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Novel role of a triglyceride-synthesizing enzyme: DGAT1 at the crossroad between triglyceride and cholesterol metabolism

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ABSTRACT

Acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) is a key enzyme in triacylglycerol (TG) biosynthesis. Here we show that genetic deficiency and pharmacological inhibition of DGAT1 in mice alters cholesterol metabolism. Cholesterol absorption, as assessed by acute cholesterol uptake, was significantly decreased in the small intestine and liver upon DGAT1 deficiency/inhibition. Ablation of DGAT1 in the intestine (I-DGAT1$^{-/-}$) alone is sufficient to cause these effects. Consequences of I-DGAT1 deficiency phenocopy findings in whole-body DGAT1$^{-/-}$ and DGAT1 inhibitor-treated mice. We show that deficiency/inhibition of DGAT1 affects cholesterol metabolism via reduced chylomicron size and increased trans-intestinal cholesterol excretion. These effects are independent of cholesterol uptake at the apical surface of enterocytes but mediated through altered dietary fatty acid metabolism. Our findings provide insight into a novel role of DGAT1 and identify a pathway by which intestinal DGAT1 deficiency affects whole-body cholesterol homeostasis in mice. Targeting intestinal DGAT1 may represent a novel approach for treating hypercholesterolemia.

1. Introduction

Acyl-CoA:diacylglycerol acyltransferase (DGAT)1 and 2 catalyze the final and rate-limiting step of triacylglycerol (TG) biosynthesis mediated mainly through the 2-monoacylglycerol and glycerol-phosphate pathways [1]. The two DGAT enzymes DGAT1 and DGAT2 are encoded by two different genes belonging to distinct acyl-transferase families, which lack significant sequence homology [2]. Highest expression levels of both genes are found in tissues active in TG synthesis, such as adipose tissue, small intestine, liver, and mammary gland [3]. In the intestine, DGAT1 is predominantly involved in the biosynthesis of TG from dietary fatty acids (FA) [4]. In the postprandial state, esterified lipids generated by intestinal DGAT1 are incorporated into chylomicron particles, secreted into the lymphatic system, and subsequently released into the peripheral circulation via the thoracic duct [5]. DGAT1 knockout (DGAT1$^{-/-}$) mice are viable, resistant to diet-induced obesity and fatty liver disease, exhibit increased energy expenditure, and normal insulin and leptin sensitivity [3,6]. Mice that express DGAT1 exclusively in the small intestine are, however, susceptible to high-fat diet-induced hepatic steatosis and obesity [7]. Loss of DGAT1 specifically in the intestine leads to reduced postprandial TG and retinyl ester concentrations due to reduced chylomicron secretion [8]. These observations implicate intestinal DGAT1 as a potential target to reduce postprandial lipemia by altering chylomicron biosynthesis [9].

Pharmacological DGAT1 inhibition studies in animal models have consistently demonstrated reduction in diet-induced weight gain and postprandial chylomicron secretion [10,11]. Pharmacological inhibition

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; ApoE, apolipoprotein E; DGAT, acyl-CoA:diacylglycerol acyltransferase; I-DGAT1, intestine-specific; Inh-DGAT1, DGAT1 inhibitor-treated; GLP-1, glucagon-like peptide 1; HF/HCD, high-fat/high-cholesterol diet; MTP, microsomal triglyceride transfer protein; NLS, neutral sterol loss; TICE, trans-intestinal cholesterol excretion.

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of DGAT1 was also shown to reduce plasma total cholesterol (TC) concentrations in mice [12]. The utility of DGAT1 inhibitors has recently been strengthened by studies in patients suffering from familial chylomicronemia [13]. Thus, DGAT1 is a potential target to improve metabolic parameters in hyperlipidemic states. Intestinal DGAT1 has been speculated to exert these effects by increasing the secretion of gut hormones, such as glucagon-like peptide-1 and peptide YY [14]. However, endocrine changes alone are unable to explain the observed postprandial phenotype upon pharmacological DGAT1 inhibition and thus the precise mechanisms regulating DGAT1 inhibitory effects still remain elusive [8].

We have previously reported that DGAT1 deficiency in apolipoprotein $E^{-/-}$ mice has an athero-protective role. These beneficial effects included lower plasma cholesterol concentrations and reduced intestinal cholesterol absorption [15]. Given its predominant role in TG biosynthesis, the profound reduction in cholesterol uptake and absorption is puzzling. Our results indicate that inhibition or ablation of DGAT1 in enterocytes is sufficient to reduce the incorporation of cholesterol esters (CE) into chylomicrons. We speculate that the reduced chylomicron size further facilitates the trans-intestinal excretion of cholesterol (TICE) upon DGAT1 inhibition conferring athero-protection.

2. Materials and methods

2.1. Animals and diets

Age-matched male mice (8–12 weeks of age) were used for all experiments unless indicated. Mice had ad libitum access to water and food and were maintained under a 12-h light/12-h dark cycle in a temperature-controlled environment. I-DGAT1$^{-/-}$ [8] and DGAT1$^{-/-}$ [16] mice were generously provided by Dr. Robert Farese, Jr. (Harvard T.H. Chan School of Public Health, Boston, MA). LDLR$^{-/-}$ mice (purchased from Jackson Laboratory, Bar Harbor, ME) were crossed with DGAT1$^{-/-}$ mice to generate DGAT1$^{-/-}$ LDLR$^{-/-}$ mice. All mice were fed either regular chow diet (11.9% caloric intake from fat; Altromin 1324, Lage, Germany) or challenged with a high-fat/high-cholesterol diet (HF/HCD; 30% caloric intake from fat plus 1% cholesterol; Ssniff, Soest, Germany) starting at the age of 8 weeks. The fatty acid composition of the HF/HCD is delineated in supplementary Table S1.

