Diet-dependent Alterations of Hepatic Scd1 Expression are Accompanied by Differences in Promoter Methylation

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Key words
- obesity
- lipid metabolism
- epigenetic gene regulation
- DNA methylation

Abstract
Obesity and alterations of lipid homeostasis are hallmarks of the metabolic syndrome and largely influenced by the dietary conditions of the individual. Although heritability is considered to be a major risk factor, the almost 40 candidate genes identified by genome-wide association studies (GWAS) so far account for only 5–10% of the observed variance in BMI in human subjects. Alternatively, diet-induced changes of epigenetic gene regulation might be involved in disturbed lipid homeostasis and weight development. The aim of this study was to investigate how a high-carbohydrate diet (HCD; 70 kcal % from carbohydrates, 10 kcal % from fat) or a high-fat diet (HFD; 20 kcal % from carbohydrates, 60 kcal % from fat) affects hepatic expression of genes involved in fatty acid metabolism and if these alterations are correlated to changes in promoter methylation. Expression of stearoyl-CoA desaturase 1 (Scd1) was lower in livers from HFD-fed C57BL/6J mice compared to HCD-fed animals and correlated inversely with the degree of DNA methylation at 2 distinct, adjacent CpG sites in the Scd1 promoter. In contrast, expression of transcription factors peroxisome proliferator activated receptor alpha and gamma (Ppara, Pparg), and sterol regulatory element binding transcription factor 1 (Srebf1) was not affected. The degree of hepatic Scd1 promoter methylation at these CpG sites correlated positively to fat mass and serum leptin levels, whereas serum ghrelin levels were inversely correlated with methylation at both CpG sites. Taken together, hepatic expression of Scd1 is differentially affected by carbohydrate- and lipid content of the diet. These differences in Scd1 expression are associated with altered promoter methylation, indicating that diets affect lipid metabolism in the liver via epigenetic mechanisms.

Introduction
Obesity is a central factor in pathologies of the metabolic syndrome and associated with increased risk for insulin resistance and type 2 diabetes [1]. A hallmark of obesity is the ectopic deposition of lipids in nonadipose tissues like the liver and skeletal muscle [2,3]. In the liver, accumulation of lipids and lipid metabolites interferes with insulin sensitivity, leading to increased hepatic glucose production even in the presence of increased insulin levels [2]. Furthermore, nonalcoholic fatty liver disease (NAFLD) is the first step on the route to nonalcoholic steatohepatitis (NASH) and eventually liver cirrhosis [4]. In order to identify the genes associated to an increased risk for obesity, numerous genome wide association studies (GWAS) have been conducted. So far, several single nucleotide polymorphisms (SNPs) in genes like FTO, MC4R, and LEPR have been identified that are associated to fat mass expansion and neuronal regulation of food intake [5]. The drawback, however, was that the sum of these polymorphisms accounts for only a minor part of the variation found in BMI as a measure for obesity. Recent studies suggest that epigenetic regulation of gene expression (DNA methylation and histone modifications) could be a major contributor to the variation of susceptibility to diseases like obesity and type 2 diabetes. For example, epigenetic mechanisms could be the reason why C57BL/6 mice display vast variability in gene expression in response to a high-fat diet (HFD), although being genetically identical [6]. In humans, methylation of the peroxisome proliferator activated receptor gamma coactivator-1 alpha (PGC-1α) promoter in skeletal muscle and pancreatic islets has been correlated to impaired glucose tolerance and type 2 diabetes [7,8]. In regard to these observations, it is hard to distin-
guish between inherited and acquired methylation marks because endogenous factors like nutrition and exercise can affect the methylation of multiple genes involved in glucose and lipid metabolism during the lifetime (reviewed in [9]). For example, studies in rats revealed that high-fat feeding interferes with hepatic glucose metabolism by methylation and repression of the genes coding for glucokinase and L-type pyruvate kinase [10]. Besides alterations in glucose metabolism, accumulation of lipids in the liver is an early event in the onset of diet-induced insulin resistance and obesity. Therefore, we aimed at investigating the effects of diet on epigenetic regulation of genes involved in fatty acid oxidation and storage.

