Modification of the fatty acid composition of an obesogenic diet improves the maternal and placental metabolic environment in obese pregnant mice

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ABSTRACT

Peri-conceptional exposure to maternal obesogenic nutrition is associated with in utero programming of later-life overweight and metabolic disease in the offspring. We aimed to investigate whether dietary intervention with a modified fatty acid quality in an obesogenic high-calorie (HC) diet during the preconception and gestational phases can improve unfavourable effects of an adipogenic maternal environment. In NMRI mice, peri-conceptional and gestational obesity was induced by feeding a HC diet (controls), and they were compared with dams on a fat-modified (Fat-mod) HC diet of the same energy content but enriched with medium-chain fatty acids (MCFAs) and adjusted to a decreased ratio of n-6 to n-3 long-chain polyunsaturated fatty acids (LC-PUFAs). Effects on maternal and placental outcomes at delivery (day 17.5 post coitum) were investigated. Despite comparable energy assimilation between the two groups of dams, the altered fatty acid composition of the Fat-mod HC diet induced lower maternal body weight, weights of fat depots, adipocyte size, and hepatic fat accumulation compared to the unmodified HC diet group. Further, there was a trend towards lower fasting glucose, insulin and leptin concentrations in dams fed the Fat-mod HC diet. Phenotypic changes were accompanied by inhibition of transcript and protein expression of genes involved in hepatic de novo lipogenesis comprising PPARG2 and its target genes Fasn, Acaca, and Fabp4, whereas regulation of

other lipogenic factors (*Srebf1*, *Nr1h3*, *Abca1*) appeared to be more complex. The modified diet led to a sex-specific placental response by upregulating PPARG-dependent fatty acid transport gene expression in female versus male placentae. Qualitative modification of the fatty acid spectrum of a high-energy maternal diet, using a combination of both MCFAs and n-3 LC-PUFAs, seems to be a promising interventional approach to ameliorate the adipogenic milieu of mice before and during gestation.

1. Introduction

To date, up to two thirds of women in the reproductive age are overweight or obese in the United States [1]. Among the various perinatal risk factors of later-life overweight and metabolic disease in the offspring including gestational diabetes and excessive gestational weight gain, peri-conceptional maternal obesity confers the strongest risk in humans [2]. An adipogenic maternal environment during pregnancy and lactation is considered to induce such adverse later-life outcomes [3,4] via mechanisms referred to as "fetal programming". Even during the earliest developmental stages peri-conceptionally, an obesogenic milieu can exert sex-specific adverse effects on adult offspring, as shown in mice [5].

To counteract these early-life risks predisposing to childhood obesity, effective primary preventive strategies are needed [6]. The nutritional composition of the maternal diet during gestation has been suggested to have an impact on fetal development in mice and humans [3,7]. Specifically, the consumption of a high-fat (60% kcal fat) diet (HFD) had strong effects on offspring outcomes in mice, irrespective of maternal obesity *per se* [8]. Compared to dietary fat consisting of mainly long-chain fatty acids (LCFAs, ≥14 carbon atoms), the use of medium-chain triglycerides (MCTs) containing medium-chain fatty acids (MCFAs, 8-12 carbon atoms) has been supposed to result in a reduction of body weight and fat mass in animals and humans [9,10]. A recent study in rats, consuming either an LCFA- or MCFA-rich diet during pregnancy, suggested an adiposity-lowering effect in offspring of MCFA-fed dams when they were exposed to HFD later in life [11].

Nutritional supplementation with omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) and thereby reducing the ratio of n-6 to n-3 LC-PUFAs has also been suggested to have a beneficial impact on important health issues including improvements of dyslipidemia, insulin resistance, and inflammation [12]. Despite advantageous effects also on body weight reduction and lowering of fat accumulation, as observed in *in vitro* and animal experiments [13,14], data on n-3 LC-PUFA supplementation during pregnancy on both maternal and offspring outcomes are scarce and inconsistent, from both animal and human studies [15,16].

In previous studies, we found that female Naval Medical Research Institute (NMRI) mice fed a high-calorie (HC) diet with 60% of energy from mainly saturated fat and a high sugar-to-starch-ratio presented with an increased weight at conception, an impaired glucose tolerance in pregnancy, and higher placental and offspring weights compared to control diet (CD)-fed dams [5]. We hypothesized that such adverse effects of a maternal obesogenic HC diet might be ameliorated or even prevented if the fat composition is altered via a combination of both an enrichment with MCFAs and a reduction of the n-6:n-3 LC-PUFA ratio. The aim was to evaluate the impact of a modification of the fatty acid quality of a HC diet during the peri-conceptional and gestational period of NMRI dams, by assessing maternal and placental outcomes at delivery.

2. Materials and methods

2.1. Experimental schedule

The experimental design with NMRI dams was reported previously by our group [5]; details are presented in the Supplementary Materials and Methods section. NMRI outbred dams were chosen because we aimed to generate a model of mild diet-induced maternal adiposity in pregnancy that develops less severe metabolic consequences of HFD feeding than standard inbred strains [17,18], and thus shows closer correspondence to the considerable share of overweight and obese women without metabolic complications during pregnancy. Briefly, 30 female NMRI mice at 3 weeks of age were randomly distributed into two groups. One group received the HC diet, an unmodified obesogenic diet (E15741, Ssniff, Soest, Germany) and served as a control group. Apart from a high-saturated fat content, the HC diet was enriched with sugar (Supplementary Table 1). The intervention group received a diet with the same high energy density and identical protein and carbohydrate composition but with a modified fat quality consisting of an increased amount of MCFAs and a reduced n-6:n-3 LC-PUFA ratio (termed "Fat-mod HC diet"; S8379-E012, Ssniff) (Supplementary Table 1). To provide the respective fatty acid patterns, beef tallow and soybean oil was used for the HC diet, whereas for the Fat-mod HC diet, hydrogenated coconut oil, marine oil (n-3), walnut oil, and lower amounts of beef tallow were applied (Supplementary Table 1). Walnut oil was used as a source of essential fatty acids, because it contains slightly higher concentrations of linolenic acid (C18:3 n-3) and lower concentrations of palmitic acid (C16:0) than soybean oil (Supplementary Table 1). The fatty acid compositions of coconut oil and marine oil (n-3) are presented in the Supplementary Table 2; additional details on stability analyses of fatty acids in the diet are provided in the Supplementary Materials and Methods section.

