Research Article

JOURNAL OF HEPATOLOGY

Hepatic oleate regulates liver stress response partially through PGC-1α during high-carbohydrate feeding

Xueqing Liu[1,†], Maggie S. Burhans[2,†], Matthew T. Flowers[1], James M. Ntambi[1,2,*]

[1]*Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706, USA;* [2]*Department of Nutritional Sciences, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706, USA*

Background & Aims: High-carbohydrate diets contribute to the development of liver stress and fatty liver disease. While saturated fatty acids are known to induce liver stress, the role of monounsaturated fatty acids (MUFA), synthesized by the stearoyl-CoA desaturase (SCD) family of enzymes, in regulation of liver function during lipogenic dietary conditions remains largely unknown. The major products of SCD-catalyzed reactions are oleate (18:1n-9) and palmitoleate (16:1n-7).

Methods: We generated mouse models with restricted exogenous MUFA supply and reduced endogenous MUFA synthesis, in which SCD1 global knockout (GKO) or liver-specific knockout (LKO) mice were fed a lipogenic high-sucrose very low-fat (HSVLF) or high-carbohydrate (HC) diet. In a gain-of-function context, we introduced liver-specific expression of either human *SCD5*, which synthesizes 18:1n-9, or mouse *Scd3*, which synthesizes 16:1n-7, into SCD1 GKO mice and fed the HSVLF diet.

Results: Lipogenic high-carbohydrate diets induced hepatic endoplasmic reticulum (ER) stress and inflammation in SCD1 GKO and LKO mice. Dietary supplementation with 18:1n-9, but not 18:0, prevented the HSVLF diet-induced hepatic ER stress and inflammation in SCD1 LKO mice, while hepatic *SCD5*, but not *Scd3*, expression reduced the ER stress and inflammation in GKO mice. Additional experiments revealed liver-specific deletion of the transcriptional coactivator PGC-1α reduced hepatic inflammatory and ER stress response gene expression in SCD1 LKO mice.

Conclusions: Our results demonstrate an indispensable role of hepatic oleate in protection against lipogenic diet-induced hepatic injury, and PGC-1α potentiates the ER stress response under conditions of restricted dietary oleate coupled to reduced capacity of endogenous hepatic oleate synthesis.

Lay summary: Susceptibility to metabolic dysfunction is influenced by genetic and environmental factors. In this study we show that modulation of two genes regulates the liver response, including ER stress and inflammation, to a high-carbohydrate low-fat diet. We reveal that hepatic availability of oleate, a monounsaturated fatty acid, is important for maintenance of liver health.

Introduction

The prevalence of obesity and associated metabolic diseases is a global epidemic. The etiology of these disorders is multifactorial and the nutritional environment is one such contributing factor [1]. High-carbohydrate diets, especially those rich in sugars, promote lipid synthesis through induction of the *de novo* lipogenesis program, which is associated with unfavorable metabolic conditions such as hyperlipidemia and nonalcoholic fatty liver disease [2]. Fatty acids and lipids are commonly implicated in mediating inflammation and endoplasmic reticulum (ER) stress [3–6] resulting in metabolic disturbances such as insulin resistance (IR) and nonalcoholic fatty liver disease (NAFLD) [7], and genetic and high-fat diet fed mouse models of obesity have been shown to develop ER stress [8]. Additionally, the transcriptional coactivator peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*Ppagrc1a*; PGC-1α), which is a master regulator of mitochondrial biogenesis and lipid metabolism, has been linked to mediating ER stress in skeletal muscle in response to exercise [9]. However, its interplay with various fatty acid species in regulation of ER stress in response to other stimuli has not been reported.

