comparisons were analyzed by Pearson correlation. Omega3_Q10, Omega3 fatty acids combined with coenzyme Q10. Adjusted for multiple comparisons using FDR control (BH method), # Q -values < 0.05 are considered statistically significant, except for CDCA, the other metabolomics indicators not significant after FDR correction.

3.6 Correlation Analysis of Gut Microbiome, Serum Metabolites, and Clinical Indexes

Correlation heat maps revealed relationships between differential metabolites, clinical indexes, and gut microbiome (Fig. 6). All associations represent correlation analyses and do not imply causality.

Metabolites vs. clinical indexes: 3-Hydroxyisovaleric acid and GLCA-3S were negatively correlated with LDL-C ($P < 0.05$); 3-Hydroxyisovaleric acid was negatively correlated with L5AB, L5PN, L5CH, L5PL, LDAB, and LDCH ($P < 0.05$). GLCA-3S and TDCA were negatively correlated with IDTG and IL-8 ($P < 0.05$); TDCA was negatively correlated with IL-1 β ($P < 0.05$). 2-Hydroxyglutaric acid, CA, and CDCA were positively correlated with LDL-C and related subfractions ($P < 0.05$); Glycylproline correlated positively with ABA1/LDHD and negatively with HDCH/TPA1 ($P < 0.05$) (Fig. 6A).

Microbiome vs. clinical indexes: Lachnospiraceae was positively correlated with LDCH, LDPL, LDAB, and lipid ratios ($P < 0.05$); Lachnospiraceae_NK4A136_group and Subdoligranulum were negatively correlated with LDL subfractions ($P < 0.05$); Escherichia-Shigella correlated positively with TG and VLTG ($P < 0.05$) (Fig. 6B).

Metabolites vs. microbiome: GLCA-3S correlated positively with Subdoligranulum and norank_f_Eubacterium_coprostanoligenes_group ($P < 0.05$); TDCA correlated positively with

norank_f_Eubacterium_coprostanoligenes_group ($P < 0.05$); GCDCA correlated positively with *Escherichia-Shigella* ($P < 0.05$) (Fig. 6C).