Experiments to determine cholesterol fluxes upon chronic pharmacological DGAT1 inhibition were performed in compliance with national laws and were approved by the respective Ethical Committees for Animal Experiments, University of Groningen, The Netherlands. For all other studies, animal experiments were approved by the Austrian Federal Ministry of Science, Research, and Economy, Vienna, Austria, in accordance with the Council of Europe Conventions.

2.2. Blood biochemical analyses

Blood was collected from 4 h (6 a.m. until 10 a.m.) fasted mice and plasma was prepared within 20 min. TG, TC, and free cholesterol (FC) concentrations were measured enzymatically according to manufacturer's instructions (DiaSys, Holzheim, Germany). Lipoprotein fractions were separated from 200 μl pooled plasma from each group using fast protein liquid chromatography (Pharmacia P-500) equipped with a Superose 6 column (Amersham Biosciences, Piscataway, NJ). TC concentrations in the isolated fractions were measured enzymatically.

2.3. Acute pharmacological DGAT1 inhibition

The DGAT1 inhibitor used in this study has been described elsewhere as compound 2 [11]. Dosage for the inhibitor treatment was chosen based on a previous report [8]. Treatments were initiated one day before start of the experiment at 8 a.m. after a 2 h fasting period and were repeated every 24 h during the course of the experiment. Mice had access to food 2 h post treatment. Wild-type mice on a C57BL/6 background were orally dosed with either vehicle composed of 0.5% (w/v) of hydroxypropylmethylcellulose (Sigma-Aldrich, St. Louis, MO) in 0.1% (v/v) Tween 80 or DGAT1 inhibitor (5 mg/kg body weight) dissolved in vehicle. On the day of cholesterol absorption experiments (described below) mice were gavaged with corn oil containing radiotracers 2 h post DGAT1 inhibitor treatment.

2.4. Short-term cholesterol absorption

Cholesterol absorption studies were performed as described previously [15]. Briefly, chow diet-fed mice were fasted for 4 h and gavaged with 200 μl corn oil containing 2 μCi [^{3}H]cholesterol (ARC Inc., St Louis, MO) and 200 μg cholesterol. After 4 h, plasma, liver, and three parts of the small intestine were isolated. Duodenum, jejunum, and ileum were rinsed with PBS to remove luminal contents. The tissues were dissolved in 1 M NaOH at 65 °C overnight for protein quantitation and analyzed by liquid scintillation counting.

2.5. Fractional cholesterol absorption

Fractional cholesterol absorption was measured by the fecal dual-isotope ratio method as described [15]. Chow diet-fed mice were fasted for 4 h and gavaged with a single dose of 200 μl corn oil containing 0.2 μCi [^{3}H]sitostanol (ARC Inc., St. Louis, MO) and 0.1 μCi [^{14}C]cholesterol (ARC Inc., St. Louis, MO). Feces were collected for 72 h and lipids were extracted using the Folch extraction method. Radioactivity in fecal samples was measured by liquid scintillation counting. Fractional cholesterol absorption was calculated using the following formula: % absorption = (dose [^{14}C]:[^{3}H] − fecal [^{14}C]:[^{3}H])/ dose [^{14}C]:[^{3}H] × 100.

2.6. Uptake and secretion of cholesterol by primary enterocytes

Primary enterocytes were isolated from overnight fasted C57BL/6 mice, suspended in 4 ml of DMEM, and incubated at 37 °C as described [17]. DMEM was supplemented with either vehicle (DMSO) or DGAT1 inhibitor (EC_{50} = 0.03 μm, dissolved in DMSO) and 1 μCi/ml [-^{3}H]cholesterol suspended in mixed lipid micelles (0.14 mM sodium cholate, 0.15 mM sodium deoxycholate, 0.17 mM phosphatidylcholine, 0.19 mM mono-oleoylglycerol). Cholesterol uptake was determined at indicated time points, after which enterocytes were washed, cellular lipids were extracted, and radioactivity was measured by liquid scintillation counting. For determination of cholesterol secretion, enterocytes were labeled for 1 h and chased with medium containing secretion micelles (1.4 mM oleic acid, 0.14 mM sodium cholate, 0.15 mM sodium deoxycholate, 0.17 mM phosphatidylcholine, 0.19 mM mono-oleoylglycerol) in the absence and presence of DGAT1 inhibitor. After 2 h of chase, enterocytes were centrifuged, lipids were extracted from the supernatant and separated by thin layer chromatography to quantify enterocyte [^{3}H]cholesterol secretion into FC and cholesteryl ester (CE) fractions [17].

2.7. Chylomicron secretion

Chylomicron secretion rate using [9, 10-^{3}H(N)]triolein (Perkin Elmer, Boston, MA) and [^{14}C]cholesterol (ARC Inc., St. Louis, MO) was assessed as previously described [8] with minor modifications. Briefly, mice fed HF/HCD for 12 weeks were fasted for 4 h starting at 6 a.m. Thereafter, they were intraperitoneally injected with Poloxamer-407 (P-407; 1 g/kg body weight, Sigma-Aldrich, St. Louis, MO) in PBS and gavaged with 200 μl corn oil containing 1 μCi [^{3}H]triolein, 0.5 μCi [-^{14}C]cholesterol, and 200 μg cholesterol. Prior to P-407 injection and post 1, 2, 3, and 4 h corn oil gavage, blood samples were collected and radioactivity was measured in the plasma. Four hours post gavage, mice were anaesthetized and lymph was surgically removed as described [18] with modifications. Briefly, anaesthetized mice were aseptically prepared for surgery and the abdomen was opened through a left