A major regulator of lipid storage in liver and adipose tissue is the enzyme stearoyl-CoA desaturase-1 (Scd1), which introduces a double bond into saturated fatty acids to produce monounsaturated fatty acids (MUFAs) [11]. This generation of MUFAs is important for the generation of triglycerides and complex lipids, but also involved in cellular signaling, differentiation, and apoptosis [12,13]. In Scd1−/− mice lipogenesis in the liver is reduced in favor of increased beta-oxidation, suggesting an important role of Scd1 in diet-induced obesity [14]. Interestingly, liver-specific knockdown of Scd1 resulted in a protection from carbohydrate-induced, but not from high-fat diet-induced obesity and hepatic steatosis [15]. Also the combined knockdown of Scd1 in liver and adipose tissue did not protect the mice from high-fat diet-induced obesity [16]. Together, these data indicate a complex regulation of Scd1 expression and function by dietary factors, particularly carbohydrates. This complexity is further illustrated by conflicting observations regarding high-fat diet-dependent regulation of Scd1 expression. While some researchers found an increase in hepatic Scd1 expression upon feeding mice a HFD [17,18], others reported a reduced expression of Scd1 [19]. A human study with obese subjects even found a positive correlation of hepatic Scd1 expression to insulin sensitivity, and a negative correlation to liver fat, suggesting a protective function of Scd1 during obesity [20].

In this study, we report diet-dependent differences in DNA methylation of the hepatic Scd1 promoter that correlate to Scd1 expression and body weight, indicating a novel mechanism of diet-dependent regulation of Scd1 expression and, thus, fatty acid metabolism.

Materials and Methods

Animals and diets
Male C57BL/6J mice from The Jackson Laboratory (Bar Harbor, USA) were housed in individually-ventilated cages at a temperature of 21 ± 1°C with a 12-h light-dark cycle (lights on at 6 AM). After weaning at day 21, the animals received either a high-carbohydrate diet (HCD, 20 kcal% from protein, 70 kcal% from carbohydrate, and 10 kcal% from fat, order number D12450B) (n = 8) or a high-fat diet (HFD, 20 kcal% from protein, 20 kcal% from carbohydrate and 60 kcal% from fat, order number D12492) (n = 8) both from Research Diets (New Brunswick, USA). The detailed diet composition is depicted in Table 1 and Fig. 1. Upon 18 weeks on these diets, the animals were sacrificed for tissue preparation and blood collection after a 6-h fasting period. Blood glucose was determined with an Ascensia ELITE XL glucose meter (Bayer Health Care, Leverkusen, Germany). Body composition (fat and lean mass) was measured by nuclear magnetic resonance with an EchoMRI Whole Body Composition Analyzer (Echo Medical Systems, Houston, TX, USA) as previously described [21]. The mice were housed and handled in accordance with good animal practice as defined by FELASA (www.felasa.eu/guidelines.php) and the national animal welfare body GV-SOLAS (www.gv-solas.de/index.html). All animal experiments were approved by the ethics committee of the State Agency of Environment, Health and Consumer Protection (State of Brandenburg, Germany).

RNA preparation, cDNA synthesis, and qRT-PCR
Total RNA from liver tissue of mice was extracted, and cDNA synthesis was performed as described previously [22]. For quantitative real-time PCR (qRT-PCR), a 7500 Fast real-time PCR system from Applied Biosystems (with 7500 software, version 2.0.1; Darmstadt, Germany) was used and TaqMan gene expression assays (Applied Biosystems) were applied. TaqMan gene expression assays are identified in Table 2. Data were normalized using the 2−ΔΔCT method and Snap23 as an endogenous control.

Western blot analysis
Liver tissue was homogenized in RIPA buffer with a TissueLyser (QIAGEN, Hilden, Germany). After centrifugation for 30 min at

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Table 1: Composition of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>HCD (D12450B)</th>
<th>HFD (D12492)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>67.3%</td>
<td>26.3%</td>
</tr>
<tr>
<td>Fat</td>
<td>4.3%</td>
<td>34.9%</td>
</tr>
<tr>
<td>Protein</td>
<td>19.2%</td>
<td>26.2%</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>20%</td>
<td>245%</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>25%</td>
<td>225%</td>
</tr>
<tr>
<td>Lard*</td>
<td>20%</td>
<td>180%</td>
</tr>
<tr>
<td>Mineral mix S10026</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>13%</td>
<td>13%</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Potassium citrate, 1 H2O</td>
<td>16.5%</td>
<td>16.5%</td>
</tr>
<tr>
<td>Vitamin mix V10001</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