Body weight and body composition were determined weekly. At age 5 weeks, individual food intake and fecal excretion were recorded over 4 days. At age 12 weeks, mice were mated and screened for vaginal plugs every 12 h. Besides screening for vaginal plugs, mating success was determined by abdominal palpation of fetuses at 7.5 days post coitum (dpc). Mice remained on their respective experimental diets during pregnancy, and food intake, body weight, and body composition were assessed every three days. At 17.5 dpc, animals were anesthetized following fasting for 12 h, bled from the retro-orbital plexus, and killed by cervical dislocation. Organs including 6 different fat depots, placentae, and fetuses were promptly dissected, blotted dry, and weighed to the nearest mg. Tissue samples were immediately processed and either frozen at −80 °C, fixed in 4% paraformaldehyde (pH 7.4), or fixed in RNALater (Qiagen, Hilden, Germany) and frozen at −20 °C. Experiments were approved by the Animal Ethics Committee (Bavaria, Germany) and are in accordance with the Council of Europe Convention ETS 123 (2010/63/EU).

2.2. Determination of body composition, energy intake, and energy assimilation

Body composition (% fat and lean mass) was assessed via whole animal body composition measurement using a time domain nuclear magnetic resonance imaging (MRI) analyzer (Minispec LF50; Bruker Optics, Ettlingen, Germany) without anesthesia as reported previously [5]. Details on analyses of energy intake, energy assimilation, and feces composition are outlined in the Supplementary Materials and Methods section.

2.3. Analysis of serum parameters of lipid and glucose metabolism in dams

To verify that the functional compounds of the Fat-mod HC diet reached the systemic circulation, serum samples of two subgroups of

Table 1
Assimilation parameters including individual food and energy intake, feces and energy output, and assimilated energy per body weight, analyzed in mice of the HC diet and Fat-mod HC diet groups at an age of 5 weeks.

Parameter	HC (control) diet $n = 13$	Fat-mod HC diet $n = 11$
Food intake (g/d)	3.61 ± 0.11	3.58 ± 0.07
Protein intake (g/d)	0.87 ± 0.03	0.86 ± 0.02
Energy intake (kJ/d)	89.5 ± 2.8	87.4 ± 1.6
Defecated feces (g/d)	0.40 ± 0.01	0.33 ± 0.01***
Energy content feces (kJ/g)	18.3 ± 0.2	15.1 ± 0.2***
Excreted energy (kJ/d)	7.4 ± 0.3	4.9 ± 0.2***
Assimilated energy (kJ/d)	82.2 ± 2.5	82.5 ± 1.6
Assimilation coefficient	91.8 ± 0.2	94.4 ± 0.2***
Assimilated energy per body weight (kJ/g body weight × day)	2.9 ± 0.1	3.0 ± 0.1

Data are presented as mean ± S.E.M; mice exhibiting extreme food spilling were excluded from the analysis; statistically significant differences between groups were determined by Mann-Whitney U test; ***$p < 0.001$. Energy intake = food intake × caloric value of the diet. Assimilated energy = energy intake − excreted energy. Food assimilation coefficient = (assimilated energy × 100) / energy intake. Fat-mod HC diet, fat-modified high-calorie diet; HC diet, high-calorie diet.

non-pregnant mice, fed either the Fat-mod HC diet ($n = 5$) or the HC diet ($n = 5$) over a period of 4 weeks preconceptionally, were analyzed for serum glycerophospholipid fatty acids by gas chromatography according to Glaser et al. [19]. At 16.5 dpc, an intraperitoneal glucose tolerance test (ipGTT) was performed after fasting for 6 h, as described [5]. Serum analyses of metabolic parameters in dams at 17.5 dpc were carried out according to Rathkolb et al. [20]; details are outlined in the Supplementary Materials and Methods section.

2.4. Histological organ analyses and determination of adipocyte size in dams

Histological liver and adipose tissue specimens were investigated by assessment of liver steatosis using a modified steatosis score and adipocyte size analysis, as reported previously [5]; details are outlined in the Supplementary Materials and Methods section.

2.5. Gene expression analyses in tissues of dams and the placenta

Total RNA was extracted from liver, adipose tissue, and pooled placenta samples, and mRNA expression of maternal and placental genes was analyzed. Following extraction of fetal DNA from the offsprings' tails (NucleoSpin Tissue kit; Macherey-Nagel, Düren, Germany), sex was determined by PCR amplification of the sex determining region of Chr Y (*Sry*), and the amplified PCR products were detected on a 1.5% agarose gel.

Placental gene expression was measured in a subgroup of 5 placenta pools per dietary group and sex [21]. For subgroup selection, litters were ranked according to the dams' weight at conception, and 5 litters were selected across the range. Placentae of each selected litter were ranked according to weight, and from each litter, 3 placentae per sex were selected across the range and used for placenta pools. This approach was chosen to account for potential differences in placental gene expression by placental weight and sex, considering that the placental response towards nutritional influences has been shown to differ by sex [22]. Each placenta was homogenized as a whole in QIAzol lysis reagent (miRNeasy Mini Kit, Qiagen), and equal amounts (10 mg) of the 3 homogenates were pooled.

