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Global deletion of lipocalin 2 does not reverse high-fat diet-induced obesity resistance in stearoyl-CoA desaturase-1 skin-specific knockout mice

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ABSTRACT

Over the past century, obesity has developed into a paramount health issue that affects millions of people worldwide. Obese individuals have an increased risk to develop other metabolic disorders, such as insulin resistance and atherosclerosis, among others. Previously we determined that mice lacking stearoyl-CoA desaturase-1 (SCD1) enzyme specifically in the skin (SKO) were lean and protected from high-fat diet induced adiposity. Additionally, lipocalin 2 (Lcn2) mRNA was found to be 27-fold higher in the skin of SKO mice compared to control mice. Given reports suggesting that Lcn2 plays a role in protection against diet-induced weight gain, adiposity and insulin resistance, we hypothesized that deletion of Lcn2 alongside the skin-specific SCD1 deficiency would diminish the obesity resistance observed in SKO mice. To test this, we developed mice lacking SCD1 expression in the skin and also lacking Lcn2 expression globally and surprisingly, these mice did not gain significantly more weight than the SKO mice under high-fat diet conditions. Therefore, we conclude that Lcn2 does not mediate the protection against high-fat diet-induced adiposity observed in SKO mice.

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1. Introduction

Stearoyl-CoA desaturase 1 (SCD1) is a Δ9 desaturase that catalyzes the formation of *cis*-monounsaturated fatty acids (MUFA) from saturated fatty acids. Palmitic acid (16:0) and stearic acid (18:0) are the preferred substrates of SCD1 and their desaturation yields palmitoleic acid (16:1n7) and oleic acid (18:1n9), respectively [1]. In mice, SCD1 is expressed at relatively high levels in skin and white adipose tissue [2]. Within the skin, in situ hybridization experiments demonstrated that SCD1 is expressed exclusively in the sebaceous gland [3]. Mice with a naturally occurring mutation or targeted deletion of the SCD1 gene (GKO) exhibit a severe deleterious skin phenotype that includes alopecia, dysfunctional sebaceous glands, atrophic meibomian glands and narrow eye fissures [3] [2]. In addition, GKO mice have increased energy expenditure, resistance to genetic and diet-induced adiposity, as well as protection against the development of hepatic steatosis and insulin resistance [4,5].

Due to the complex and extensive phenotypes of GKO mice and to understand the tissue-specific roles of SCD1, we generated a number of tissue-specific SCD1 knockout mouse models using Cre-loxP technology. We have subsequently demonstrated that while skin-specific knockout mice (SKO) are resistant to high-fat diet (HFD) induced obesity and associated metabolic disorders [6], liver-specific knockout, adipose-specific knockout and liver/adipose double knockout mice are not [7,8]. In addition, SKO mice exhibit all of the hair, skin and eye phenotypes of GKO mice [6]. Taken together, the results from these metabolic studies have emphasized the importance of skin SCD1 in mediating protection against HFD-induced adiposity. In follow-up experiments, we discovered that retinol metabolism is dysregulated in the skin of

Abbreviations: DKO, Lcn2−/−;SCD1 SKO; HFD, high-fat diet; SCD1, stearoyl-CoA desaturase 1; GKO, SCD1 global knockout; MUFA, monounsaturated fatty acids; RAR, retinoic acid receptor; SKO, SCD1 skin knockout; TG, triglycerides; WAT, white adipose tissue.

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SKO mice, as retinol, retinoic acid and retinyl esters were all significantly increased, and microarray analysis of skin mRNA revealed that the altered retinol metabolism in SKO mice is associated with upregulated expression of retinoic acid receptor (RAR) target genes [9]. Lipocalin 2 (*Lcn2*) was one of the most highly induced genes in skin of SKO mice identified by microarray analysis and has been shown by others to be upregulated in human sebocytes upon retinoic acid treatment [10]. Prolonged treatment (72 h) of human SEB-1 cells with retinoic acid and A939572, an SCD1 inhibitor, resulted in 9-fold upregulation in Lcn2 mRNA and protein and similar upregulation in other retinoic acid-induced target genes; these changes in expression and protein synthesis implicate SCD1 as an important mediator of retinol metabolism, at least *in vitro* [9]. In SKO mice, retinoic acid-regulated genes were significantly upregulated in the skin within 23 days of birth despite the fact that skin SCD1 expression typically remains low in the skin until around 8 days after birth. These early changes demonstrated that irregularity in skin retinoic acid metabolism precede adulthood and suggest the possibility that poorly controlled retinoic acid levels may underlie the systemic metabolic changes observed in SKO mice [9].