* Typical analysis of cholesterol in lard = 0.95 mg/g. Cholesterol (mg)/4057 kcal = 19. Cholesterol (mg)/kg = 18

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Schwenk RW et al. Scd1 Expression and Promoter Methylation … Horm Metab Res 2013; 45: 786–794
18,000×g and 4°C, supernatants were collected. Proteins (15 μg) from the supernatants were separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. For immunchemical detection, membranes were incubated with primary antibodies against mouse SCD1 (sc-14719 from Santa Cruz, Dallas, USA) and AKT (# 9272 from Cell Signaling, Danvers, USA) for 16 h at 4°C, and subsequently with corresponding HRP-linked secondary antibodies (Dianova, Hamburg, Germany) for 1 h at room temperature.

Isolation of genomic DNA and bisulfite sequencing PCR (BSP)
DNA was isolated using the Invisorb Genomic DNA Kit II from Stratatec (Berlin, Germany) according to manufacturer’s instructions. Bisulfite conversion of gDNA was performed using the Cells-to-CpG Kit from Applied Biosystems (Carlsbad, USA) following the manufacturer’s instructions. Briefly, 1 μg of genomic DNA was mixed with Denaturation Reagent and incubated at 50°C for 10 min. Upon addition of bisulfite-containing Conversion Reagent, the nonmethylated cytosins of the DNA were converted to thymine using a thermal cycler and multiple heating (95°C) and cooling (65°C) steps. After desulfonation of the samples, the DNA was washed and purified. Subsequently, the bisulfite-converted DNA (bsDNA) was subjected to PCR using bisulfite-specific primers (Table 3). Upon purification with the QIAquick PCR Purification Kit from Qiagen (Hilden, Germany) and sequencing PCR using the BigDye® Terminator v3.1 Ready Reaction Mix from Applied Biosystems, the samples were sequenced on a 3130 xl sequencer (Applied Biosystems). For quantification of the degree of methylation per CpG in each PCR fragment, the peak height of cytosine (ph_c) was divided by the sum of the cytosine and thymine (ph_c + th) and multiplied by 100 (ph_c/ph_c + th × 100) [23].

Serum levels of leptin, ghrelin, and insulin
Blood samples were collected after a 6-h fasting period at time of sacrifice from vena cava and immediately chilled on ice. After 15 min of centrifugation at 8000×g and 4°C, serum was collected and stored at −80°C. Serum leptin levels were measured using a murine ELISA kit from DRG International (Mountainside, USA). For ghrelin measurements blood samples were immediately centrifuged after the addition of AEBSF and EDTA. Plasma was collected and acidified with aqueous 1 N HCl. For quantification of total ghrelin levels a commercially available RIA for Rat/Mouse from Millipore (Billerica, USA) was used. Active ghrelin was measured using the Rat/Mouse Ghrelin ELISA from Milli-
pore. Serum insulin levels were measured with the Mouse Ultraselective Insulin ELISA kit (ALPCO, Salem, USA). All assays were performed according to the manufacturer’s instructions.

Statistics
All data are presented as means ± SEM. Statistical analysis was performed by Student’s t-test or linear regression analysis using the software Prism 5 from GraphPad Software (La Jolla, USA). Significance levels were set for p-values of less than 0.05 (*), 0.01 (**) and 0.001 (***)