Major tissue lipids such as triglycerides (TG), cholesterol esters (CE), phospholipids (PL), wax esters and nonesterified free fatty acids (FFA) possess essential biological functions including maintenance of membrane integrity, cellular signaling regulation, and energy storage, among others. Synthesis of these lipids requires saturated fatty acids (SFA) and unsaturated fatty acids, with monounsaturated fatty acids (MUFA) being particularly essential [10,11]. MUFA are synthesized endogenously from SFA by the stearoyl-CoA desaturase (SCD) family of enzymes, and the major products of SCD-catalyzed reactions are oleate (18:1n-9) and palmitoleate (16:1n-7). There are four known

Keywords: ER stress; Inflammation; Oleate; PGC-1α; Stearoyl-CoA desaturase.
Received 11 May 2015; received in revised form 24 February 2016; accepted 1 March 2016; available online 11 March 2016

* Corresponding author. Address: Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706, USA. Tel.: +1 6082399074.
E-mail address: ntambi@biochem.wisc.edu (J.M. Ntambi).
† These authors contributed equally as joint first authors.

Abbreviations: ER, endoplasmic reticulum; TG, triglycerides; CE, cholesterol esters; PL, phospholipids; FFA, free fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; SCD, stearoyl-CoA desaturase; GKO, global knockout; HSVLF, high-sucrose very low-fat; LKO, liver-specific knockout; GLS5, GKO liver-specific *SCD5*; GLS3, GKO liver-specific *Scd3*; HC, high sucrose, high-carbohydrate; WT, wild-type; TLC, thin layer chromatography; GLC, gas liquid chromatography; ALT, alanine aminotransferase; UPR, unfolded protein response; DLKO, double liver knockout; ROS, reactive oxygen species.

isoforms of SCD in mice (SCD1-4) and two known isoforms in humans (SCD1 and 5). Despite high dietary abundance of 18:1n-9, the expression of the ubiquitously expressed isoform SCD1 is highly regulated by developmental, dietary, hormonal, and environmental factors. Because of the involvement of MUFA in the regulation of diverse processes including signal transduction, cell differentiation and neuronal development, SCD1 is regarded as an important enzyme in the regulation of normal and patho-physiological processes [12–14].

While SFA are known to induce lipotoxicity, insulin resistance, liver stress and inflammation [15,16], the specific role of hepatic MUFA in regulation of liver function has not been thoroughly studied. We previously demonstrated that hepatic stress and inflammation are highly induced in SCD1 global knockout mice (GKO) fed a lipogenic high-sucrose very low-fat (HSVLF) diet despite dramatic protection against hepatic steatosis and adiposity induced by this high-carbohydrate diet [17]. To investigate the role specifically of hepatic MUFA in protection against high-carbohydrate diet-induced liver stress and inflammation, we utilized a model in which SCD1 liver knockout mice (LKO) were fed the HSVLF diet such that the exogenous MUFA supply and the endogenous capacity to induce MUFA synthesis were restricted. We demonstrate that liver-specific SCD1 deficiency is sufficient to induce hepatic ER stress and inflammation despite protection against diet-induced hepatic steatosis [18]. Mechanistically, using liver-specific transgenic desaturase mice in the SCD1 GKO background we demonstrate differential effects of endogenously synthesized hepatic oleate and palmitoleate, two MUFA species, in regulation of lipogenic diet-induced stress and inflammation. We also reveal a role for PGC-1α in mediating hepatic ER stress under high-carbohydrate feeding. Taken together, these results reveal that hepatic oleate, either through exogenous dietary supply or endogenous *de novo* synthesis, is essential in the prevention or resolution of hepatic stress and inflammation induced by a lipogenic diet.

Materials and methods

Animals and diets

All mice were of the C57BL/6 background. *Scd1*$^{lox/lox}$ (Lox) mice and *Scd1*$^{lox/lox}$; Albumin Cre/+ (*SCD1* LKO) mice were generated previously [18]. For studies on the *de novo* synthesis of oleate and palmitoleate, two liver-specific transgenic lines were generated by cloning either the human *SCD5* or mouse *Scd3* cDNA sequence into the pLiv.LE6 vector construct (a gift from John Taylor, Gladstone Institute). The *SCD5* and *Scd3* transgenes were crossed into the SCD1 GKO (*Scd1*$^{-/-}$) background to produce GKO liver-specific *SCD5* (GLS5) and *Scd3* (GLS3) transgenic mice, as previously described [19]. To study the role of PGC-1α in SCD1 LKO mice, we crossed *Ppargc1a*$^{lox/lox}$ mice (a gift from Dr. Bruce Spiegelman) with *Scd1*$^{lox/lox}$ mice to generate *Ppargc1a*$^{lox/+}$; *Scd1*$^{lox/+}$ mice, which were then bred to generate *Ppargc1a*$^{lox/lox}$; *Scd1*$^{lox/lox}$ mice. We then bred in the Cre recombinase allele, driven by the albumin promoter, to generate *Ppargc1a*$^{lox/lox}$; *Scd1*$^{lox/lox}$; Albumin Cre/+ mice, in which both PGC-1α and SCD1 are deleted only from the liver (double liver knockout mice, DLKO).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis with three or more groups was done using one-way ANOVA with Tukey's post hoc test, and the difference between two groups was tested by two-tailed, unpaired Student's *t* test. Significance was considered when $p < 0.05$.

