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Stearoyl-CoA desaturase 1 deficiency reduces lipid accumulation in the heart by activating lipolysis independently of peroxisome proliferator-activated receptor α

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ABSTRACT

Stearoyl-CoA desaturase 1 (SCD1) has recently been shown to be a critical control point in the regulation of cardiac metabolism and function. Peroxisome proliferator-activated receptor α (PPARα) is an important regulator of myocardial fatty acid uptake and utilization. The present study used SCD1 and PPARα double knockout ($SCD1^{-/-}$/$PPAR\alpha^{-/-}$) mice to test the hypothesis that PPARα is involved in metabolic changes in the heart that are caused by SCD1 downregulation/inhibition. SCD1 deficiency decreased the intracellular content of free fatty acids, triglycerides, and ceramide in the heart of $SCD1^{-/-}$ and $SCD1^{-/-}$/$PPAR\alpha^{-/-}$ mice. SCD1 ablation in $PPAR\alpha^{-/-}$ mice decreased diacylglycerol content in cardiomyocytes. These results indicate that the reduction of fat accumulation in the heart associated with SCD1 deficiency occurs independently of the PPARα pathway. To elucidate the mechanism of the observed changes, we treated HL-1 cardiomyocytes with the SCD1 inhibitor A939572 and/or PPARα inhibitor GW6471. SCD1 inhibition decreased the level of lipogenic proteins and increased lipolysis, reflected by a decrease in the content of adipose triglyceride lipase inhibitor G0S2 and a decrease in the ratio of phosphorylated hormone-sensitive lipase (HSL) at Ser565 to HSL (pHSL[Ser565]/HSL). PPARα inhibition alone did not affect the aforementioned protein levels. Finally, PPARα inhibition decreased the phosphorylation level of 5′-adenosine monophosphate-activated protein kinase, indicating lower mitochondrial fatty acid oxidation. In summary, SCD1 ablation/inhibition decreased cardiac lipid content independently of the action of PPARα by reducing lipogenesis and activating lipolysis. The present data suggest that SCD1 is an important component in maintaining proper cardiac lipid metabolism.

1. Introduction

The transcription factor peroxisome proliferator-activated receptor α (PPARα) is highly expressed in tissues that have a high capacity for fatty acid (FA) oxidation, including the liver, skeletal muscles, and the heart. PPARα activation promotes FA oxidation, ketone body synthesis, and glucose sparing [1]. PPARα-deficient mice exhibit lower rates of FA oxidation and consequently cardiac lipid accumulation [2], but they are protected from the development of diabetes-induced cardiac hypertrophy [3]. Cardiac-specific PPARα over-expression in mice has been shown to cause insulin resistance and an increase in FA oxidation in the heart [4]. Moreover, mice with cardiac-specific PPARα over-expression exhibit intracellular triglyceride (TG) and ceramide accumulation, which is associated with left ventricular hypertrophy and diastolic and systolic dysfunction [3,5]. Chronic PPARα activation in the heart in mice with cardiac-specific PPARα over-expression drives the nearly complete oxidation of intracellular TG-derived free FAs (FFAs) through greatly accelerated TG turnover rates. This mechanism of the preferential oxidation of intracellular TGs vs. exogenous FAs is driven at least partially by PPARα through regulation of the expression of enzymes

Abbreviations: ACC, Acetyl-CoA carboxylase; ACO, Acyl-CoA oxidase; AMPK, 5′-Adenosine monophosphate-activated protein kinase; ATGL, Adipose triglyceride lipase; BSA, Bovine serum albumin; CPT1, Carnitine palmitoyltransferase 1; DAG, Diacylglycerol; FA, Fatty acid; FFA, Free FA; FAS, Fatty acid synthase; FATP1, Fatty acid transport protein 1; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; G0S2, G0/G1 switch protein 2; HSL, Hormone-sensitive lipase; PPARα, Peroxisome proliferator-activated receptor α; SCD1, Stearoyl-CoA desaturase 1; SREBP-1, Sterol regulatory element-binding protein 1; TG, Triglyceride.

