Research Article

JOURNAL OF HEPATOLOGY

S-Adenosylmethionine increases circulating very-low density lipoprotein clearance in non-alcoholic fatty liver disease

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Background & Aims: Very-low-density lipoproteins (VLDLs) export lipids from the liver to peripheral tissues and are the precursors of low-density-lipoproteins. Low levels of hepatic S-adenosylmethionine (SAMe) decrease triglyceride (TG) secretion in VLDLs, contributing to hepatosteatosis in methionine adenosyltransferase 1A knockout mice but nothing is known about the effect of SAMe on the circulating VLDL metabolism.

We wanted to investigate whether excess SAMe could disrupt VLDL plasma metabolism and unravel the mechanisms involved.

Methods: Glycine *N*-methyltransferase (*GNMT*) knockout (KO) mice, *GNMT* and perilipin-2 (*PLIN2*) double KO (*GNMT-PLIN2*-KO) and their respective wild type (WT) controls were used. A high fat diet (HFD) or a methionine deficient diet (MDD) was administrated to exacerbate or recover VLDL metabolism, respectively. Finally, 33 patients with non-alcoholic fatty-liver disease (NAFLD); 11 with hypertriglyceridemia and 22 with normal lipidemia were used in this study.

Results: We found that excess SAMe increases the turnover of hepatic TG stores for secretion in VLDL in *GNMT*-KO mice, a model of NAFLD with high SAMe levels. The disrupted VLDL assembly resulted in the secretion of enlarged, phosphatidylethanolamine-poor, TG- and apoE-enriched VLDL-particles; special features that lead to increased VLDL clearance and decreased serum TG levels. Re-establishing normal SAMe levels restored VLDL secretion, features and metabolism. In NAFLD patients, serum TG levels were lower when hepatic GNMT-protein expression was decreased.

Conclusions: Excess hepatic SAMe levels disrupt VLDL assembly and features and increase circulating VLDL clearance, which will cause increased VLDL-lipid supply to tissues and might contribute to the extrahepatic complications of NAFLD.

Keywords: S-Adenosylmethionine; Very-low density-lipoproteins; Glycine *N*-methyltransferase; Non-alcoholic fatty-liver disease.

Received 15 April 2014; received in revised form 5 September 2014; accepted 9 October 2014; available online 18 October 2014

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Abbreviations: apo, apolipoprotein; CD, control diet; DGAT, diacylglycerol O-acyltransferase; DG, diglyceride; DZA, 3-deazaadenosine; FA, fatty acid; FC, free cholesterol; GNMT, glycine N-methyltransferase, KB, ketone bodies; KO, knockout; MAT, methionine adenosyltransferase; MDD, methionine deficient diet; MTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty-liver disease; NASH, non-alcoholic steatohepatitis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; PLIN, perilipin; qRT-PCR, real-time polymerase chain reaction; SAH, S-adenosylhomocysteine; SAMe, S-adenosylmethionine; TG, triglyceride; TGL, triglyceride lipase; VLDL, very-low-density lipoprotein; WT, wild type.

Introduction

The liver plays a central role in whole body metabolic homeostasis. It can obtain lipids from the circulation, synthesize them and secrete them in lipoproteins into the blood stream. Very-low-density lipoproteins (VLDL) transport triglycerides (TG) from the liver to peripheral tissues, providing an energy source. Plasma levels of VLDL are defined by the rate of clearance from plasma and the rate of hepatic secretion, so an imbalance between these two processes will lead to dyslipidemia, highly associated with increased risk of cardiovascular disease [1,2], which is one of the extrahepatic complications of non-alcoholic fatty-liver disease (NAFLD) [3].

Individuals with NAFLD exhibit disrupted VLDL metabolism [4,5]. In fact, abnormalities in the hepatic uptake of lipoproteins and/or secretion of VLDL can lead to hepatosteatosis [6,7].

The hepatic VLDL secretion rate is regulated by a variety of factors that must guarantee release of adequate amounts of TGs from the liver. To ensure VLDL secretion, apolipoprotein (apo) B should be translocated in the lumen of the endoplasmic reticulum (ER) during its translation [8], where it interacts with the microsomal TG transfer protein (MTP), whose lipid transfer activity is one of the major determinants in VLDL secretion [9]. ApoE facilitates ApoB maturation and VLDL assembly and secretion [10,11], and it mediates cellular uptake of several lipoproteins from the circulation [12,13].

Most of VLDL-TG (60–70%) is derived from intracellular stores [14,15]; therefore, the mobilization of lipids from cytosolic lipid droplets towards the ER represents a potentially regulated step in VLDL production and secretion [15,16].

