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

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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-

Ingredient	HCD (D12450B)		HFD (D12492)	
	gm%	kcal%	gm%	kcal%
Carbohydrate	67.3	70	26.3	20
Fat	4.3	10	34.9	60
Protein	19.2	20	26.2	20
	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800
l-Cystine	3	12	3	12
Corn starch	315	1260	0	0
Maltodextrin 10	35	140	125	500
Sucrose	350	1400	68.8	275.2
Cellulose, BW200	50	0	50	0
Soybean Oil	25	225	25	225
Lard*	20	180	245	2205
Mineral mix S10026	10	0	10	0
Dicalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate, 1 H_2O	16.5	0	16.5	0
Vitamin mix V10001	10	40	10	40
Choline bitartrate	2	0	2	0

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

Table 1 Composition of the experimental diets.

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-vented 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) and TaqMan gene expression assays (Applied Biosystems) were applied. TaqMan gene expression assays are identified in **Table 2**. Data were normalized using the $2^{-\Delta\Delta CT}$ 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

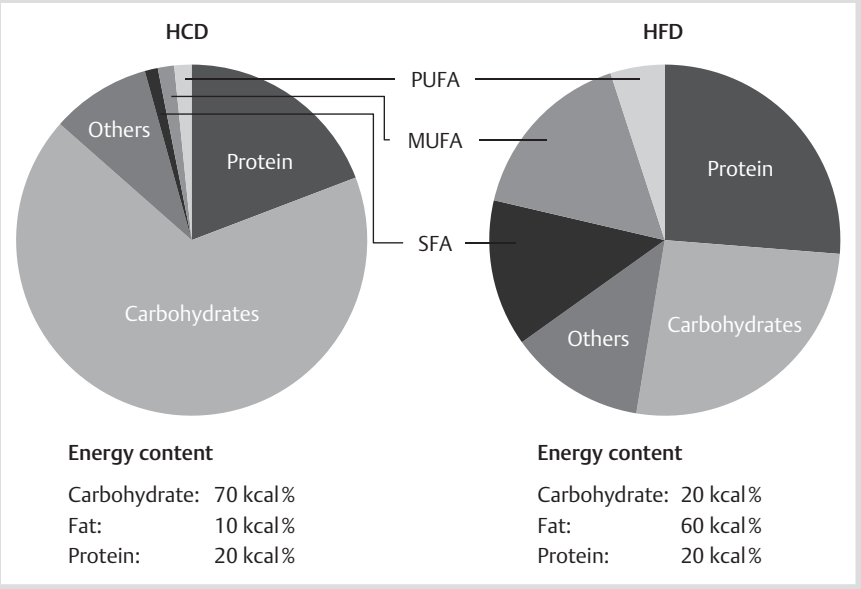

Fig. 1 Composition of the experimental diets. Amounts of carbohydrates, saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA) of the high-carbohydrate diet (HCD) and the high-fat diet (HFD), respectively, depicted in percent of weight (%gm).

Gene	Description	TaqMan Assay
Acaca	Acetyl-Coenzyme A carboxylase alpha	Mm01304277_m1
Acsl1	Acyl-CoA synthetase long-chain family member 1	Mm00484217_m1
Cpt1a	Carnitine palmitoyltransferase 1a, liver isoform	Mm01231183_m1
Fasn	Fatty acid synthase	Mm00662319_m1
Mlycd	Malonyl-CoA decarboxylase	Mm01245665_m1
Ppara	Peroxisome proliferator activated receptor alpha	Mm00627559_m1
Pparg	Peroxisome proliferator activated receptor gamma	Mm00440945_m1
Scd1	Stearoyl-Coenzyme A desaturase 1	Mm01197142_m1
Snap23	Synaptosomal-associated protein 23	Mm01330351_mH
Srebf1	Sterol regulatory element binding transcription factor 1	Mm00550338_m1

Table 2 TaqMan gene expression assays applied to determine mRNA expression levels by qRT-PCR.