For detailed experimental methods, please see the supplementary material.

Results

Hepatic MUFA levels are reduced in SCD1 LKO mice fed a high-sucrose very low-fat diet

Lox and SCD1 LKO mice were fed a standard chow diet or a lipogenic, HSVLF diet for 10 days to study the role of hepatic MUFA on liver function, including stress and inflammation in the context of a highly lipogenic dietary regimen. Among chow-fed Lox and SCD1 LKO mice there were no differences in the fatty acid composition of liver or plasma fatty acids (Fig. 1A–D). In HSVLF-fed mice however, the levels of oleate and palmitoleate, the major products of the SCD1 reaction, were lower in hepatic TG, FFA and CE fractions in SCD1 LKO as compared to Lox mice (Fig. 1A–C). Palmitate and stearate levels were not different in TG and FFA (Fig. 1A, B) but were significantly higher in the CE fraction in SCD1 LKO mice (Fig. 1C), likely due to a switch to SFA as substrates for cholesterol esterification to reduce the accumulation of free cholesterol. In plasma FFA, nonesterified oleate was significantly reduced in SCD1 LKO mice but there were no differences in SFA (Fig. 1D). Plasma albumin levels exhibited no genotypic difference (Supplementary Fig. 1), suggesting that any reductions in plasma fatty acids were not due to differences in fatty acid transport capacity. Overall, these data confirm that HSVLF-fed SCD1 LKO mice provide a model in which to study restricted hepatic MUFA availability, with dietary and endogenous supplies of MUFA to the liver significantly reduced in the absence of changes in SFA levels.

HSVLF-fed SCD1 LKO mice maintain normal MUFA levels in adipose tissue

We next analyzed epididymal white adipose tissue TG fatty acids. There were no significant genotypic differences in either MUFA or SFA under chow or HSVLF dietary conditions (Fig. 2A). Additionally, hepatic SCD1 deficiency did not alter *Scd1* mRNA or SCD1 protein levels in this tissue during HSVLF dietary feeding (Fig. 2B, C). The relative mRNA levels of other lipogenic genes such as *Srebf1*, *Acaca* and *Fasn* were also unchanged (Fig. 2B), suggesting that *de novo* fatty acid synthesis in adipose tissue of SCD1 LKO mice was not altered. Chow-fed Lox and SCD1 LKO mice also did not show differences in white adipose tissue lipogenic gene expression (data not shown). Thus, alterations in tissue fatty acid levels due to liver-specific SCD1 deficiency during a lipogenic diet are not extended to white adipose, and despite intact SCD1 expression in extrahepatic tissues, liver MUFA composition was not normalized.

SCD1 LKO mice fed HSVLF exhibit liver injury and inflammation

We previously reported that SCD1 GKO mice develop liver dysfunction with stress and inflammation when fed the lipogenic HSVLF diet for 10 days [17]. However, whether these phenotypes are specifically due to impaired hepatic MUFA synthesis is unknown. To address this issue, we assessed markers of stress and inflammation in HSVLF-fed SCD1 LKO mice. Plasma alanine aminotransferase (ALT) activity, a marker for liver damage, was increased nearly 20-fold in SCD1 LKO mice relative to Lox but was not altered in chow-fed SCD1 LKO mice (Fig. 3A). Necrotic foci were also evident in liver sections of SCD1 LKO mice but not in Lox or chow-fed SCD1 LKO mice (Fig. 3B). In parallel with