Low liver S-adenosylmethionine (SAMe) in methionine adenosyltransferase 1A (*MAT1A*)-KO mice decreases TG secretion into VLDL particles, which contributes to hepatosteatosis [6]. Glycine *N*-methyltransferase (GNMT) drives the catabolism of SAMe and its deficiency in mice results in a marked increase in hepatic SAMe content and rapid NAFLD development [17]. SAMe is the methyl donor required for the methylation of phosphatidylethanolamine (PE). In hepatocytes, around 30% of phosphatidylcholine (PC) is synthesized by the sequential methylation of PE, in a reaction catalysed by the enzyme PE *N*-methyltransferase (PEMT) [18]. We have found that the flux from PE to PC and its catabolism into diglycerides (DGs) and conversion into TGs is stimulated in the liver of *GNMT*-KO mice [19]. There is a requirement for PEMT in the liver to ensure normal VLDL secretion [18,20]. Thus, we have evaluated if high levels of hepatic SAMe will disrupt VLDL assembly and as a consequence VLDL features and plasma metabolism.

Materials and methods

Human samples

This study comprised 33 non-diabetic patients with cholelithiasis and a clinical diagnosis of NAFLD without necroinflammation or fibrosis (Supplementary Tables 1 and 2) and 36 patients with asymptomatic cholelithiasis in whom a liver biopsy was taken during programmed laparoscopic cholecystectomy (Supplementary Table 1). Inclusion criteria for patients are detailed in the Supplementary data section. The study was performed in agreement with the Declaration of Helsinki and with local and national laws. The Human Ethics Committee of the University Hospital Santa Cristina and the University of Basque Country approved the study procedures and written informed consent was obtained from all patients before inclusion in the study.

Animals

3-month-old male *GNMT*-KO mice, *GNMT-PLIN2*-KO mice, *PLIN2*-KO mice and their WT littermates were produced in the animal facility of CIC bioGUNE. They were maintained on different diets detailed in supplemental information. Animal procedures were approved by the University of the Basque Country and CIC bioGUNE Animal Care and Use Committees.

Preparation of labelled VLDL particles and in vivo clearance

Labelled human VLDL particles were obtained by a method previously described [21,22] and as detailed in the Supplementary Materials and methods.

Quantification of lipids

Lipids were extracted and quantified as described before [23]. TGs were quantified using a commercially available kit (A. Menarini Diagnostics). PE, PC, and DG were separated by thin layer chromatography and quantified as detailed elsewhere [24].

Statistical analysis

Data were represented as means ± SEM. Differences between groups were tested using the Student's *t* test and two way ANOVA. Significance was defined as $p < 0.05$. The baseline characteristics of the patients studied were compared using the unpaired *t* test or Mann-Whitney *U* test. These analyses were performed using SPSS version 15.0 software and GraphPad Version 5.03.

Additional methods are detailed in the Supplementary Materials and methods section.

Results

Deletion of GNMT disrupts VLDL assembly, VLDL features and decreases circulating VLDL levels in serum

GNMT-KO mice show steatosis and fibrosis with increased serum aminotransferases [17] and no signs of insulin resistance [19]. In *GNMT*-KO mice VLDL-TG secretion was increased whereas VLDL-PE was decreased as compared to their wild-type (WT) controls (Fig. 1A). No changes were observed with regard to VLDL-PC secretion (Fig. 1A), while the VLDL size was augmented in *GNMT*-KO mice (Fig. 1A). All these compositional and physical VLDL features restored after feeding a methionine deficient diet (MDD) for 3 weeks (Fig. 1A). Consistent with the increased VLDL-TG secretion, we found increased turnover of the hepatocyte TG lipid stores (Fig. 1B) and increased MTP and diacylglycerol O-acyltransferase (DGAT) activity while no changes in TG lipase activity (Supplementary Fig. 1A) were observed. The increased hepatocyte TG secretion was re-established after inhibition of PEMT with 3-deazaadenosine (DZA) (Fig. 1C).

One of the factors that define the levels of circulating TGs in blood is the hepatic secretion rate. To our surprise, *GNMT* deletion in mice resulted in a decrease of serum TG (Fig. 1D), due to most of all VLDL and some LDL subfractions (Fig. 1D). The decrease in VLDL and LDL was also evident when ApoB levels were analysed in serum (Supplementary Fig. 1B). Re-establishing SAMe hepatic levels, by feeding MDD, restored serum TG and ApoB levels in *GNMT*-KO mice (Fig. 1D and Supplementary Fig. 1B).

In patients with NAFLD (n = 33) serum TG levels ranged from 54 to 317 mg/dl showing a high heterogeneity among subjects (Supplementary Table 1). In order to investigate whether altered SAMe metabolism could be linked with increased VLDL clearance in patients with NAFLD, we classified the individuals into two groups depending on serum TG levels (Supplementary Table 2). In a first group, (NAFLD-1), we introduced those subjects with serum TG levels higher than the mean of TGs in NAFLD patients (130.1 mg/dl) (n = 11) and in a second group (NAFLD-2) we included those with TG levels below 130.1 mg/dl (n = 22). We quantified TG levels in serum and analysed TG distribution in lipoproteins in the serum of the two groups (Fig. 1E) and found that decreased TG levels corresponded to most of all VLDL and some LDL subfractions (Fig. 1E). We also found that in NAFLD-2 patients there is a shift to the left of the maximum peak of VLDL, indicating that VLDL particles are enlarged (Fig. 1E). High hepatic SAMe levels in *GNMT*-KO mice were linked with enlarged VLDL