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Medium-chain triglycerides promote macrophage reverse cholesterol transport and improve atherosclerosis in ApoE-deficient mice fed a high-fat diet

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ABSTRACT

We previously observed that medium-chain triglycerides (MCTs) could reduce body fat mass and improve the metabolism of cholesterol. We hypothesized that MCTs can improve atherosclerosis by promoting the reverse cholesterol transport (RCT) process. Therefore, the objective of this study was to investigate the roles of MCTs in macrophage RCT and the progression of atherosclerosis. To test this hypothesis, 30 4-week-old ApoE-deficient ($ApoE^{-/-}$) mice were randomly divided into 2 groups and fed a diet of 2% MCTs or long-chain triglycerides (LCTs) for 16 weeks. Ten age- and sex-matched C57BL/6J mice were fed a diet of 2% LCTs as the control. Macrophage-to-feces RCT was assessed in vivo by intraperitoneal injection of RAW 264.7 macrophages containing ^{3}H-labeled cholesterol, and atherosclerotic plaques were measured. The mRNA and protein expressions were determined by reverse transcriptase polymerase chain reaction and Western blot analyses, respectively. There was a greater decrease in body fat mass, atherosclerotic plaques, and an improvement in serum lipid profiles. In addition, the MCT mice group showed an increase in ^{3}H-tracer in the feces and a decrease in the liver. Significantly higher levels of mRNA and protein expression of hepatic ATP-binding cassette transporter A1, ATP-binding cassette transporter G5, cholesterol 7α-hydroxylase, and intestinal ATP-binding cassette transporter G8, as well as lower levels of expression of intestinal Niemann-Pick C1-like 1, were found in the MCT group. These results suggest that MCTs could obviously promote macrophage RCT and improve atherosclerosis in $ApoE^{-/-}$ mice, indicating that MCTs have the potential to prevent cardiovascular disease.

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG5, ATP-binding cassette transporter G5; ABCG8, ATP-binding cassette transporter G8; ApoA1, apolipoprotein A1; $ApoE^{-/-}$, ApoE-deficient; BSA, bovine serum albumin; CVD, cardiovascular disease; CYP7A1, cholesterol 7α-hydroxylase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; HDL, high-density lipoprotein; HDL-C, high density lipoprotein cholesterol; LCTs, long chain triglycerides; LDL-C, low density lipoprotein cholesterol; LSC, liquid scintillation counter; MCFAs, medium chain fatty acids; MCTs, medium chain triglycerides; NPC1L1, Niemann-Pick C1-like 1; ox-LDL, oxidized low density lipoprotein; PCR, polymerase chain reaction; PMA, phorbol-12-myristate-13-acetate; PUFAs, polyunsaturated fatty acid; RCT, reverse cholesterol transport; SFAs, saturated fatty acids; SR-BI, scavenger receptor class B type I; TC, total cholesterol; TG, triglyceride.

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1. Introduction

Atherosclerosis is reported to be the main pathological cause of cardiovascular disease (CVD). Dyslipidemia, including higher serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and lower high-density lipoprotein cholesterol (HDL-C), is a major risk factor for atherosclerosis [1]. Dietary fat is considered one of the most important factors associated with blood lipid metabolism and plays significant a role in the cause and prevention of atherosclerosis. Saturated fatty acids (SFAs) could increase plasma LDL-C and promote the progression of atherosclerosis [2]. Decreasing SFAs and replacing them with polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids could reduce the risk of atherosclerosis [3,4]. However, not all SFAs have similar effects, and the health effects of SFAs depend on their carbon chain length, the distribution of fatty acids in triacylglycerols and the degree of saturation of fatty acid. The proportion of different fatty acids in diets plays an important role in metabolism of glucose and lipids [5]. Medium-chain fatty acids (MCFAs) are saturated and have chain lengths between 6 to 12 carbons. MCFAs occur naturally as medium-chain triglycerides (MCTs) in milk fat and in various feed materials, such as coconut, palm oils, and cuphea seed oils [6–8]. Many studies have demonstrated the role of MCTs and MCFAs in reducing body weight, particularly in reducing body fat accumulation [9–11]. Some studies have reported that MCTs and MCFAs could decrease TC and LDL-C and increase HDL-C [12–14]. Our previous studies suggested that compared to oil containing long-chain fatty acids (LCFAs), MCTs could reduce serum TC and LDL-C and increase HDL-C in hypertriglyceridemic subjects [10,15], and we have observed similar effects in C57BL/6J mice [16,17]. A growing body of evidence suggests that, even though MCFAs are SFAs, they may improve the metabolism of cholesterol.