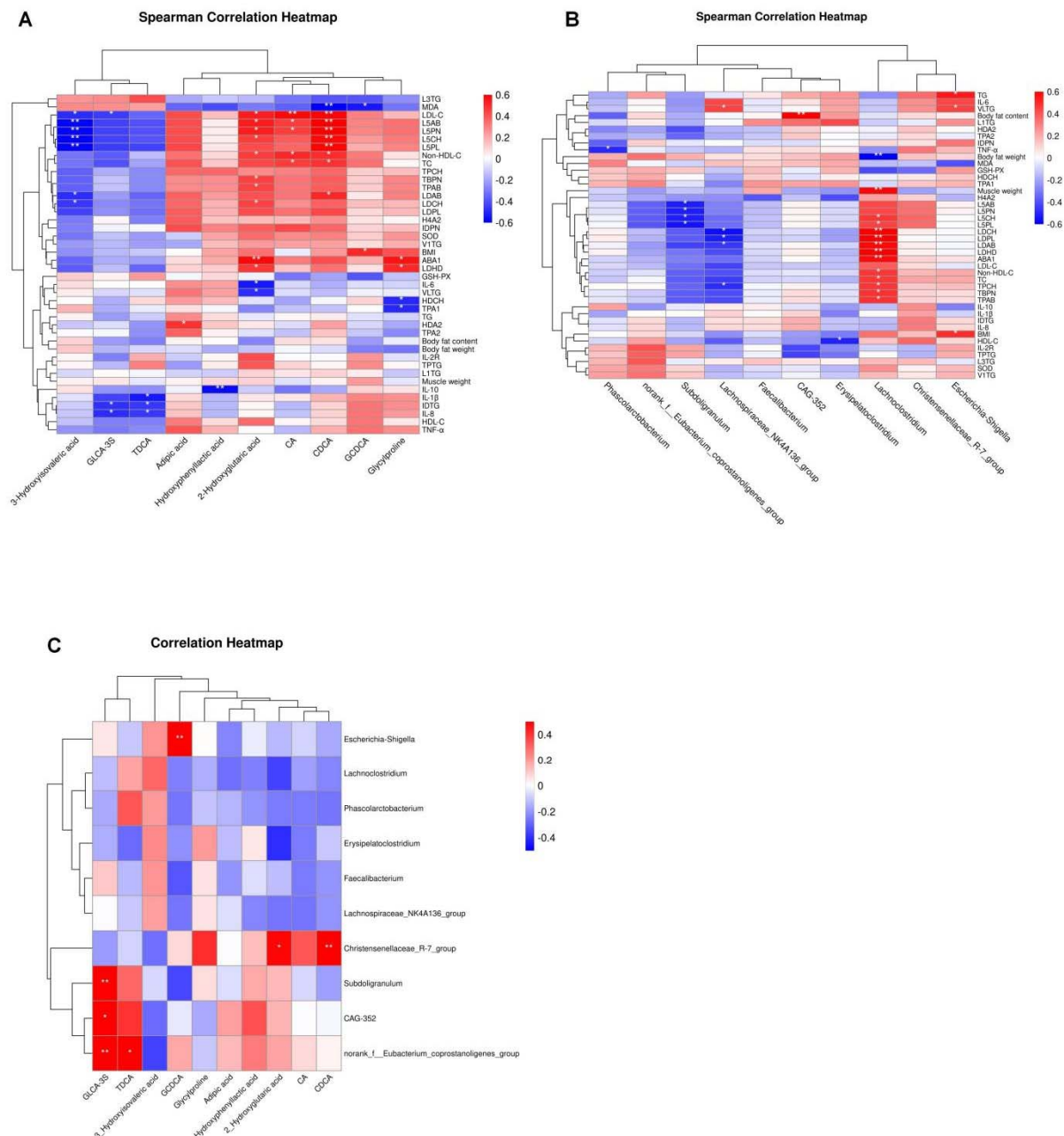


Figure 6. The overall correlation analysis of the gut microbiome, serum metabolites, and clinical indexes. (A) The correlation heat map of the metabolites and clinical indexes; (B) The correlation heat map of the gut microbiota and clinical indexes. (C) The correlation heat map of the gut microbiome and differential metabolites; Comparisons were analyzed by Spearman correlation. * $P < 0.05$, ** $P < 0.01$.

4. Discussion

This 12-week open-label randomized controlled trial observed that combined supplementation with ALA and CoQ10 was associated with short-term changes in lipid-related and metabolic markers in individuals with borderline dyslipidemia. Exploratory analyses revealed concurrent changes in selected lipoprotein subfractions, gut microbiota composition, bile acid-related metabolites, and antioxidant markers. In the context of an open-label, non-placebo-controlled design, the observed changes cannot be entirely attributed to

the ALA and CQ10 supplements themselves; they may also be influenced by behavioral interventions, expectation effects, or other unmeasured confounding factors inherent to open-label designs.

Research results revealed inconsistencies. Although significant differences in change-from-baseline values (Δ TG and Δ HDL-C) were observed between groups, within-group trend analysis indicated that triglyceride (TG) levels increased and high-density lipoprotein cholesterol (HDL-C) levels decreased in both groups after intervention. However, the magnitude of TG increase was lower in the Omega-3_Q10 group than in the control group, while the magnitude of HDL-C decrease was greater in the Omega-3_Q10 group than in the control group. Potential explanations for these phenomena include poor lifestyle adherence. Although both groups received standardized dietary and exercise guidance, objective dietary intake data showed that energy and fat intake decreased from baseline to week 12, with no significant differences between groups, suggesting partial non-adherence to dietary recommendations, which is a common challenge in clinical trials for mild dyslipidemia. Additionally, the 12-week intervention period may have been insufficient to induce significant changes in lipid metabolism.

In addition, the decline in Δ HDL-C was more pronounced in the Omega3_Q10 group than in the control group. This finding should be interpreted with caution. Previous studies have discussed that changes in HDL-C levels may be related to metabolic coordination, antioxidant processes, or lipid transport pathways, rather than directly indicating HDL dysfunction [12,18–20]. In the present study, although TC, LDL-C, and non-HDL-C showed greater reductions in change-from-baseline values in the intervention group, the TC/HDL-C ratio remained stable. NMR lipidomic analyses showed concurrent changes in several lipoprotein-related parameters (e.g., TPC, HDL-C, Apo-A1/Apo-A2), which have been reported in the literature in relation to HDL composition and function [14]. However, HDL functional properties, such as cholesterol efflux capacity, were not directly measured in this study. Therefore, the clinical significance of the observed reduction in HDL-C remains unclear. Further studies with longer follow-up and direct functional assessments are needed to clarify the implications of this finding.