Lcn2 is a 25 kDa secreted protein [11] and a member of the lipocalin family of proteins that bind and transport a number of small molecules [12]. Lcn2 has been shown to have low binding affinity for retinoic acid [13] while it is unresolved whether Lcn2 binds retinol [13,14]. Lcn2 also binds iron-containing siderophores and possesses anti-microbial properties [12]. In mouse models, Lcn2 has been proposed to play a prominent role in obesity-associated inflammation as well as related metabolic phenotypes including insulin sensitivity and level of adiposity, although the directionality of the involvement of Lcn2 in these events remains unclear. Results from Lcn2 knockout mouse models have been conflicting, where Lcn2 has been demonstrated to promote [15], protect against [16] or have minimal effect on insulin resistance [17]. In addition to effects on insulin sensitivity, Guo et al. also demonstrated that Lcn2 knockout mice have significantly greater body weight and adipose tissue weight as compared to wild type mice [16].

With the dramatic 27-fold upregulation in skin Lcn2 in SKO mice [9] and the suggested role of Lcn2 in protecting against diet-induced weight gain, adiposity and insulin resistance, we sought to determine if Lcn2 might mediate the metabolic state of SKO mice. To address our objective, we crossed Lcn2 knockout mice with SKO mice and fed a high-fat diet (HFD) for up to 15 weeks. The results from this study reveal that Lcn2 does not mediate the protection against HFD-induced weight gain, adiposity, hepatic steatosis and glucose tolerance in SKO mice.

2. Materials and methods

2.1. Animals and diets

SCD1$^{flox/flox}$ (Lox) mice were generated as described previously [7] and were used as control mice for all experiments. Skin-specific SCD1 knockout mice (SKO) were generated by crossing Lox mice with transgenic mice expressing Cre recombinase under the control of the human keratin 14 promoter, as previously described [6]. Lcn2−/− mice were previously described [16]. To generate mice with global deficiency of Lcn2−/− and skin-specific deficiency of SCD1 (double knockout mice, DKO), Lcn2−/− mice were crossed with Lox mice to generate Lcn2$^{+/-}$;SCD1$^{flox/+}$. Male and female Lcn2$^{+/-}$;SCD1$^{flox/+}$ mice were then crossed to generate Lcn2$^{-/-}$;SCD1$^{flox/flox}$ mice. Subsequently, male SKO mice were crossed with female Lcn2$^{-/-}$;SCD1$^{flox/flox}$ mice to generate Lcn2$^{+/--}$;SCD1$^{flox/flox}$;Ker-Cre/+ mice. DKO mice were then generated by crossing male Lcn2$^{+/-}$;SCD1$^{flox/flox}$;Ker-Cre/+ mice with female Lcn2$^{-/-}$;SCD1$^{flox/flox}$ mice.

All mice were maintained on a 12 h light–dark cycle (6PM–6AM) and had free access to food and water unless specified otherwise. Breeders were fed Purina 5015 diet. Offspring were weaned at 21 days and fed a chow diet (Purina #5008). Mice were individually caged at 7 weeks of age and fed a lard-based, high-fat diet (HFD; 60% kcal from fat; Research Diets #D12492) starting at 8 weeks of age. Male mice were fed the HFD for 10–15 weeks while females were fed the diet for 15 weeks. All mice were nonfasted at euthanization by isoflurane overdose. Blood was collected via cardiac puncture, tissues were weighed and immediately snap-frozen in liquid nitrogen and stored at −80 °C. All experimental animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

2.2. Western blot analysis

Whole tissue lysate from liver and microsomal protein from skin was used for immunoblot analysis of Lcn2, SCD1 and GAPDH or CREB as loading controls. Whole tissue was homogenized in RIPA buffer and protein was quantified by BCA protein assay (Pierce). Microsomal protein was prepared as previously described [8]. For immunoblots, 50 μg of protein per sample was separated by 12% SDS–PAGE and transferred to PVDF membrane (Millipore). Membranes were incubated overnight with anti-SCD1 (Santa Cruz Biotechnology, #sc-14720), Lcn2 (R & D Systems), GAPDH or CREB (Santa Cruz Biotechnology, #sc-58) primary antibodies, followed by IgG-horseradish peroxidase-conjugated secondary antibody. Proteins were visualized with ECL Prime Detection Reagent (Amersham).

2.3. Quantitative real-time PCR

Liver total RNA was extracted using TRI reagent. Subsequently, total RNA was treated with Turbo DNase (Ambion) and then reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Relative mRNA expression levels were quantified by cDNA amplification with gene-specific forward and reverse primers and Power SYBR Green PCR Master Mix on an ABI 7500 Fast RT PCR system. Data were normalized to Arbp using the ΔΔCt method. Primer sequences available upon request.

2.4. Hepatic triglyceride analysis

Total lipids were extracted from 10 mg liver tissue. Liver TG were measured using a colorimetric enzymatic assay (Wako Chemicals, USA).

2.5. Glucose tolerance tests

Glucose tolerance tests were performed with male mice after 9 weeks of HFD consumption. Lox, SKO and DKO mice were dosed with a 20% glucose solution at 2 g/kg body weight via intraperitoneal injection. Blood was collected from the tail vein and glucose was measured at 0, 30, 60, 90 and 120 min post-injection using a blood glucose meter and glucose test strips (One Touch Ultra, Diabetic Express).

2.6. Statistical analyses

All results are expressed as mean ± SEM. Variables that did not follow a Gaussian distribution were log transformed for statistical

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