Reverse cholesterol transport (RCT) is thought to be a protective mechanism against atherosclerosis. In this pathway, excess cholesterol is transported from peripheral cells (such as macrophages on atherosclerotic plaques) to high-density lipoprotein (HDL), which transfers cholesterol to the liver for excretion in the form of bile acids or free cholesterol. Several studies conducted in the past few years have found that omega-3 fatty acids, such as the docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) that is abundant in fish oil, could improve macrophage-to-feces RCT in C57BL/6J mice [18] and hamsters [19]. Our previous study found that MCFAs could reduce blood cholesterol by promoting the excretion of fecal cholesterol and cholic acid [20]. However, the mechanisms by which this occurs are still unclear.

Based on our previous studies, we hypothesize that MCTs are involved in modulating the RCT-associated messenger RNA (mRNA) and protein, and promoting the process of RCT, and improve atherosclerosis. Therefore, we investigated the effect of MCTs on macrophage-to-feces RCT and the progression of atherosclerosis in ApoE-deficient ($ApoE^{-/-}$) mice. Macrophage RCT was assessed by injecting ^{3}H cholesterol-labeled RAW 264.7 macrophages intraperitoneally into mice. Body mass, epididymal fat mass, serum lipid profiles, and aorta atherosclerotic plaques were measured, and RCT-associated mRNA and protein were determined by reverse transcriptase polymerase chain reaction and Western blot analyses, respectively. In addition, we also observed the effects of MCFAs on the cholesterol efflux from THP-1 macrophages.

2. Methods and materials

2.1. Materials

MCTs (consisting of caprylic acid and capric acid) and LCTs (soybean oil) were donated by Nisshin Oillio (Tokyo, Japan). Fetal bovine serum (FBS) and RPMI 1640 culture medium were purchased from Gibco (Grand Island, Nebraska, USA). Human LDL, apolipoprotein A-1(ApoA1), phorbol-12-myristate-13-acetate (PMA), Oil Red O, bovine serum albumin (BSA), caprylic acid (8:0), capric acid (10:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and alpha linolenic acid (18:3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ^{3}H-cholesterol was purchased from Perkin- Elmer Life Science. Other reagents for which a vendor is not mentioned were purchased from Sigma-Aldrich.

2.2. Animals

Thirty male $ApoE^{-/-}$ mice (4-week-old) and 10 male wild-type C57BL/6J mice (4-week-old) were purchased from the Shanghai Biomodel Organism Science & Technology Development Co., LTD (license no. SCXK: JING 2009–0023) and housed in polycarbonate cages (5 animals in each cage) with a 12-hour light/dark cycle. The room temperature was maintained at 22 ± 1 °C, and humidity was kept between 40% and 60%. All experimental procedures were approved by the Animal Care and Use Committee of the Chinese PLA General Hospital.

2.3. Diets, dietary lipids, and experimental design

All diets were made from a basal diet purchased from the Academy of Military Medical Sciences that was based on the AIN-93G diet. The experimental diets contained 77.7% AIN-93G, 10% lard, 10% yolk powder, 0.3% bile salts, 2% MCTs, or 2% LCTs. The ingredient, nutrient, and fatty acid compositions of the experimental diets are shown in Table 1. The dietary nutrients were tested by Beijing Institute of Nutrition.

The dietary lipids were analyzed by gas-liquid chromatography [21]. Briefly, 20% heptadecanoic acid as internal standard was added into 50 mg diets powder, and the fatty acid methyl esters (FAMEs) were obtained from the base-catalyzed methanolysis of the glycerides, after dissolving the lipid extract in high-performance liquid chromatography-quality hexane. The total fatty acid profile was recorded by analyzing the FAMEs by gas-liquid chromatography (Shimadzu GC 2010, Kyoto, Japan) with an autoinjector (AOC-20i) Split/Splitless equipped with a flame ionization detector and a CP Sil 88 fused-silica capillary column (100 m × 0.25 mm × 0.2 μm). The FAMEs were identified by comparing their retention times to those of commercial standards. The values were expressed as a percentage of the total FAMEs.