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that determine the rates of TG synthesis and lipolysis [6]. Adipose triglyceride lipase (ATGL) catalyzes the rate-limiting step in TG hydrolysis in the heart, increasing the activity of PPARα to promote FA oxidation [7]. These findings underscore the important role that PPARα plays in the regulation of lipid metabolism in the heart and development of lipotoxic cardiomyopathy.

Recent studies have shown that stearoyl-CoA desaturase 1 (SCD1), an enzyme that is involved in the biosynthesis of monounsaturated FAs, induces the reprogramming of cardiomyocyte metabolism, thereby playing an important role in the regulation of cardiac function [8–10]. The lack of SCD1 expression decreases FA uptake and oxidation and increases glucose transport and oxidation in the heart [8]. Disruption of the SCD1 gene improves cardiac function in obese leptin-deficient ob/ob mice by correcting systolic and diastolic dysfunction [9]. This improvement is associated with a reduction of the expression of genes that are involved in FA transport and lipid synthesis within the heart, together with decreases in cardiac FFA, diacylglycerol (DAG), TG, and ceramide levels and a reduction of cardiomyocyte apoptosis [9]. Additionally, recent studies have shown that physiological hypertrophy that is induced by endurance training is accompanied by higher expression of SCD1 and SCD2 [10].

The metabolic changes that are observed in SCD1-deficient mice include significant decreases in the expression of PPARα and its target genes (i.e., carnitine palmitoyltransferase 1 [CPT1] and acyl-CoA oxidase [ACO]) [8]. The downregulation of PPARα activity in the SCD1-deficient heart is likely caused by a 30% reduction of intracellular polyunsaturated FA content [8], which is one of the main regulators of PPARα expression [11]. Moreover, dietary or de novo-synthesized oleate by SCD increased the expression and activity of PPARα in the heart [12]. The endogenously produced monounsaturated lipid oleoylethanolamide increased epididymal adipose tissue lipolysis in a PPARα-dependent manner [13]. Furthermore, palmitoleic acid, a monounsaturated n − 7 fatty acid (16:1n7) that is synthesized by the desaturation of palmitic acid catalyzed by SCD1, has been shown to act systemically in peripheral tissues to modulate important metabolic processes through a mechanism that requires functional PPARα [14]. Palmitoleic acid increases PPARα binding to its DNA consensus sequence (PPRE), which is indicative of PPARα activation [14].

The objective of the present study was to explore the relationships between SCD1 and PPARα in the control of lipid metabolism processes in the heart. To test the hypothesis that PPARα is involved in the reduction of lipid content that is observed in the heart in SCD1$^{-/-}$ mice, we generated SCD1 and PPARα double-knockout (SCD1$^{-/-}$/PPARα$^{-/-}$) mice and murine HL-1 cardiomyocyte models with inhibited SCD1 and/or PPARα activity. We showed that SCD1 deficiency decreased lipid accumulation by regulating lipogenesis and lipolysis in cardiomyocytes independently of PPARα.

2. Materials and methods

2.1. Materials

CPT1, fatty acid synthase (FAS), fatty acid transport protein 1 (FATP1), G0/G1 switch protein 2 (G0S2), hormone-sensitive lipase (HSL), and sterol regulatory element-binding protein 1 (SREBP-1) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ATGL, 5′-adenosine monophosphate-activated protein kinase (AMPK), phosphorylated AMPK at Thr172 (pAMPK), phosphorylated HSL at Ser563 (pHSL[Ser563]), phosphorylated HSL at Ser565 (pHSL[Ser565]), phosphorylated acetyl-CoA carboxylase at Ser79 (pACC), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were obtained from Cell Signaling (Hartsfordshire, UK). Horseradish peroxidase-conjugated streptavidin was purchased from Pierce (Rockford, IL, USA). The other chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