CpG island	Forward primer	Reverse primer	Product size
1	TGTTTAGGGTTAGTGTGGGTTTAATA	TAATATATCCCTTCAACCAACATCC	316
2	GGATGTTGGTTGAAGGGATATATTA	AATACCACCTTATCCTACCTTTTCC	396
3+4	GTTTGTTTTATTTTTTAAAATTTTAGTTTA	AAAACTCATCTACCCAAATTACAATCTAC	383

Table 3 Primers for bisulfite sequencing PCR (BSP) of CpG islands in the *Scd1* promoter.

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 immunochemical 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 Stratec (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+t}), and multiplied by 100 ($ph_c/ph_{c+t} \times 100$) [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-

Table 4 Body parameters of HCD- and HFD-fed mice.

	HCD		HFD		
Parameter	**Mean**	**SEM**	**Mean**	**SEM**	**p-Value**
Body weight (g)	28.4	1.22	42.8	0.73	<0.0001
Lean mass (g)	22.8	1.01	29.5	0.49	0.0003
Fat mass (g)	7.2	0.52	18.1	0.82	<0.0001
Liver weight (g)	1.19	0.072	1.27	0.069	0.4146
Liver triglycerides (µg)	0.39	0.10	0.89	0.28	0.075

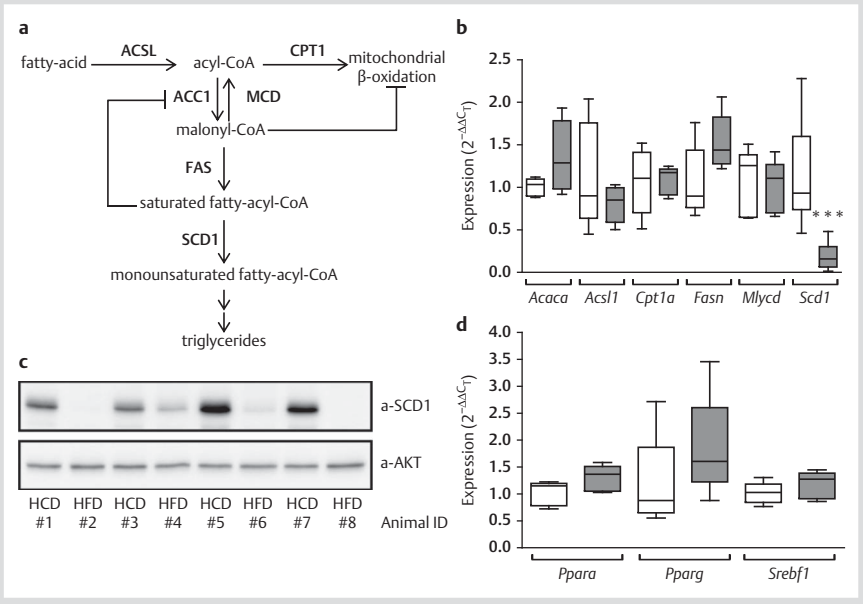

Fig. 2 Expression of genes involved in fatty acid metabolism. **a** Proteins and their function in fatty acid metabolism. **b** Expression of genes involved in fatty acid metabolism in livers of male C57BL/6J mice upon 18 weeks of high-carbohydrate diet (HCD, white bars) and high-fat diet (HFD, grey bars), respectively. **c** Western blot analysis of *SCD1* protein abundance in livers from mice on HCD and HFD. Detection of AKT served as loading control. **d** Expression of transcription factors involved in hepatic fatty acid metabolism. Data are mean values ± SEM of 8 animals per group (n = 8). *** Statistically different from corresponding basal value with p < 0.001.

pore. Serum insulin levels were measured with the Mouse Ultrasensitive 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 (*Acsl1*), 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* 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 differences in methylation of 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