ALA, an essential n-3 fatty acid, serves as a precursor to EPA and DHA. However, its conversion to these longer-chain fatty acids is inefficient and influenced by multiple regulatory factors. Previous studies have reported that high dietary ALA intake may modulate Δ -6 desaturase activity, redirecting metabolism from the linoleic acid-arachidonic acid pathway toward ALA-EPA synthesis, thereby potentially influencing EPA production [21]. Research indicates that each 1 g/day increase in dietary ALA intake has been associated with reductions in TG, TC, and LDL-C by 0.002 mmol/L, 0.007 mmol/L, and 0.006 mmol/L, respectively, with greater efficacy observed in Asian populations and individuals with hyperlipidemia [22]. In the present study, dietary omega-3 fatty acid intake was increased through nutritional guidance and supplementation with 1.6 g/day ALA to adjust fatty acid ratios. Previous studies have reported that ALA intervention may increase PUFA levels and lower the n-6/n-3 ratio, contributing to optimized lipid profiles [23]. Additionally, ALA has been shown to improve lipid profiles and inflammatory status in patients with chronic kidney disease [24]. The primary mechanisms underlying ALA's lipid-lowering effects involve increased n-3 PUFA content,

reduced inflammatory markers, and improved fatty acid balance, none of which can be confirmed within the current study design.

This investigation emphasized the assessment of combined ALA and CoQ10 supplementation on lipid subgroups. NMR analysis detects hydrogen atoms' nuclear magnetic resonance signals in plasma lipoprotein particles and employs algorithms (including the partial least squares regression model PLSR) to predict lipoprotein subcomponent concentration and structure[25]. This technique enables precise quantification of 65 to 116 lipoprotein subcomponents, encompassing VLDL, LDL, and HDL, including LDL1-5 and VLDL subclasses[26]. This study employed a 12-week short-term intervention. Due to the limited follow-up duration, endpoint indicators such as cardiovascular events could not be assessed. Consequently, intermediate endpoints—including blood lipids and lipoprotein subcomponents—were selected as intermediate markers related to cardiovascular risk. Sd-LDL-C and TG are recognized as critical risk markers and targets for residual risk management [27]. Reductions in LDL-C or sd-LDL-C have been associated with decreased long-term cardiovascular risk, with the magnitude of risk reduction varying by intervention duration and intensity [28,29]. The changes in intermediate indicators (LDL-C, and sd-LDL-C) observed in this study may have potential clinical relevance; however, long-term data verification is necessary.

NMR studies have revealed that diabetic patients exhibit Non-LDL lipoprotein abnormalities, including increased sdLDL and altered HDL function, which independently correlate with elevated cardiovascular risk[30]. Furthermore, LDL cholesterol subcomponents, particularly LDL3-5, serve as independent CVD risk markers[31]. Mediterranean diet adherence or increased nut consumption can enhance the NMR lipoprotein profile by reducing large VLDL particles and increasing large HDL particles, creating an anti-atherosclerotic pattern[32,33]. Omega-3 PUFAs typically reduce TG by 15~30% through enhanced hepatic β -oxidation and suppressed lipogenesis[34], while CoQ10 supplementation decreases TC and LDL-C, particularly at doses ≤ 100 mg/day during short-term interventions[35]. While previous omega-3 PUFA studies primarily examined TG reduction[34], and CoQ10 research rarely investigated lipoprotein subfractions[36]. This NMR-based analysis provides exploratory observations of lipid distribution at the subfraction level, highlighting the importance of advanced lipid profiling in nutritional interventions.

In the present study, changes in bile acid-related metabolites and lipid parameters were observed in the intervention group, including reductions in primary bile acids (CA and CDCA) and increases in secondary bile acids (GLCA-3S, TDCA). Such patterns of bile acid alteration have been discussed in previous studies examining the interaction between dietary fatty acids and bile acid metabolism, although the underlying regulatory pathways remain incompletely understood. Previous studies have reported that omega-3 PUFAs may influence bile acid synthesis and composition through hepatic and intestinal regulatory pathways, including FXR-related signaling; however, these effects are context-dependent and vary across populations and study designs[19, 37, 38]. In clinical settings, alterations in the omega-6/omega-3 PUFA ratio have been associated with imbalances in primary and secondary bile acid profiles, suggesting a close relationship between fatty acid composition and bile acid homeostasis[39]. In addition, CoQ10 supplementation has been

reported to affect hepatic lipid metabolism and endothelial function in other populations, potentially in association with changes in lipid metabolism enzyme activity[20, 40]. In the present trial, the concurrent changes in bile acid-related metabolites, together with KEGG pathway enrichment in primary bile acid biosynthesis, are interpreted as exploratory observations rather than evidence of specific mechanistic pathways (Supplementary material: Table S9 and Fig. S1).