Total RNA was extracted from RNAlater-conserved frozen liver and omental adipose tissue samples using the Nucleospin RNA II kit (Macherey-Nagel); RNA from placenta pools was extracted using the QIAzol method and purified (miRNeasy Mini Kit, Qiagen). 1 μg RNA was reverse transcribed into cDNA, and quantitative real-time reverse
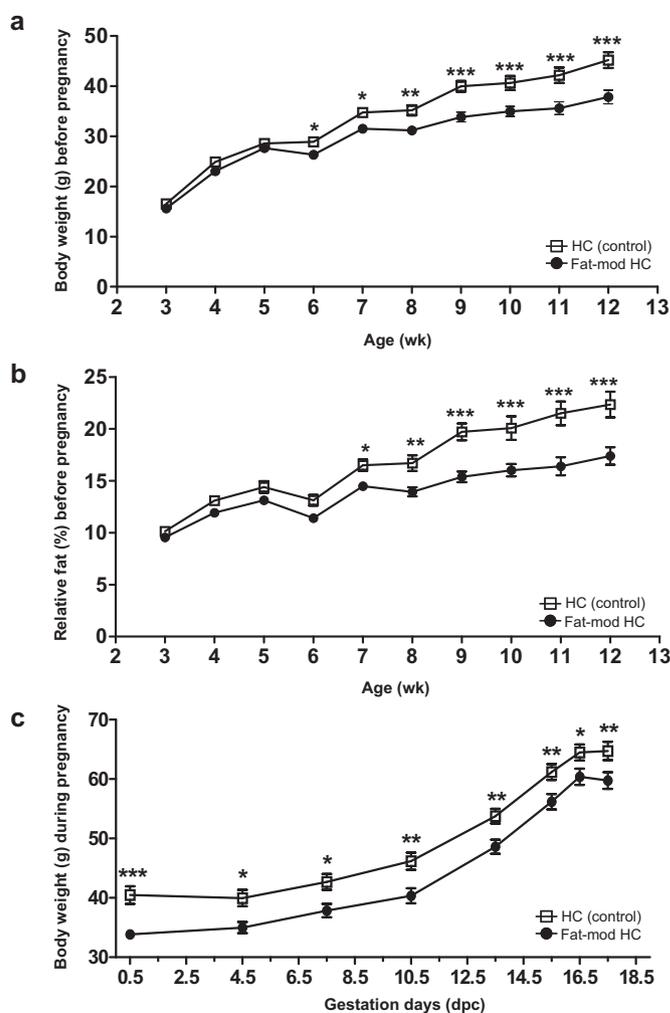
Fig. 1. Effects of fat modification of an obesogenic high-calorie diet on maternal body composition before and during pregnancy. Preconceptional analysis of (a) body weight and (b) relative body fat mass of dams fed the Fat-mod HC diet or the HC (control) diet from the age of 3 to 12 weeks ($n = 15$/group). Data for body fat mass are presented until conception (age 12 weeks), when dams became too large for analysis of body composition by the MRI analyzer (Minispec LF50; Bruker Optics). ANOVA revealed significant effects of the diet ($p < 0.001$), age ($p < 0.0001$), and diet*age interaction ($p < 0.0001$) for preconceptional body weight and relative body fat. Significance of individual differences between the groups was tested by Tukey's post hoc test. (c) Measurement of weight gain during pregnancy (Fat-mod HC diet: $n = 12$; HC diet: $n = 11$). ANOVA revealed significant effects of the diet ($p < 0.01$) and gestation days ($p < 0.0001$); differences between groups were analyzed by Tukey's post hoc test. Data are presented as mean ± S.E.M; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ANOVA, analysis of variance; dpc, day post coitum; Fat-mod HC, fat-modified high-calorie diet; HC, high-calorie diet; MRI, magnetic resonance imaging.

transcription PCR was performed as described previously [5]. All measurements were done in duplicates. To normalize expression data, we tested and validated several reference genes per tissue, as detailed in the Supplementary Materials and Methods section. mRNA expression levels were calculated relative to the mean of the reference gene peptidylprolyl isomerase B (*Ppib*) in liver, ubiquitin-conjugating enzyme E2D 2 (*Ube2d2*) in adipose tissue, or TATA box binding protein (*Tbp*) in placenta.

2.6. Western blot analysis

Proteins were extracted from tissues using CelLytic MT lysis buffer with protease and phosphatase inhibitor cocktails (Sigma-Aldrich Chemie, Munich, Germany). The protein concentration of lysates was determined using the bicinchoninic acid method (Pierce BCA Protein

Assay Kit; Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein (15 μg for liver and 8 μg for adipose tissue per lane) were loaded onto NuPAGE Novex 4–12% Bis-Tris Mini (for proteins < 160 kDa) or NuPAGE Novex 3–8% Tris-Acetate Protein Gels (for proteins > 160 kDa) (Thermo Fisher Scientific) and transferred electrophoretically to a nitrocellulose membrane (Bio-Rad Laboratories, Munich, Germany). After blocking with skimmed milk, the membranes were probed with primary antibody and were immunodetected by BM Chemiluminescence Blotting Substrate (Roche, Mannheim, Germany) using appropriate alkaline phosphatase-conjugated secondary antibodies (GE Healthcare, Freiburg, Germany). Images were taken on a ChemiDoc Touch Imaging System, and densitometry was performed using the Image Lab Software V 5.2.1 (both Bio-Rad Laboratories). Information on primary antibodies and their targets is provided in the Supplementary Table 3.

2.7. Statistical analysis

Data for body weight and body composition were statistically analyzed using analysis of variance (ANOVA) (linear mixed models; PROC MIXED; SAS release 8.2, SAS Institute, Cary, NC, USA), taking into account the fixed effects of diet, age/dpc, the interaction of diet*age/dpc and the random effect of animal [23]. Square root-transformed data of placental gene expression were analyzed using PROC GLM (general linear models; SAS release 8.2), taking into account the fixed effects of diet, sex, and the interaction of diet*sex. Significance of individual differences was tested by Tukey's post hoc test. The remainder of the statistics was performed in GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA) using Mann-Whitney U test to assess differences in body and organ weights at 17.5 dpc, fetal and placenta weights, litter size, assimilation parameters, area under the glucose curve of the ipGTT, serum parameters, transcript abundance, hepatic steatosis score, and adipocyte size between the two groups of animals. For densitometric results, the two dietary groups were compared using the two-tailed unpaired Student's t-test. Data are presented as mean ± standard error of the mean (S.E.M.). $P < 0.05$ was considered significant.

3. Results

3.1. Effects of diet modification on serum levels of functional constituents

Profiling of serum fatty acids reflected the fatty acid composition of the respective diets (Supplementary Table 4). In serum of the Fat-mod HC diet group, higher concentrations of myristic acid (C14:0) were detected compared to the HC diet group, consistent with the assimilation of coconut oil as a dietary component (Supplementary Table 1). In addition, increased concentrations of the n-3 LC-PUFAs linolenic acid (C18:3 n-3), eicosapentaenoic acid (EPA; C20:5 n-3), and docosahexaenoic acid

(DHA; C22:6 n-3) were identified in serum of the Fat-mod HC diet mice, reflecting the intake of n-3 LC-PUFAs via marine oil (n-3) and walnut oil (Supplementary Table 1). Docosapentaenoic acid (C22:5 n-3) concentrations were lower in the Fat-mod HC diet group, possibly due to retro-conversion to EPA [24]. Concentrations of arachidonic acid (C20:4 n-6) and oleic acid (C18:1 n-9) were lower than in serum of the HC diet group, all together resulting in a marked reduction of the serum n-6:n-3 LC-PUFA ratio in Fat-mod HC diet mice.

3.2. Influence of the fat-modified obesogenic diet on energy assimilation and maternal body weight gain before and during pregnancy

The analysis of the daily food intake and feces excretion of individual mice at age 5 weeks, after being fed the diets for 2 weeks, revealed a similar food and energy intake in both groups (Table 1). The assimilation coefficient was higher in the Fat-mod HC diet group, resulting from a decreased energy excretion. This finding was consistent with both lower fecal weight and energy content per gram feces. Further analysis of feces composition by Fourier-transformed infrared spectroscopy was performed, and information in the region of wavenumbers 2829–2970 cm^{-1} resulted in a complete separation of the diet groups in a hierarchical cluster analysis. Peak heights in this region suggested that fecal fat content in the Fat-mod HC diet-fed mice was reduced (data not shown). Overall, there was no difference in the daily assimilated energy per body weight between the two dietary groups (Table 1).