Results

Carbohydrate and fat content of diet modifies expression of genes involved in hepatic lipid metabolism
In order to investigate the effect of diet composition on hepatic fatty acid metabolism, male C57BL/6j mice received either a high-carbohydrate diet (HCD) or a high-fat diet (HFD) for 18 weeks. Animals receiving HCD had a significantly lower lean and fat mass compared to the animals fed the HFD (Table 4). While liver weight did not differ between the groups, total amounts of liver triglycerides were elevated in the HFD group, but did not reach statistical significance. The amounts of dietary carbohydrates and fatty acids largely influence lipid metabolism and might contribute to differential methylation and expression of genes involved in fatty acid metabolism. Therefore, we first investigated the expression of genes involved in fatty acid oxidation [acyl-CoA synthetase long-chain family member 1 (Acaca)], malonyl-CoA decarboxylase (Mlycd) and carnitine palmitoyltransferase 1a (Cpt1a) and fat storage [acetyl-Coenzyme A carboxylase alpha (Acaca), fatty acid synthase (Fasn), and stearoyl-CoA desaturase 1 (SCD1)] (Fig. 2a). From these genes, Scd1 expression was elevated in livers of the HCD group compared to the HFD group (p<0.001) (Fig. 2b). Western blot analysis confirmed this observation; SCD1 protein levels were higher in livers of mice on the HCD than in livers of the HFD group (Fig. 2c). Since Scd1 expression is regulated by the lipogenic transcription factor sterol regulatory element binding protein-1c (SREBP-1c, Srebf1) [24] we analyzed the mRNA levels of different key transcription factors involved in lipid metabolism. Interestingly, we did not find any differences in expression of peroxisome proliferator activated receptor alpha and gamma (Ppara, Pparg), or sterol regulatory element binding transcription factor 1 (Srebf1) (Fig. 2d), indicating that hepatic Scd1 expression is not regulated by differential expression of its key transcription factor Srebf1 in response to a high-carbohydrate diet, but rather by other mechanisms.

Altered CpG methylation in the Scd1 promoter in liver tissue
Exogenous factors like environmental stress and nutrition can affect DNA methylation and persistently influence expression of genes involved in whole body energy homeostasis [25,26]. Therefore, we tested if the differential hepatic Scd1 expression in HCD versus HFD-fed mice was associated to differential methylation in its promoter region. Using MethPrimer software [27] we depicted representative CpG-enriched fragments (CpG islands) in the promoter region of Scd1 (Fig. 3a). Next, we

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCD</th>
<th>SEM</th>
<th>HFD</th>
<th>SEM</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>28.4</td>
<td>1.22</td>
<td>42.8</td>
<td>0.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lean mass (g)</td>
<td>22.8</td>
<td>1.01</td>
<td>29.5</td>
<td>0.49</td>
<td>0.0003</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>7.2</td>
<td>0.52</td>
<td>18.1</td>
<td>0.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.19</td>
<td>0.072</td>
<td>1.27</td>
<td>0.069</td>
<td>0.4146</td>
</tr>
<tr>
<td>Liver triglycerides (μg)</td>
<td>0.39</td>
<td>0.10</td>
<td>0.89</td>
<td>0.28</td>
<td>0.075</td>
</tr>
</tbody>
</table>
investigated if methylation of single CpGs in these islands was different between the 2 groups. Three fragments containing 4 CpG islands in the promoter region of Scd1 were amplified for bisulfite sequencing (Fig. 3a). In the first CpG island (position –916 to –614 relative to transcription start site (TSS)), cytosine methylation at position –838 and –833 was higher (1.3- and 1.5-fold, respectively, p < 0.001) in the HFD-fed group (Fig. 3b). In the second CpG island (–614 to –230 bp relative to TSS) there was a general low methylation level that was not different between the diet groups (Fig. 3c). In the third and fourth CpG island (+105 to +483 bp relative to TSS), a single cytosine (position +384) in the fourth CpG island was 2.1-fold (p < 0.05) higher in the HFD group (Fig. 3d).

Methylation of CpG –838 and CpG –833 negatively correlated with the expression of Scd1 in the livers of all mice (R² = 0.378 and 0.454, respectively) (Fig. 4a, b). Furthermore, methylation of CpG –838 and CpG –833 correlated positively with body weight, but stronger with fat mass (R² = 0.549 and 0.554, respectively) than with lean mass (R² = 0.335 and 0.321, respectively). Methylation of CpG +384 did not correlate with expression of Scd1 (R² = 0.006) (Fig. 4c). Accordingly, it displayed no correlation to lean and fat mass (R² = 0.259 and 0.284, respectively) (Fig. 4c). Taken together, Scd1 gene expression and body weight development is largely correlated to methylation at 2 distinct CpG sites in the liver Scd1 promoter.