To aid interpretation of these exploratory findings, Figure 7 presents a conceptual framework integrating gut microbiome composition, bile acid-related metabolites, antioxidant indices, and lipid parameters. Within this framework, differences in the relative abundance of several gut microbial taxa were observed between groups, including *Faecalibacterium*, *Subdoligranulum*, *Lachnospiraceae_NK4A136_group*, *Escherichia-Shigella*, and *Lachnoclostridium*. Previous experimental and clinical studies have suggested that some of these taxa may be linked to short-chain fatty acid production, inflammatory responses, or lipid metabolism [41-49]. For example, SCFA-producing genera such as *Faecalibacterium* and *Subdoligranulum* have been discussed in the literature in relation to intestinal barrier function and metabolic homeostasis, whereas increased abundance of *Escherichia-Shigella* and *Lachnoclostridium* has been associated with inflammatory conditions [46-49]. Correlation analyses in the present study indicated associations between specific bacterial genera, lipid subfractions, inflammatory markers, and triglyceride-rich lipoproteins. These correlations are descriptive in nature and do not establish causal relationships, and they may reflect complex host-microbiota interactions influenced by dietary intake, metabolic status, and other unmeasured factors.

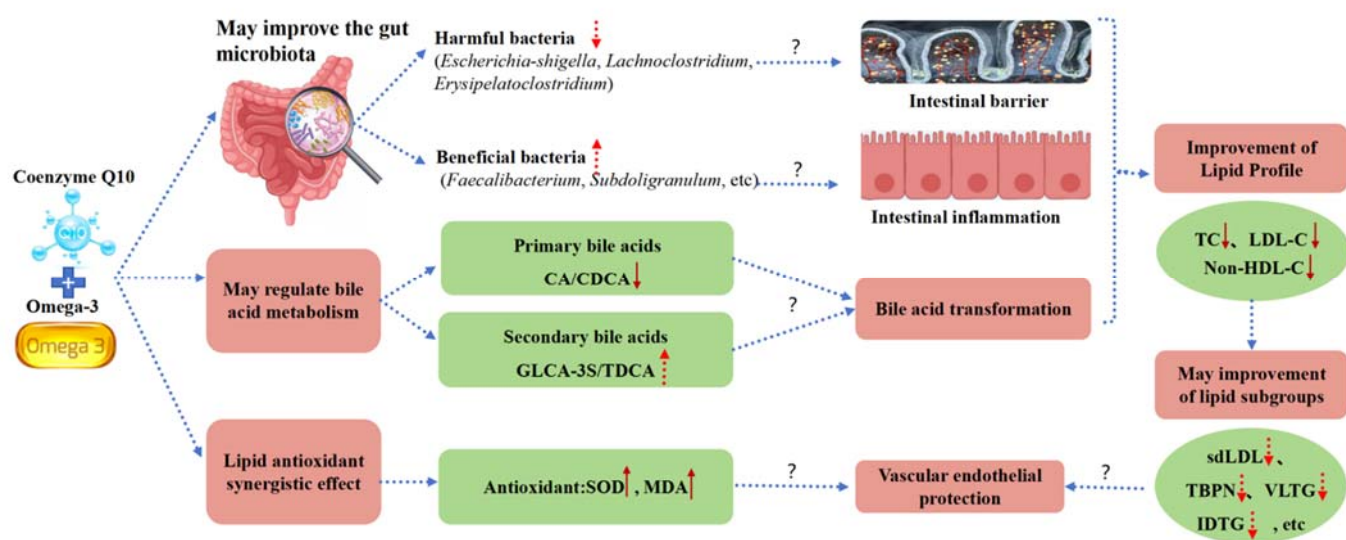


Figure 7: Exploratory integration of multi-omics findings. This schematic diagram summarizes potential associations among gut microbiota composition, bile acid-related metabolites, antioxidant indices, and lipid parameters observed in this study. The figure is intended solely as a conceptual framework to aid interpretation of exploratory and correlational results and does not imply validated mechanistic pathways or causal relationships. Abbreviations are provided for clarity of the schematic illustration only and do not imply validated mechanistic involvement. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; GLCA-3S, glucuronidated cholic acid-3-sulfate; TDCA, taurodeoxycholic acid; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; sdLDL, small dense low-density lipoprotein; TBNP, total bilayer phosphatidylcholine; VLTG, VLDL-triglycerides; SOD, superoxide dismutase; MDA, malondialdehyde.

Omega-3 PUFAs and CoQ10 have been reported to influence metabolic processes through multiple pathways, including fatty acid oxidation, mitochondrial energy metabolism, and redox balance [34,35,50,51].

In the current study, changes in antioxidant-related indicators were observed alongside alterations in lipid parameters, providing additional context for interpreting the multi-omics findings. Rather than demonstrating direct mechanistic effects, the current multi-omics analysis provides an integrated description of concurrent changes across microbiota, metabolite, and lipid datasets. This integrative perspective is consistent with prior studies that have examined gut microbiota, bile acids, or lipid metabolism in isolation, and it highlights potential links along the gut–liver axis that warrant further investigation [52].