Despite comparable energy assimilation, mice of the Fat-mod HC diet group had a lower preconceptional body weight and fat mass from the age of 6 to 7 weeks onwards (Fig. 1a, b). Body weight remained lower in the Fat-mod HC diet group also during the gestational period (Fig. 1c) and at delivery at 17.5 dpc (Fat-mod HC diet: 59.8 ± 1.4 g; HC diet: 64.7 ± 1.5 g, $p = 0.02$), whereas body length at 17.5 dpc did not vary between the groups (Fat-mod HC diet: 10.6 ± 0.2 cm; HC diet: 10.5 ± 0.1 cm, $p = 0.28$).

### 3.3. Effects of dietary fatty acid modification on maternal liver fat metabolism

Liver weights of the Fat-mod HC diet group were reduced compared to the HC diet-fed dams at 17.5 dpc (Fig. 2a, Supplementary Table 5). In addition, steatosis staging identified a lower grade of hepatic steatosis following fat modification (Fig. 2b, c), also confirmed by liver fat staining (data not shown). There was no histological or clinical chemical evidence of inflammation or liver injury (Table 2).

Regulation of gene transcripts of relevant liver metabolic pathways was compared between the two dietary groups (Fig. 2d). mRNA expression of fatty acid synthase (Fasn) and acetyl-Coenzyme A carboxylase alpha (Acaca), two key enzymes of de novo lipogenesis, was reduced in the Fat-mod HC diet group, along with a downregulation of sterol

Fig. 2. Impact of the peri-conceptional fat-modified high-calorie diet on maternal liver fat accumulation at the end of gestation. (a) Liver weight of dams fed the Fat-mod HC diet ($n = 12$) or the HC (control) diet ($n = 11$) relative to their body weight prior to delivery at 17.5 dpc. (b) Hepatic fat accumulation in both dietary groups (Fat-mod HC diet: $n = 12$; HC diet: $n = 11$) determined by a semi-quantitative steatosis score as described previously [5]. (c) Maternal liver histology, representative sections of dams of the Fat-mod HC diet group and the HC diet group at 17.5 dpc. In the HC diet group, micro-vesicular vacuolization of more than 66% of hepatocytic cytoplasm (indicating lipid storage) was observed (FFPE, HE; asterisks mark central veins of liver lobules; scale bars represent 50 μm), amounting to a steatosis score of 2.04 ± 0.08 (mean ± S.E.M) as shown in (b). In the Fat-mod HC diet group, less than 33% of hepatocytic cross-sections were affected by microvesicular fat, resulting in a markedly reduced score of 0.59 ± 0.16 (mean ± S.E.M). (d) mRNA expression of genes involved in de novo lipogenesis, fatty acid storage, transport and β-oxidation, and HDL formation relative to the reference gene Ppib in liver of dams fed the Fat-mod HC diet ($n = 12$) at 17.5 dpc. Results are presented in relation to the mean relative mRNA expression of the HC diet group ($n = 11$), as indicated by the dotted line. (e, f) Representative Western blot and densitometric analyses of liver proteins that were transcriptionally altered by the Fat-mod HC diet as compared to the HC diet. Protein lysates were isolated from liver of three dams per dietary group, and 15 μg protein were loaded per lane. GAPDH was used as a loading control. The anti-SREBP1 antibody detects both the membrane bound precursor (125 kDa) and the mature nuclear form of SREBP1 (65 kDa). All data are presented as mean ± S.E.M. Significant differences between the Fat-mod HC diet group and the HC diet group were determined by the Mann-Whitney U test and for the densitometric data by the two-tailed unpaired Student's t-test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abca1, ATP-binding cassette, sub-family A, member 1; ABCA1, ATP-binding cassette, sub-family A, member 1; Acaca, acetyl-Coenzyme A carboxylase alpha; ACACA, acetyl-CoA carboxylase 1; Cd36, Cd36 antigen; Cpt1, carnitine palmitoyltransferase 1; dpc, day post coitum; FA, fatty acid; Fasn, fatty acid synthase; FAS, fatty acid synthase; Fat-mod HC, fat-modified high-calorie diet; Fabp4, fatty acid binding protein 4; FABP4, fatty acid-binding protein, adipocyte; FFPE, formalin-fixed, paraffin-embedded; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HC, high-calorie diet; HE, hematoxylin-eosin; Nr1h3, nuclear receptor subfamily 1, group H, member 3; NR1H3, oxysterols receptor LXR-alpha; Plin2, perilipin 2; Ppara, peroxisome proliferator activated receptor alpha; Ppargc1a, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; Pparg2, peroxisome proliferator activated receptor gamma 2; PPARG2, peroxisome proliferator-activated receptor gamma 2; Ppib, peptidylprolyl isomerase B; Srebf1, sterol regulatory element binding transcription factor 1; n-SREBP1, sterol regulatory element-binding protein 1, nuclear; p-SREBP1, sterol regulatory element-binding protein 1, precursor.