Serum leptin and ghrelin levels correlate to methylation of the hepatic Scd1 promoter

The anorexigenic hormone leptin is known to inhibit Scd1 expression, while its counterpart the orexigenic hormone ghrelin is an activator of Scd1 gene expression [17,28]. Furthermore, insulin has been shown to enhance Scd1 expression via activation of SREBP-1c [29]. Because (i) hepatic Scd1 expression correlated with fat mass and (ii) leptin is produced and secreted by the adipose tissue, we tested if serum leptin levels correlate to the degree of CpG –838 and CpG –833 methylation. As expected,
serum leptin and insulin levels of the HCD-fed group were low compared to the HFD-fed group (p < 0.01) (Fig. 5a), indicating impaired insulin sensitivity in the HFD group. On the other hand, levels of total ghrelin, but not active ghrelin, were higher in the HFD group compared to the HFC group (p < 0.05) (Fig. 5a). Serum leptin levels correlated positively to methylation of CpG–838 ($R^2 = 0.4819$) (Fig. 5b) and CpG–833 ($R^2 = 0.470$) (Fig. 5c), and ghrelin levels correlated negatively to methylation of both CpG sites ($R^2 = 0.392$ and 0.424, respectively) (Fig. 5b, c). In contrast to leptin and ghrelin, there was no correlation of serum insulin levels to methylation at any of the 2 CpG sites ($R^2 = 0.1193$ and 0.1632, respectively) (Fig. 5a). Taken together, serum leptin and ghrelin levels, affected by dietary carbohydrate and fat content, are indicative for the methylation and expression of hepatic Scd1.

### Discussion

Hepatic expression of genes involved in fatty acid metabolism is differentially affected by dietary content of carbohydrates, saturated and unsaturated fatty acids. Amongst these genes we found Scd1, the gene coding for stearoyl-Coenzyme A desaturase 1, to be substantially weaker expressed in livers from animals that were fed a HFD for 18 weeks, compared to the HCD-fed group. The differences in Scd1 expression were independent of mRNA levels of the transcription factors Ppara, Pparg, and Srebf1. However, the magnitude of Scd1 expression was inversely correlated to the degree of methylation of CpG–838 and CpG–833 in the Scd1 promoter, suggesting that methylation of these 2 CpG sites is involved in the regulation of Scd1 expression by macronutrient composition of the diet. This hypothesis was further strengthened by the observation that total body weight (especially fat mass) is also highly correlated with Scd1 promoter methylation and expression. A possible link between fat mass and Scd1 promoter methylation is leptin, which is known to inhibit Scd1 expression and correlated to methylation of both CpG sites. Hence, methylation of CpG–838 and CpG–833 presents a novel mechanism how macronutrients and hormones might affect hepatic Scd1 expression.

Major dietary inducers of Scd1 expression in the liver are carbohydrates, while poly-unsaturated fatty acids (PUFAs) are able to blunt this effect [30]. Carbohydrate-dependent induction of Scd1 expression is complex and partly mediated by SREBP-1c and liver X receptor (LXR) [31, 32]. SREBP-1c is also transcriptionally activated at elevated insulin levels in a phosphatidylinositol-3-kinase (PI3K)-dependent manner [29]. Although insulin levels were increased in the HFD group, Srebf1 expression was not altered, indicating impairment of insulin sensitivity by feeding the HFD. This impaired insulin sensitivity could also serve as an explanation why hepatic Scd1 expression was not increased in the hyperinsulinenic HFD group. Besides SREBP-1c, carbohydrate responsive element-binding protein (ChREBP) is activated by acetylation in response to dietary carbohydrates, and subsequently induces Scd1 expression to promote lipogenesis [33]. In contrast to carbohydrates, PUFAs impair the expression of SREBP-1c by inhibiting the binding of LXR to the Srebf1 promoter [34]. Furthermore, LXR response ele-

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**Fig. 4** Correlation of Scd1 promoter methylation with body parameters. Degree of methylation of CpG–838 a, CpG–833 b, and CpG +384 c was correlated to hepatic Scd1 expression, lean and fat mass.

**Table 1** Correlation of CpG methylation and body parameters. Degree of methylation of CpG–838 and CpG–833 was correlated to hepatic Scd1 expression, lean and fat mass.