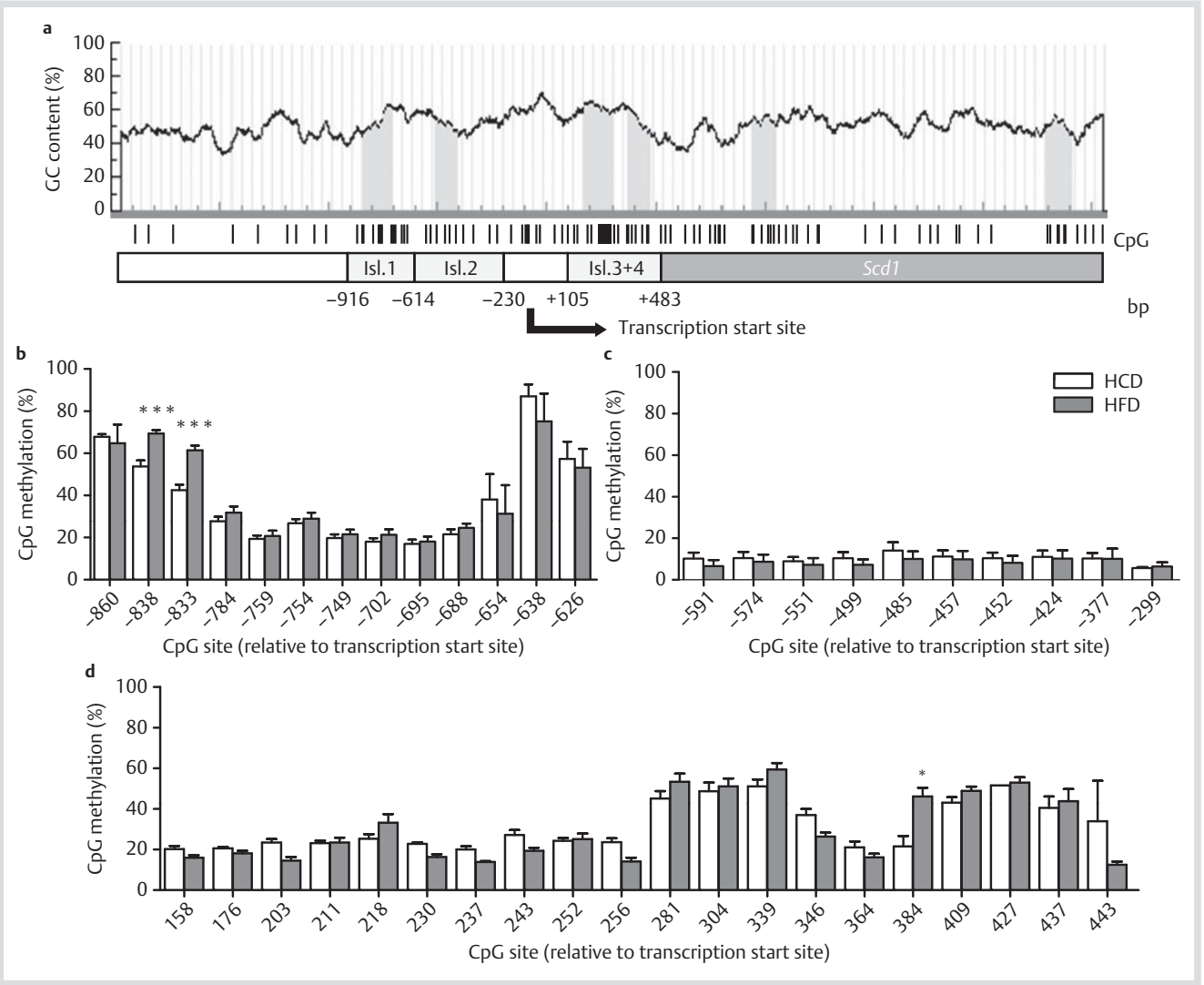

Fig. 3 Methylation of the hepatic *Scd1* promoter. **a** CpG islands of the *Scd1* promoter. CpG content of the promoter region (2000 bp upstream of transcription start), the 5′-UTR and the CDS was analyzed using MethPrimer Software (http://www.urogene.org/methprimer). Areas with a length of more than 200 bp, a GC content of more than 50 % and observed vs. expected CpG ratio of more than 60 % were defined as CpG islands. Numbers indicate the positions relative to transcription start site. **b**–**d** Single CpG methylation in CpG island 1 **b**, 2 **c**, and 3 plus 4 **d** of the *Scd1* promoter upon HCD-feeding (white bars) and HFD-feeding (grey bars), respectively. CpG islands were selectively amplified by BSP and sequenced. Methylation was calculated as the amount of unconverted vs. converted cytosine. Data are mean values ± SEM of 8 animals per group (n = 8). * Statistically different from corresponding basal value with $p < 0.05$, *** statistically different from corresponding basal value with $p < 0.001$.