regulatory element binding transcription factor 1 (*Srebf1*) and its transcriptional activator nuclear receptor subfamily 1, group H, member 3 (*Nr1h3*, also known as *Lxra*, liver X receptor alpha). mRNA abundance of the steatosis regulator peroxisome proliferator activated receptor gamma 2 (*Pparg2*) was also lower in this group, together with one of its target genes fatty acid binding protein 4 (*Fabp4*), whereas expression of hepatic genes essential for mitochondrial fatty acid β-oxidation was unchanged.
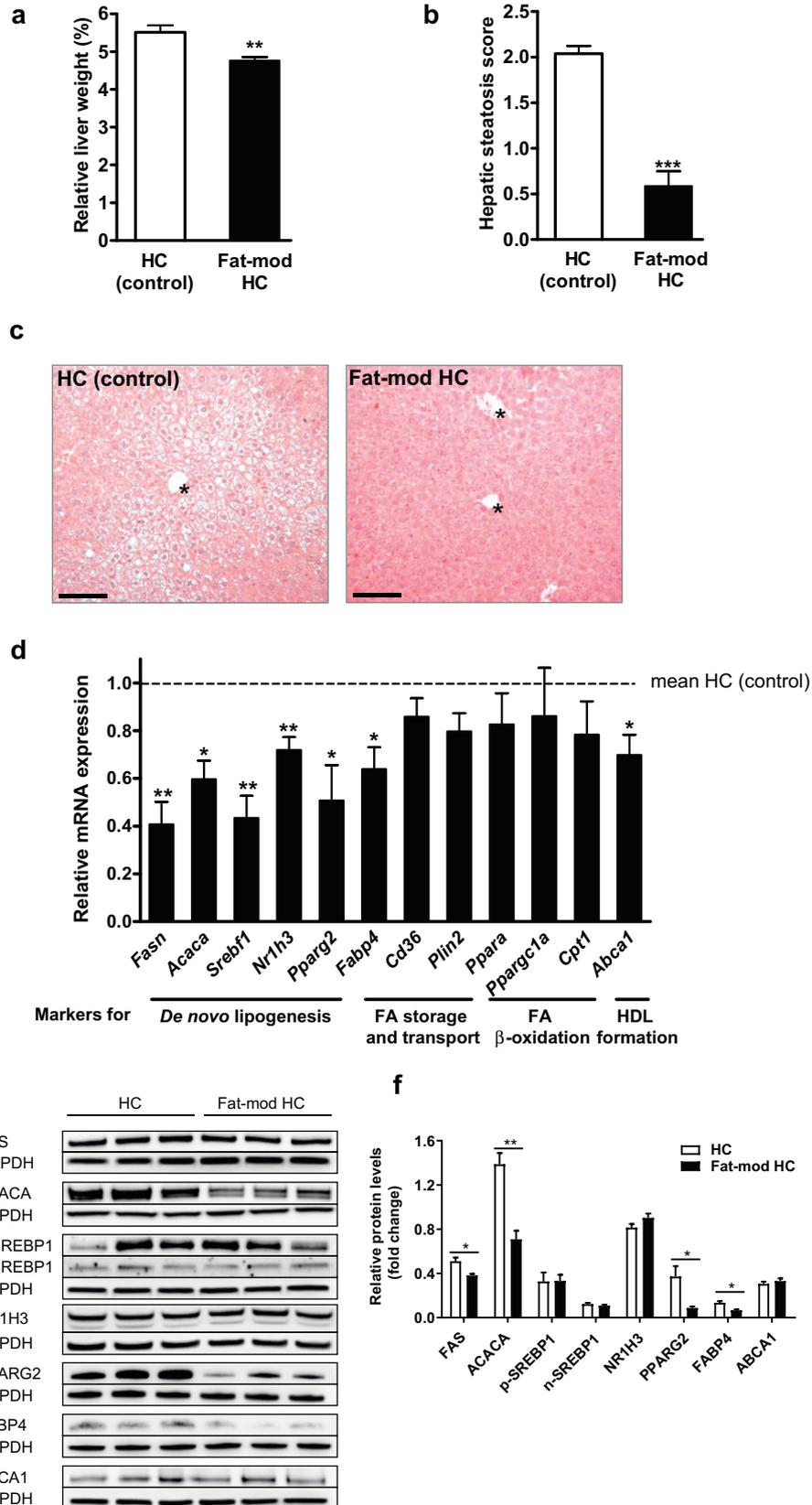
A simultaneous decrease in protein expression in liver of Fat-mod HC diet-fed dams was identified for PPARG2 and its targets FAS, ACACA, and FABP4, all involved in fatty acid synthesis and transport (Fig. 2e, f). In contrast, protein abundances of sterol regulatory element binding protein 1 (SREBP1), predominantly present in liver as the inactive precursor form, and NR1H3 were not altered by the Fat-mod HC diet, potentially due to post-transcriptional regulatory mechanisms compensating for the reduced gene expression. A similar divergence between mRNA and protein abundances was obtained for the main modulator of high-density lipoprotein (HDL) biogenesis, hepatic ATP-binding cassette, sub-family A, member 1 (*Abca1*), which is a target gene of a transcription cascade involving PPARG-mediated interaction with *Nr1h3* (Fig. 2d, e, f). We found lower concentrations of HDL cholesterol, the major cholesterol-transporting lipoprotein in mice [25], in serum of Fat-mod HC diet-fed dams (Table 2). Besides, there was a downward trend in blood fasting glucose and insulin levels, although not statistically significant, and no difference in the AUC of an ipGTT in pregnancy between groups (Table 2).

3.4. Effects of dietary fatty acid modification on maternal adipose tissue

Weights of 5 white adipose tissue depots were decreased in dams of the Fat-mod HC diet versus the HC diet group at 17.5 dpc, whereas the interscapular brown adipose tissue weight was unaffected (Fig. 3a, Supplementary Table 5). Analysis of adipocyte size and size distribution indicated a smaller mean fat cell size, and a leftward shift in the histogram confirming a higher proportion of adipocytes of smaller sizes in the omental fat of the Fat-mod HC diet versus the HC diet group (Fig. 3b, c, d).

Regulation of genes underlying lipid metabolism pathways was analyzed in the omental adipose tissue (Fig. 3e). In the Fat-mod HC diet group, a suppressive effect was identified for *Srebf1* mRNA expression, and, although not statistically significant, a trend pointing to a lower protein abundance of SREBP1 (predominantly as the nuclear active form) was found (Supplementary Fig. 1). Though not reaching significance, there was also a tendency towards downregulation of the other nutrient sensor genes *Nr1h3* ($p = 0.09$) and *Pparg1* ($p = 0.07$), but not for *Pparg2*, the nutritionally regulated isoform in adipose tissue [26]. Strongly decreased mRNA expression levels were identified for mesoderm-specific transcript/imprinted paternally expressed gene 1

(*Mest*, also known as *Peg1*) and leptin (*Lep*), which are involved in mechanisms influencing adipocyte size [27] and adipose mass regulation [28]. For LEP protein that is secreted by the adipocyte, we found a trend towards lower serum concentrations in the Fat-mod HC diet-fed versus the HC diet-fed dams (Table 2).

3.5. Effects of dietary fatty acid modification on fetal growth and placental regulation

There were no adverse effects of the modified dietary fat quality of dams on both mating success (Fat-mod HC diet: 80.0% [12/15]; HC diet: 73.3% [11/15]) and litter size (mean ± S.E.M; Fat-mod HC diet: 15 ± 0.6; HC diet: 14 ± 0.8, $p = 0.37$). Also, fetal weights (mean ± S.E.M: Fat-mod HC diet: 0.98 ± 0.03 g, HC diet: 1.01 ± 0.04 g, $p = 0.93$) and placental weights (mean ± S.E.M: Fat-mod HC diet: 0.12 ± 0.004 g, HC diet: 0.12 ± 0.003 g; $p = 0.41$) at 17.5 dpc were not altered.