<table>
<thead>
<tr>
<th>CpG Site</th>
<th>Lean Mass (g)</th>
<th>Fat Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CpG–838</td>
<td>$R^2 = 0.328$</td>
<td>$R^2 = 0.258$</td>
</tr>
<tr>
<td>CpG–833</td>
<td>$R^2 = 0.298$</td>
<td>$R^2 = 0.238$</td>
</tr>
</tbody>
</table>

Schwenk RW et al. Scd1 Expression and Promoter Methylation ... Horm Metab Res 2013; 45: 786–794
ments (LXRE) are also present in the Scd1 promoter and PUFAs might affect Scd1 expression independent of SREBP-1c [35]. However, LXR-dependent inhibition of gene transcription is not likely to be the mechanism behind our observations, as Srebf1 expression was not different between the 2 diet groups. This observation is in line with published data that report a decreased Scd1 expression upon HFD-feeding, independent of Srebf1 expression [19]. A possible mechanism might include leptin, which has been shown to inhibit Scd1 expression independently of Srebf1 [17].

Methylation at CpG−838 and CpG−833 adds another layer, how diet and/or body weight might regulate expression of Scd1. Epigenetic regulation of Scd1 expression in the liver has not yet been studied, however, diet-dependent alterations of promoter methylation are known to affect gene expression, for example, of hepatic glucokinase and L-type pyruvate kinase [10]. Diet-induced alterations of promoter methylation are not liver specific and have also been reported for adipose tissue (e.g., leptin) and brain (e.g., melanocortin-4 receptor, tyrosine hydroxylase, and dopamine transporter) [25,36,37]. Importantly, the degree of single CpG methylation correlated with Scd1 expression and body weight only for CpG−838 and CpG−833, while the third differentially methylated CpG site (CpG−834) did not show a clear correlation to Scd1 expression or body weight, suggesting a predominant role in Scd1 expression only for the first 2 CpG sites. Sequence-based analysis of the proximity of CpG−838 and CpG−833 identified recognition sites for hepatic nuclear factors (HNF/FoxA) and mouse Krueppel like factors (MOK) at these CpG sites. How these 2 families of transcription factors are involved in Scd1 expression is not established yet and will require further investigation.

Increased fat mass is associated with increased serum levels of adipocyte-derived leptin, which is a major regulator of satiety [38]. Besides its anorexigenic function, leptin has also been shown to inhibit Scd1 expression in liver and adipose tissue to promote fatty acid oxidation [17,39]. So far, the exact mechanism is not known and effects of leptin on hepatic Scd1 have been suggested to be both indirect via the central nervous system as well as direct on the hepatocyte [40]. In accordance to the literature, serum leptin levels correlated inversely with expression of Scd1 in the livers of HCD- and HFD-fed mice. However, further research is needed to clarify if increased circulating levels of leptin and methylation of the hepatic Scd1 promoter are independent observations or mechanistically linked. Ghrelin, the orexigenic counterpart of leptin, activates Scd1 expression in adipose tissue via the sympathetic nervous system [28].

**Fig. 5** Serum leptin, ghrelin, and insulin levels. (a) Serum levels of leptin, total ghrelin, active ghrelin, and insulin in livers from HCD- and HFD-fed mice. (b, c) Correlation of serum leptin, ghrelin and insulin levels to methylation of CpG−838 b and CpG−833 c.
we have reported a similar correlation of circulating ghrelin levels to hepatic Scd1 gene expression and, additionally, to methylation of its promoter. On neuronal level, ghrelin has been shown to activate Sirtuin1 (Sirt1)/p53 and mTOR signaling pathways [41, 42]. If these pathways are involved in regulating Scd1 expression in the liver is currently not known. Nevertheless, recent studies indicate a strong and opposing effect of leptin and ghrelin on hepatic steatosis and fibrosis, emphasizing their impact on hepatic metabolism independent of their central-nervous function [43, 44].

Taken together, these data show that diet composition and body weight development affect hepatic Scd1 promoter methylation and gene expression. Furthermore, promoter methylation was tightly linked to serum leptin and ghrelin levels, suggesting a novel mechanism, in which both hormones and macronutrients could regulate Scd1 expression and, thus, ectopic lipid accumulation in the liver.

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Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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