Based on these exploratory observations, several hypotheses can be proposed for future research, drawing directly from the patterns observed in the present study, including potential associations between (i) gut microbiota composition and lipid-related inflammatory markers, (ii) bile acid profiles and lipid distribution patterns, (iii) lipoprotein subfraction characteristics and triglyceride-rich particles, and (iv) antioxidant status and metabolic regulation. These hypotheses are intended to provide a structured framework for subsequent mechanistic and interventional studies, and they should not be interpreted as confirmed pathways derived from the present trial.

This study presents several limitations. (1) Extrapolation of results should be done with caution. This study used an open-label, non-placebo-controlled design and differences in participant compliance and expectation-related bias cannot be excluded. (2) The effective dose of EPA and DHA derived from ALA supplementation was likely insufficient because of the low conversion efficiency of ALA (<5%). No significant changes in circulating EPA or DHA were observed, and the contribution of EPA/DHA-related mechanisms cannot be clearly determined. (3) The study was conducted at a single center with a relatively small overall sample size ($n = 82$), which may limit generalizability. In addition, the gut microbiota and metabolomics analyses were based on a small subsample (10 participants per group) and should be interpreted as exploratory. (4) The initial analyses of high-dimensional omics data did not include correction for multiple comparisons. After applying false discovery rate (FDR) correction, most associations did not remain statistically significant, indicating a risk of false-positive findings. These results therefore require validation in larger cohorts. (5) Some findings showed inconsistent patterns, including a decrease in HDL-C and upward within-group trends in TC and TC/LDL-C. In addition, cardiovascular risk was evaluated only using short-term changes in intermediate biomarkers. No clinical cardiovascular endpoints, such as atherosclerotic plaque progression or cardiovascular events, were assessed. As a result, conclusions regarding cardiovascular risk reduction cannot be drawn from this study. Further confirmation would require placebo-controlled, double-blind designs with longer follow-up and clinically relevant endpoints..

5. Conclusion

Combined supplementation of ALA (1.6 g/day) and coenzyme Q10 (136 mg/day) was associated with short-term changes in selected lipid-related markers and metabolic profiles in individuals with borderline dyslipidemia. These findings indicate that this nutritional combination warrants further investigation as a potential non-pharmacological intervention, while the underlying biological mechanisms remain to be further clarified. Given the short intervention duration and the absence of clinical cardiovascular endpoints, the

long-term impact of this intervention on cardiovascular risk cannot be determined and requires validation in large-scale prospective studies with well-defined clinical outcomes.

Conflicts of interest

No potential conflicts of interest were reported.

Funding

This work was supported by the Key special research Project of the China Military Security Committee (22BJZ20).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://>

References

1. C.Y. Hou. Four areas of health innovation boosted by the pandemic, *Nat. Med.* 28(2022) 1326–1328. <https://doi.org/10.1038/s41591-022-01844-w>.
2. S. Brandhorst, V.D. Longo. Dietary restrictions and nutrition in the prevention and treatment of cardiovascular disease, *Circ. Res.* 124(2019) 952–965. <https://doi.org/10.1161/circresaha.118.313352>.
3. S.M. Grundy, N.J. Stone, A.L. Bailey, et al., 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, *J. Am. Coll. Cardiol.* 73(2019) e285–e350. <https://doi.org/10.1016/j.jacc.2018.11.003>.
4. F. Mach, C. Baigent, A.L. Catapano, et al., 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, *Eur. Heart J.* 41(2020) 111–188. <https://doi.org/10.1093/eurheartj/ehz455>.
5. M.V. Holmes, I.Y. Millwood, C. Kartsonaki, M.R. Hill, et al., Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke, *J. Am. Coll. Cardiol.* 71(2018) 620–632. <https://doi.org/10.1016/j.jacc.2017.12.006>.
6. K.K. Berneis, R.M. Krauss. Metabolic origins and clinical significance of LDL heterogeneity, *J. Lipid Res.* 43(2002) 1363–1379. <https://doi.org/10.1194/jlr.r200004-jlr200>.
7. G.J. Blake, J.D. Otvos, N. Rifai, et al., Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women, *Circulation* 106(2002) 1930–1937. <https://doi.org/10.1161/01.cir.0000033222.75187.b9>.
8. P. Soininen, A.J. Kangas, P. Würtz, et al., Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics, *Circ. Cardiovasc. Genet.* 8(2015) 192–206. <https://doi.org/10.1161/circgenetics.114.000216>.
9. A.C. Skulas-Ray, P.W.F. Wilson, W.S. Harris, et al., Omega-3 fatty acids for the management of hypertriglyceridemia: a science advisory from the American Heart Association, *Circulation* 140(2019) e673–e691. <https://doi.org/10.1161/cir.0000000000000709>.
10. A. Gupta, M. Alkhalil. The emerging role of icosapent ethyl in patients with cardiovascular disease: mechanistic insights and future applications, *J. Clin. Med.* 12(15)(2023) 5178. <https://doi.org/10.3390/jcm12113758>.
11. K. Retterstøl, F. Rosqvist. Fat and fatty acids - a scoping review for Nordic Nutrition Recommendations 2023, *Food Nutr. Res.* 68(2024) 2442237. <https://doi.org/10.29219/fnr.v68.9980>.
12. A. Jayedi, S. Soltani, A. Emadi, et al., Dietary intake, biomarkers and supplementation of fatty acids and risk of coronary events: a systematic review and dose-response meta-analysis of randomized controlled trials and prospective observational studies, *Crit. Rev. Food Sci. Nutr.* 64(2024) 12363–12382. <https://doi.org/10.1080/10408398.2023.2251583>.