In placenta pools of the Fat-mod HC diet dams, ANOVA revealed that *Pparg1* mRNA abundance was significantly affected by both the maternal diet and the interaction of diet*offspring sex (Table 3), accompanied by a significant effect of the maternal diet on the sex-specific expression of retinoid X receptor alpha (*Rxra*), the functional dimerization partner of PPARG. Accordingly, the maternal diet had similar effects on the fatty acid transporters solute carrier family 27, member 1 (*Slc27a1*, also known as *Fatp1*, fatty acid transport protein 1), and member 4 (*Slc27a4*, also known as *Fatp4*, fatty acid transport protein 4), two PPARG target genes involved in fatty acid uptake. Upregulation of PPARG/RXRA-activated fatty acid transport gene transcription was only evident in female placenta pools exposed to the fat modification (Fig. 4). In contrast, the PPARG target gene *Fabp4*, involved in intracellular trafficking of fatty acids in the placenta, was downregulated by the Fat-mod HC diet exposure, irrespective of sex. There was no effect on the mRNA expression of other PPARG target genes involved in placental lipid metabolism (Table 3, Fig. 4).

4. Discussion

Our data indicate that the unfavorable effects of an obesogenic high-energy diet on the maternal and gestational environment can be attenuated by a modification of the dietary fatty acid composition, applying a combination of MCFAs and n-3 LC-PUFAs before and during pregnancy. The change in fat quality – without altering calorie content – led to a reduction in adiposity as reflected by lower maternal fat depots and adipocyte size as well as decreased hepatic fat accumulation. This beneficial phenotype was accompanied by a diet-specific regulation of genes involved in hepatic *de novo* lipogenesis, lipid accumulation in adipocytes, and a sex-specific placental response by upregulating the expression of fatty acid transport genes. Both dietary groups, the HC and the Fat-mod HC diet dams, had a similar food intake, resulting in a comparable intake of macro-/micronutrients, and assimilated similar amounts of energy. Thus, the observed improvements, following long-term intervention over 11.5 weeks involving the preconception and gestation phases, can be attributed to the qualitative fatty acid alterations, as reflected in maternal serum.

The application of a high-calorie diet with a fatty acid composition altered in both chain length and its degree of unsaturation expands previous studies in rodents, which investigated the consequences of interventions with single fat components [29–32]. A major difference between the fatty acid compositions of the two high-calorie diets we applied relates to a marked enrichment of MCFAs from coconut oil in the Fat-mod HC diet (36% of total fatty acids; >230-fold increase compared to HC diet). In both animal and human studies, there is evidence suggesting that MCFA-rich diets induce a reduction of body weight and fat mass [9,10]. In contrast to LCFAs, MCFAs are absorbed directly from the diet into the hepatic portal venous system and have the advantage of being rapidly utilized by the β-oxidation spiral, as they are transported into the mitochondria independently of the carnitine

Table 2
Fasting serum metabolic parameters of dams of the HC diet and Fat-mod HC diet groups at delivery (17.5 dpc) and results of an ipGTT (16.5 dpc).

Metabolic parameter	HC (control) diet $n = 11$	Fat-mod HC diet $n = 11$	P value
AUC$_{ipGTT}$ (mmol/L * min)[a]	1129.6 ± 60.6	1096.9 ± 58.4	0.608
Fasting glucose (mmol/L)[a]	5.5 ± 0.3	4.8 ± 0.3	0.150
Insulin (pmol/L)	136.7 ± 29.9	98.8 ± 26.5	0.316
Leptin (μg/L)	244.2 ± 80.4	56.3 ± 10.9	0.099
Total cholesterol (mmol/L)	1.5 ± 0.1	1.2 ± 0.08	0.088
LDL cholesterol (mmol/L)	0.3 ± 0.03	0.3 ± 0.02	0.935
HDL cholesterol (mmol/L)	1.1 ± 0.1	0.9 ± 0.04*	0.047
NEFAs (mmol/L)	0.7 ± 0.06	0.7 ± 0.06	0.641
Triglycerides (mmol/L)	2.5 ± 0.2	3.0 ± 0.3	0.151
Alanine transaminase (U/L)	72.5 ± 16.8	62.6 ± 5.2	0.617
Aspartate transaminase (U/L)	266.4 ± 27.3	315.5 ± 22.2	0.112
Uric acid (μmol/L)	86.0 ± 6.1	90.0 ± 4.3	0.468
Urea (mmol/L)	4.6 ± 0.2	4.8 ± 0.2	0.530

Data are presented as mean ± S.E.M; area under the glucose curves (AUC) were calculated with the trapezoidal method in GraphPad Prism software; statistically significant differences between groups were determined by Mann-Whitney U test; *$p < 0.05$. dpc, day post coitum; Fat-mod HC diet, fat-modified high-calorie diet; HC diet, high-calorie diet; HDL, high-density lipoprotein; ipGTT, intraperitoneal glucose tolerance test; LDL, low-density lipoprotein; NEFAs, non-esterified fatty acids.