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 ($R^2 = 0.378$ 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 ($R^2 = 0.549$ and 0.554, respectively) than with lean mass ($R^2 = 0.335$ and 0.321, respectively). Methylation of CpG_{+384} did not correlate with expression of *Scd1* ($R^2 = 0.006$) (**Fig. 4c**). Accordingly, it displayed no correlation to lean and fat mass ($R^2 = 0.259$ 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,

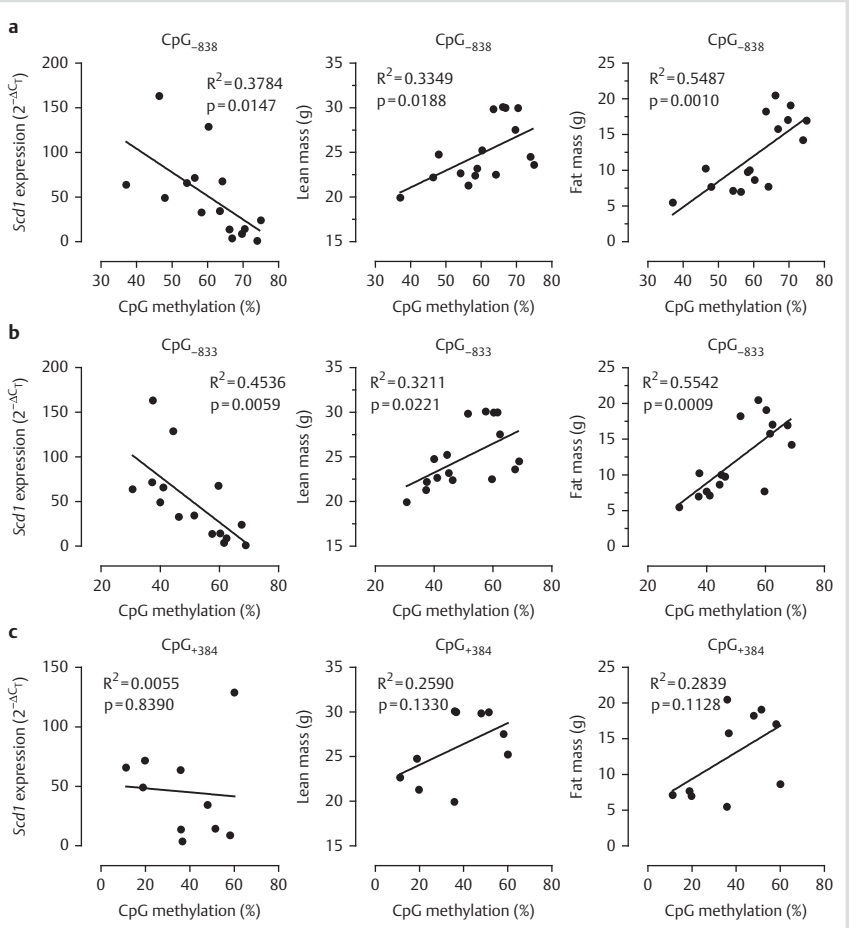

Fig. 4 Correlation of *Scd1* promoter methylation with body parameters. Degree of methylation of CpG_{-838} **a**, CpG_{-833} **b**, and CpG_{+384} **c** was correlated to hepatic *Scd1* expression, lean and fat mass.