13. J.P. Fladerer, S. Grollitsch. Comparison of coenzyme Q10 (ubiquinone) and reduced coenzyme Q10 (ubiquinol) as supplement to prevent cardiovascular disease and reduce cardiovascular mortality, *Curr. Cardiol. Rep.* 25(2023) 1759–1767. <https://doi.org/10.1007/s11886-023-01992-6>.
14. S. Hou, Z. Tian, D. Zhao, Y. Liang, et al., Efficacy and optimal dose of coenzyme Q10 supplementation on inflammation-related biomarkers: a GRADE-assessed systematic review and updated meta-analysis of randomized controlled trials, *Mol. Nutr. Food Res.* 67(2023) e2200800. <https://doi.org/10.1002/mnfr.202200800>.
15. Z. Liu, Z. Tian, D. Zhao, et al., Effects of coenzyme Q10 supplementation on lipid profiles in adults: a meta-analysis of randomized controlled trials, *J. Clin. Endocrinol. Metab.* 108(2022) 232–249. <https://doi.org/10.1210/clinem/dgac585>.
16. B. Jiménez, E. Holmes, C. Heude, et al., Quantitative lipoprotein subclass and low molecular weight metabolite analysis in human serum and plasma by (1)H NMR spectroscopy in a multilaboratory trial, *Anal. Chem.* 90(2018) 11962–11971. <https://doi.org/10.1021/acs.analchem.8b02412>.
17. T. Miao, X. Zhang, C. Zhang, et al., Type 3 resistant starch from *Canna edulis* reduce lipid levels in patients with mild hyperlipidemia through altering gut microbiome: a double-blind randomized controlled trial, *Pharmacol. Res.* 205(2024) 107232. <https://doi.org/10.1016/j.phrs.2024.107232>.
18. A.B. Andraski, F.M. Sacks, M. Aikawa, et al., Understanding HDL Metabolism and Biology Through In Vivo Tracer Kinetics. *Arterioscler Thromb Vasc Biol.* 44(2024) 76–88. <https://doi.org/10.1161/ATVBAHA.123.319742>.
19. H. Xu, F. Fang, K. Wu, et al., Gut microbiota-bile acid crosstalk regulates murine lipid metabolism via the intestinal FXR-FGF19 axis in diet-induced humanized dyslipidemia. *Microbiome.* 11(2023) 262. <https://doi.org/10.1186/s40168-023-01709-5>.
20. E. Vrentzos, I. Ikonomidis, G. Pavlidis, et al., Six-month supplementation with high dose coenzyme Q10 improves liver steatosis, endothelial, vascular and myocardial function in patients with metabolic-dysfunction associated steatotic liver disease: a randomized double-blind, placebo-controlled trial. *Cardiovasc Diabetol.* 23(2024) 245. <https://doi.org/10.1186/s12933-024-02326-8>.
21. R. Martínez, C. Mesas, A. Guzmán et al., Bioavailability and biotransformation of linolenic acid from basil seed oil as a novel source of omega-3 fatty acids tested on a rat experimental model. *Food Funct.* 13(2022) 7614–7628. <https://doi.org/10.1039/d2fo00672c>.
22. H. Yue, B. Qiu, M. Jia, et al., Effects of α -linolenic acid intake on blood lipid profiles: a systematic review and meta-analysis of randomized controlled trials. *Crit Rev Food Sci Nutr.* 61(2021) 2894–2910. <https://doi.org/10.1039/d2fo00672c>.
23. Z. He, J. Wang, Y. Wei, et al., Glycerophospholipid fatty acid remodeling optimizes lipid nutrition and volatile organic compound composition in freshwater fish with linolenic acid intervention. *Food Chem X.* 32(2025) 103264. <https://doi.org/10.1016/j.fochx.2025.103264>.
24. A.M. de Abreu, C.L.K. Copetti, D.B. Hauschild, et al., Effects of supplementation with vegetable sources of alpha-linolenic acid (ALA) on inflammatory markers and lipid profile in individuals with chronic kidney disease: A systematic review and meta-analysis. *Clin Nutr.* 41(2022) 1434–1444. <https://doi.org/10.1016/j.clnu.2022.02.013>.
25. B. Khakimov, H.C.J. Hoefsloot, N. Mobaraki, et al., Human blood lipoprotein predictions from (1)H NMR spectra: protocol, model performances, and cage of covariance, *Anal. Chem.* 94(2022) 628–636. <https://doi.org/10.1021/acs.analchem.1c01654>.
26. R. Ma, Y. Wang, Y. Li, et al., Quantitative lipoprotein subclass analysis in pig plasma by (1)H NMR spectroscopy and stability assessment, *Anal. Chem.* 97(2025) 6509–6516. <https://doi.org/10.1021/acs.analchem.4c05881>.
27. M. Balling, B.G. Nordestgaard, A. Varbo, et al., Small Dense Low-Density Lipoprotein Cholesterol and Ischemic Stroke. *Ann Neurol.* 93(2023) 952–964. <https://doi.org/10.1002/ana.26598>.
28. E. Mayerhofer, R. Malik, L. Parodi, et al., Genetically predicted on-statin LDL response is associated with higher intracerebral haemorrhage risk. *Brain.* 145(2022) 2677–2686. <https://doi.org/10.1093/brain/awac186>.
29. N.W. Andersson, G. Corn, T.L. Dohlmann, et al., LDL-C Reduction With Lipid-Lowering Therapy for Primary Prevention of Major Vascular Events Among Older Individuals. *J Am Coll Cardiol.* 82(2023) 1381–1391. <https://doi.org/10.1016/j.jacc.2023.07.027>.
30. A.J. Amor, E. Castelblanco, M. Hernández, et al., Advanced lipoprotein profile disturbances in type 1 diabetes mellitus: a focus on LDL particles, *Cardiovasc. Diabetol.* 19(2020) 126. <https://doi.org/10.1186/s12933-020-01099-0>.