[a] At 16.5 dpc, an ipGTT was performed in $n = 12$ dams of the Fat-mod HC diet group and $n = 11$ dams of the HC diet group. Because of failed blood sampling in one of the dams of the Fat-mod HC diet group at 17.5 dpc, only $n = 11$ dams were available for analysis of serum metabolic parameters.
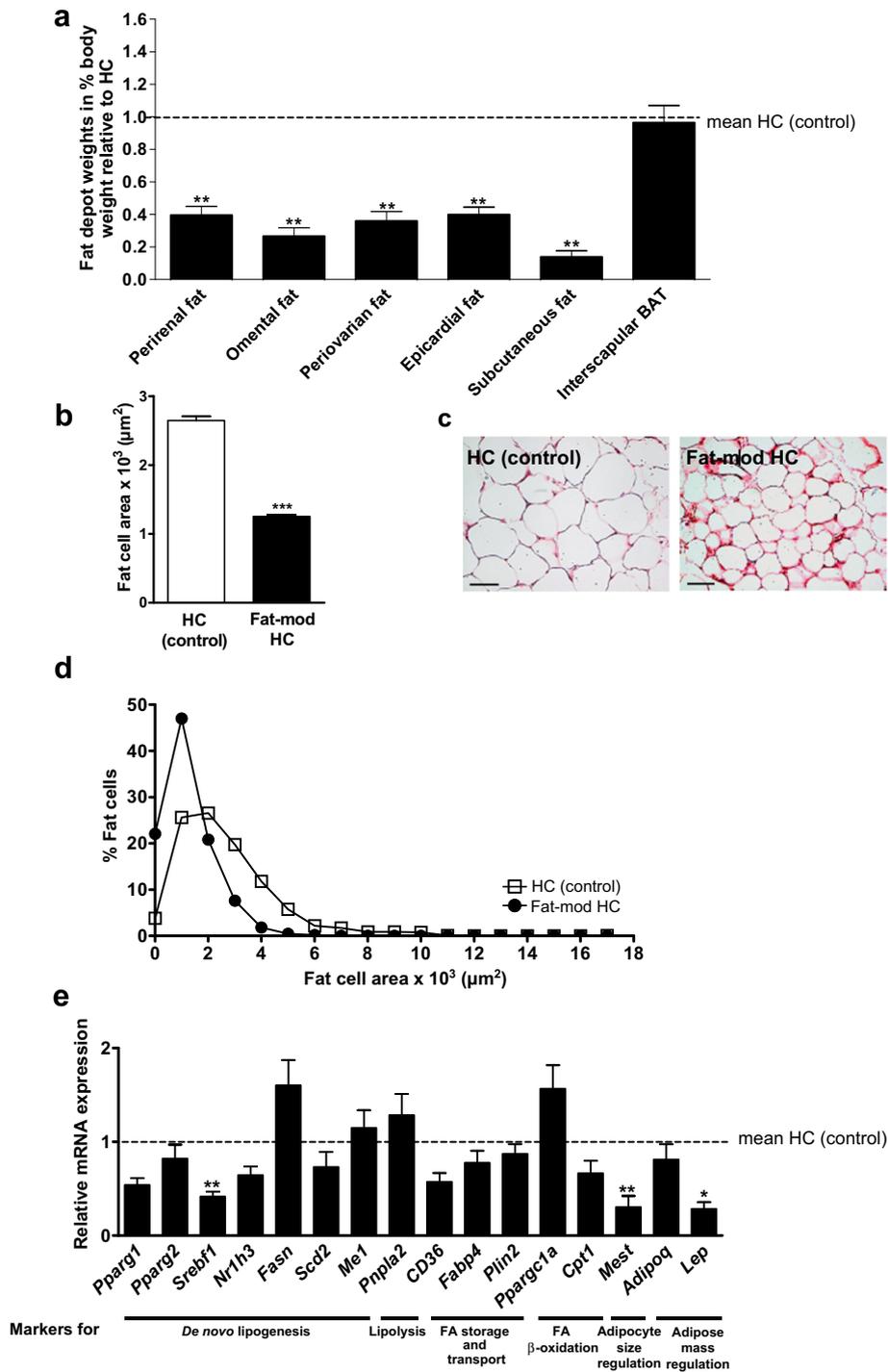
Fig. 3. Impact of the peri-conceptional fat-modified high-calorie diet on maternal adipose tissue at the end of gestation. (a) Fat was dissected out of the perirenal, omental, periovarian, epicardial, and subcutaneous depots. In addition, interscapular brown adipose tissue was dissected. Relative fat pad weights (% body weight prior to delivery) at 17.5 dpc of dams fed the Fat-mod HC diet ($n = 12$) are presented in relation to the mean relative fat pad weight of the HC (control) diet group ($n = 11$), as indicated by the dotted line. (b) Adipocyte size of omental adipose tissue of mice at 17.5 dpc (Fat-mod HC diet: $n = 12$; HC diet: $n = 11$). (c) Omental fat pad histology, representative sections of dams of the Fat-mod HC diet and HC diet groups at 17.5 dpc (FFPE, HE, scale bars represent 50 μm). (d) Fat cell area distribution of omental fat pads at 17.5 dpc expressed as percentage of cells (Fat-mod HC diet: $n = 12$; HC diet: $n = 11$; 100 adipocytes per animal). (e) mRNA expression of genes involved in fatty acid metabolism, adipocyte size determination, and adipose mass regulation relative to the reference gene *Ube2d2* in omental adipose tissue of dams of the Fat-mod HC diet group ($n = 9$) at 17.5 dpc. Results are presented in relation to the mean relative mRNA expression of the HC diet group ($n = 11$), as indicated by the dotted line. Data are presented as mean ± S.E.M. Significant differences between the Fat-mod HC diet group and the HC diet group were determined by the Mann-Whitney U test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. *Adipoq,* adiponectin, C1Q and collagen domain containing; BAT, brown adipose tissue; *Cd36*, Cd36 antigen; *Cpt1*, carnitine palmitoyltransferase 1; dpc, day post coitum; FA, fatty acid; *Fabp4*, fatty acid binding protein 4; *Fasn*, fatty acid synthase; Fat-mod HC, fat-modified high-calorie diet; FFPE, formalin-fixed, paraffin-embedded; HC, high-calorie diet; HE, hematoxylin-eosin; *Lep*, leptin; *Me1*, malic enzyme 1; *Mest*, mesoderm specific transcript/ imprinted paternally expressed gene 1; *Nr1h3*, nuclear receptor subfamily 1, group H, member 3; *Plin2*, perilipin 2; *Ppargc1a*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *Pnpla2*, patatin-like phospholipase domain containing 2; *Pparg*, peroxisome proliferator activated receptor gamma; *Scd2,* stearoyl-Coenzyme A desaturase 2; *Srebf1*, sterol regulatory element binding transcription factor 1; *Ube2d2*, ubiquitin-conjugating enzyme E2D 2.

Table 3

Influence of the maternal diet, offspring sex, and the interaction of maternal diet*offspring sex on placental gene expression levels (data represent p values obtained by ANOVA; p < 0.05 was considered significant as indicated in boldface type).

| Genes	Maternal diet	Sex	Maternal diet * sex
Pparg1	**0.0095**	0.2203	**0.0279**
Rxra	0.0647	0.3931	**0.0303**
Slc27a1 (Fatp1)	0.0868	0.7415	**0.0437**
Slc27a4 (Fatp4)	0.1184	0.7119	**0.0235**
Got2 (Fabp-pm)	0.1192	0.1922	0.1407
Fabp4	**0.0009**	0.8091	0.9404
Dgat1	0.7842	0.5267	0.0676
Plin2	0.3442	0.9950	0.3765
Cd36	0.5907	0.5612	0.0962
Abca1	0.8972	0.5105	0.3625

$N = 5$ placenta pools per dietary group and sex were analyzed. For subgroup selection, litters were ranked according to the dams' weight at conception, and 5 litters were selected across the range. Placentae of each selected litter were ranked according to weight, and from each litter, 3 placentae per sex were selected across the range and used for placenta pools. mRNA expression levels were calculated relative to the mean of the reference gene Tbp in placenta.