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 HCD group compared to the HFD group ($p < 0.05$) (**Fig. 5a**). Serum leptin levels correlated positively to the 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 ($R^2 = 0.355$ 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. 5b, c**). 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 hyperinsulinemic 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-

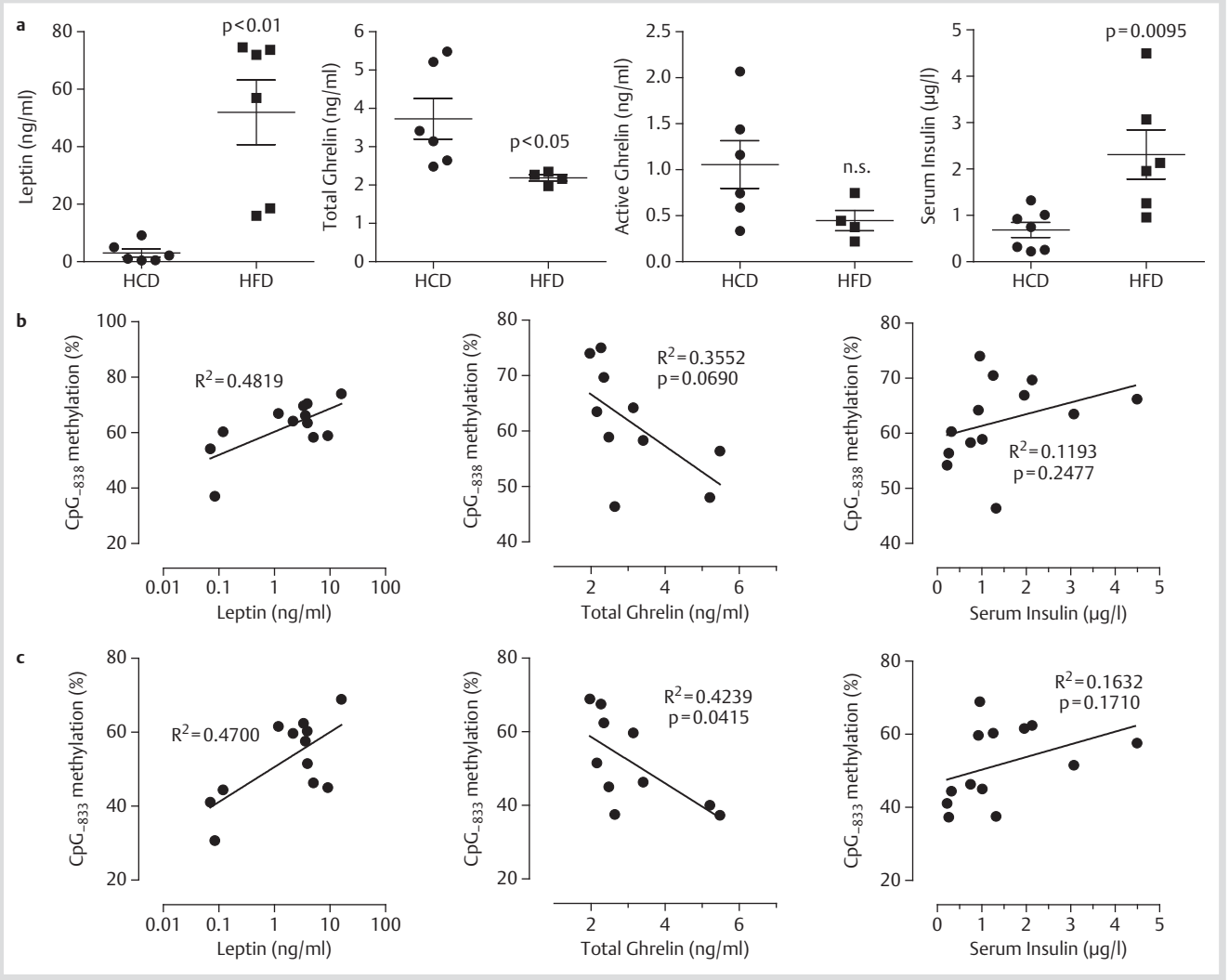

Fig. 5 Serum leptin, ghrelin, and insulin levels. **a** Serum levels of leptin, total ghrelin, active ghrelin, and insulin in livers from HCD- and HFD-fed mice. **b**, **c** Correlation of serum leptin, ghrelin and insulin levels to methylation of CpG_{-838} **b** and CpG_{-833} **c**.

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_{+384}) 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]. Here,

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, how 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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