31. Y. Ye, B. Markussen, S.B. Engelsens, et al., The quality, uniqueness, and causality of NMR-based prediction models for low-density lipoprotein cholesterol subfractions in human blood plasma, *Comput. Biol. Med.* 184(2025) 109379. <https://doi.org/10.1016/j.combiomed.2024.109379>.
32. S.R. Millar, P. Navarro, J.M. Harrington, et al., Comparing dietary score associations with lipoprotein particle subclass profiles: a cross-sectional analysis of a middle-to older-aged population, *Clin. Nutr.* 40(2021) 4720–4729. <https://doi.org/10.1016/j.clnu.2021.06.005>.
33. J.F. García-Gavilán, M.A. Connelly, N. Babio, et al., Nut consumption is associated with a shift of the NMR lipoprotein subfraction profile to a less atherogenic pattern among older individuals at high CVD risk, *Cardiovasc. Diabetol.* 21(2022) 189. <https://doi.org/10.1186/s12933-022-01624-3>.
34. P.M. Kris-Etherton, W.S. Harris, L.J. Appel. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association, *Arterioscler. Thromb. Vasc. Biol.* 23(2003) 151–152. <https://doi.org/10.1161/01.atv.0000057393.97337.ae>.
35. S. Bangalore, A. Singh, B. Toklu, et al., Efficacy of cilostazol on platelet reactivity and cardiovascular outcomes in patients undergoing percutaneous coronary intervention: insights from a meta-analysis of randomised trials, *Open Heart* 1(2014) e000068. <https://doi.org/10.1136/openhrt-2014-000068>.
36. V.Y. Mareev, Y.V. Mareev, Y.L. Begrambekova. Coenzyme Q-10 in the treatment of patients with chronic heart failure and reduced left ventricular ejection fraction: systematic review and meta-analysis, *Kardiologiya* 62(2022) 3–14. <https://doi.org/10.18087/cardio.2022.6.n2050>.
37. J.Y.L. Chiang. Bile acid metabolism and signaling in liver disease and therapy, *Liver Res.* 1(2017) 3–9. <https://doi.org/10.1016/j.livres.2017.05.001>.
38. W. Zeng, M. Sun, J. Cao, et al., Triterpenoids from *ilicis rotundae* cortex ameliorate hyperlipidemia by affecting bile acids-hepatointestinal FXR axis, *Phytomedicine* 139(2025) 156537. <https://doi.org/10.1016/j.phymed.2025.156537>.
39. S. Zhao, H. Fu, T. Zhou, et al., Alteration of bile acids and omega-6 PUFAs are correlated with the progression and prognosis of drug-induced liver injury, *Front. Immunol.* 13(2022) 772368. <https://doi.org/10.3389/fimmu.2022.772368>.
40. S. Xie, L. Yuan, Y. Sui, et al., NME4 mediates metabolic reprogramming and promotes nonalcoholic fatty liver disease progression, *EMBO Rep.* 25(2024) 378–403. <https://doi.org/10.1038/s44319-023-00012-6>.
41. J. Li, Z. Zhang, Y. Xu, et al., *Limosilactobacillus fermentum* HNU312 alleviates lipid accumulation and inflammation induced by a high-fat diet: improves lipid metabolism pathways and increases short-chain fatty acids in the gut microbiome, *Food Funct.* 15(2024) 8878–8892. <https://doi.org/10.1039/d4fo02390k>.
42. C. Tang, Y. Wang, D. Chen, et al., Natural polysaccharides protect against diet-induced obesity by improving lipid metabolism and regulating the immune system, *Food Res. Int.* 172(2023) 113192. <https://doi.org/10.1016/j.foodres.2023.113192>.
43. J. Tan, C. McKenzie, M. Potamitis, et al., The role of short-chain fatty acids in health and disease, *Adv. Immunol.* 121(2014) 91–119. <https://doi.org/10.1016/b978-0-12-800100-4.00003-9>.
44. Y. Li, Z. Yu, Y. Liu, et al., Dietary α -linolenic acid-rich flaxseed oil ameliorates high-fat diet-induced atherosclerosis via gut microbiota-inflammation-artery axis in ApoE (–/–) mice, *Front. Cardiovasc. Med.* 9(2022) 830781. <https://doi.org/10.3389/fcvm.2022.830781>.
45. Y. Tian, Y. Zhou, W. Liao, et al., Flaxseed powder supplementation in non-alcoholic fatty liver disease: a randomized controlled clinical trial, *Food Funct.* 16(2025) 1389–1406. <https://doi.org/10.1039/d4fo05847j>.
46. Z. Dong, S. Liu, Q. Deng, et al., Role of iron in host-microbiota interaction and its effects on intestinal mucosal growth and immune plasticity in a piglet model, *Sci. China Life Sci.* 66(2023) 2086–2098. <https://doi.org/10.1007/s11427-022-2409-0>.
47. P. Chandrasekaran, Y. Han, C.S. Zerbe, T. Heller, et al., Intestinal microbiome and metabolome signatures in patients with chronic granulomatous disease, *J. Allergy Clin. Immunol.* 152(2023) 1619–1633.e1611. <https://doi.org/10.1016/j.jaci.2023.07.022>.
48. Q. Lin, L. Zheng, W. Pan, et al., Fucoidan derived from *Saccharina japonica*: structural characterization and amelioration of high-fat-diet induced obesity in male C57BL/6 J mice via modulating the gut microbiota and lipid metabolites, *Int. J. Biol. Macromol.* 310(2025) 143026. <https://doi.org/10.1016/j.ijbiomac.2025.143026>.