2.2. Animals

The PPARα$^{-/-}$ mice were a generous gift from Frank J. Gonzalez (National Cancer Institute, Bethesda, MD, USA). The generation of SCD1$^{-/-}$ mice on a 129S6/SvEv background and PPARα$^{-/-}$ mice on a 129/Sv background was described previously [15,16]. The animals were individually housed in a pathogen-free facility at room temperature under a 12 h/12 h light/dark cycle and fed a standard chow diet (Purina Formulab 5008). All of the animals were allowed ad libitum access to water and food. The animals were sacrificed at 16 weeks of age. The left ventricle of the heart was excised and frozen in liquid nitrogen. All of the studies were approved by the Animal Care Research Committee of the University of Wisconsin, Madison.

2.3. Blood and tissue sampling

The mice were fasted for 16 h and sacrificed by CO_2 asphyxiation and/or cervical dislocation. Blood was collected aseptically by direct cardiac puncture and centrifuged at 13,000 × *g* at 4 °C for 5 min to collect plasma. Plasma cholesterol and TG levels were measured using commercial kits (Roche Applied Science, Indianapolis, IN, USA). Plasma FFA levels were measured using the NEFA-HR(2) Kit (Wako, Richmond, VA, USA). Retroperitoneal, reproductive, mesenteric, and subcutaneous fat pads were used to determine total fat pad content.

2.4. Culture of HL-1 cardiomyocytes

HL-1 cardiomyocytes were obtained from Dr. W.C. Claycomb (Louisiana State University, New Orleans, LA, USA). Cells were cultured on a gelatin (0.02% [wt/vol])/fibronectin (10 μg/ml) matrix and maintained in Claycomb medium supplemented with 10% (vol/vol) fetal bovine serum, 2 mM/l glutamine, 0.1 mM/l norepinephrine, 100 U/ml penicillin, and 100 U/ml streptomycin [17]. To evaluate the effects of SCD1 and/or PPARα inhibition on lipid metabolism, the cells were preincubated with 2 μM of the SCD1 inhibitor A939572 (Biofine International, Blain, WA, USA) and/or 1 μM of the PPARα inhibitor GW6471 for 4 h and then co-supplemented with 0.2 mM 18:0-bovine serum albumin (BSA) conjugate for 16 h.

2.5. Isolation and analysis of RNA

Total RNA was isolated from mice left ventricle and from HL-1 cells using TRIzol reagent (Life Technologies, Carlsbad, CA, USA). DNase-treated RNA was reverse-transcribed with SuperScript III (Life Technologies), and real-time quantitative polymerase chain reaction (PCR) was performed using an ABI Prism 7900 HT Fast Instrument. Fast SYBR green (Thermo Scientific, Pittsburgh, PA, USA) was used for the detection and quantification of genes that were expressed as mRNA, and the level was normalized to β-actin using the ΔΔCt method.

2.6. Western blot

HL-1 cells were collected and lysed for 30 min in ice-cold buffer (50 mM Tris-HCl [pH 7.4], 5 mM ethylenediaminetetraacetic acid [EDTA], 1% Triton X-100, and 150 mM NaCl) that contained protease (10 μg/μl leupeptin, 5 μg/μl pepstatin A, 2 μg/μl aprotinin, and 1 mM phenylmethylsulfonyl fluoride) and phosphatase (1 mM sodium orthovanadate and 10 mM sodium fluoride) inhibitors. After centrifugation at 12,000 × *g* at 4 °C for 15 min, the supernatants were used as whole-cell lysates for further analyses. The left ventricle samples from SCD1$^{-/-}$ and wildtype mice were homogenized and centrifuged at 10,500 × *g* for 20 min in ice-cold 50 mM HEPES buffer (pH 7.4) that contained 150 mM NaCl, 10 mM sodium pyrophosphate, 2 mM Na_3VO_4, 10 mM NaF, 2 mM EDTA, 2 mM phenylmethane sulfonyl fluoride, 5 μg/ml leupeptin, 1% Nonidet P-40, and 10% glycerol. The protein content was determined using the Bio-Rad Protein Assay (Bio-Rad,