49. X. Zhang, C. Zhang, L. Xiao, et al., Digestive characteristics of extracellular polysaccharide from *Lactiplantibacillus plantarum* T1 and its regulation of intestinal microbiota, *Int. J. Biol. Macromol.* 259(2024) 129112. <https://doi.org/10.1016/j.ijbiomac.2023.129112>.
50. I. Manolaras, A. Del Bondio, O. Griso, et al., Mitochondrial dysfunction and calcium dysregulation in COQ8A-ataxia Purkinje neurons are rescued by CoQ10 treatment, *Brain* 146(2023) 3836–3850. <https://doi.org/10.1093/brain/awad099>.
51. Y. Liu, Y. Yao, W. Tao, et al., Coenzyme Q10 ameliorates BPA-induced apoptosis by regulating autophagy-related lysosomal pathways, *Ecotoxicol. Environ. Saf.* 221(2021) 112450. <https://doi.org/10.1016/j.ecoenv.2021.112450>.
52. D. Li, H. Cai, G. Liu, et al., *Lactiplantibacillus plantarum* FRT4 attenuates high-energy low-protein diet-induced fatty liver hemorrhage syndrome in laying hens through regulating gut-liver axis, *J. Anim. Sci. Biotechnol.* 15(2024) 31. <https://doi.org/10.1186/s40104-023-00982-6>.