Abca1, ATP-binding cassette, sub-family A, member 1; ANOVA, analysis of variance; Cd36, Cd36 antigen; Dgat1, diacylglycerol O-acyltransferase 1; Got2, glutamatic-oxaloacetic transaminase 2 (also known as Fabp-pm, plasma membrane fatty acid binding protein); Fabp4, fatty acid binding protein 4; Slc27a1, solute carrier family 27 (fatty acid transporter), member 1 (also known as Fatp1, fatty acid transport protein 1); Slc27a4, solute carrier family 27 (fatty acid transporter), member 4 (also known as Fatp4, fatty acid transport protein 4); Plin2, perilipin 2; Pparg1, peroxisome proliferator activated receptor gamma 1; Rxra, retinoid X receptor alpha; Tbp, TATA box binding protein.

palmitoyltransferase 1 (CPT1) transport system [33]. Also in our model, there was no altered expression of fatty acid-regulated genes of liver lipid catabolism including Cpt1 following Fat-mod HC diet feeding, which appears to support this pathway. Furthermore, MCFAs are not preferentially deposited as esterified lipids. In our study, the Fat-mod HC diet dams excreted less fecal energy and fat, a potential indirect clue for the more efficient absorption of MCFAs compared to LCFAs. All these biochemical characteristics of MCFAs are considered to contribute to the adiposity-lowering effects of the Fat-mod HC diet on dams.

The effect on liver fat accumulation is thought to depend on the dose and type of MCFAs in the obesogenic diet. In HFD-fed C57BL/J6 mice, a partial replacement of LCFAs with MCFAs from hydrogenated coconut oil (C8:0–C12:0; 50% of total fatty acids) increased hepatic fat accumulation [34]. However, a HFD with a similar proportion of energy from fat (45% kcal) but mainly consisting of MCFAs from MCT oil (C8:0–C12:0; >90% of total fatty acids) is reported to reduce liver lipid storage [29]. Also in our model of pregnant NMRI dams, we found reduced liver fat accumulation following feeding of the Fat-mod HC diet compared to the HC diet. The observed differences among studies may not only be related to varying animal models, but also be due to the quantity and quality of the remaining fatty acids contributing to the overall fatty acid composition.

We further modified the fatty acid profile by adding n-3 LC-PUFAs as fish oil to decrease the dietary ratio of n-6:n-3 fatty acids from 7:1 to 2:1. In humans, a dietary n-6:n-3 fatty acid ratio of ideally 1:1 to 2:1 has been reported to influence the risk of cardiovascular disease [35], and ameliorating n-3 LC-PUFA deficiencies is suggested to beneficially modulate conditions associated with insulin resistance, liver steatosis,
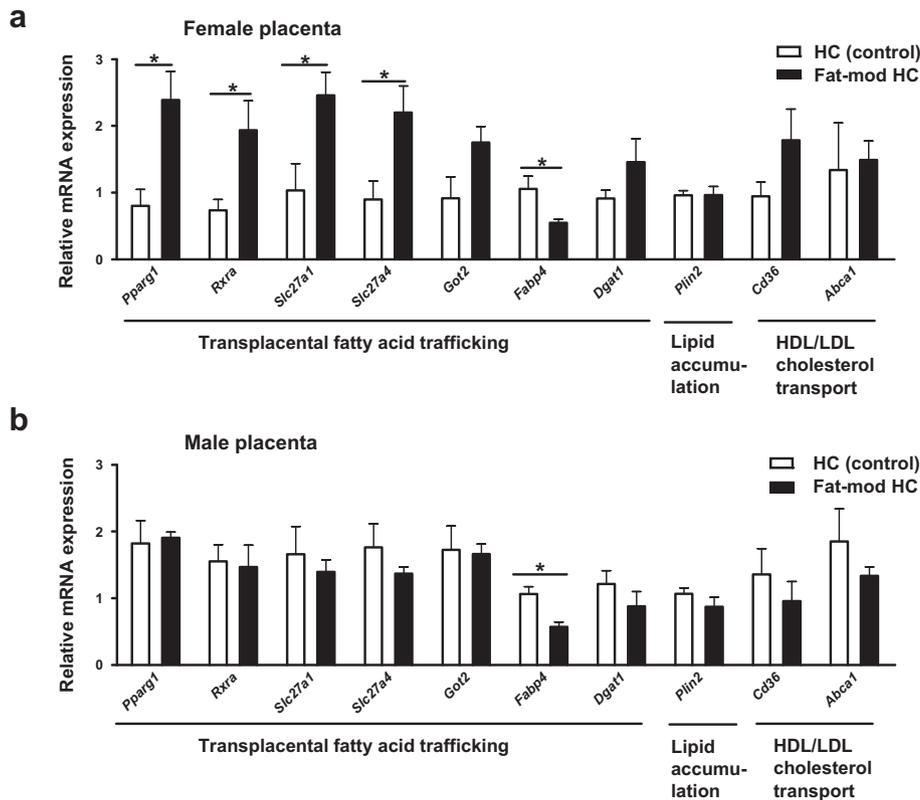
Fig. 4. Impact of the peri-conceptional fat-modified high-calorie diet on placental gene expression at the end of gestation. mRNA expression of genes involved in placental fatty acid transport and storage relative to the reference gene Tbp in (a) placenta pools of female offspring exposed to the Fat-mod HC diet or the HC (control) diet (n = 5/group) and (b) placenta pools of male offspring exposed to the Fat-mod HC diet or the HC (control) diet (n = 5/group) at 17.5 dpc. Data are presented as mean ± S.E.M. Significant differences between the Fat-mod HC diet group and the HC diet group were determined by Mann-Whitney U test; *p < 0.05. Abca1, ATP-binding cassette, sub-family A, member 1; Cd36, Cd36 antigen; Dgat1, diacylglycerol O-acyltransferase 1; dpc, day post coitum; Fat-mod HC, fat-modified high-calorie diet; Fabp4, fatty acid binding protein 4; Got2, glutamatic-oxaloacetic transaminase 2; HC, high-calorie diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Plin2, perilipin 2; Pparg1, peroxisome proliferator activated receptor gamma 1; Slc27a1, solute carrier family 27 (fatty acid transporter), member 1; Slc27a4, solute carrier family 27 (fatty acid transporter), member 4; Rxra, retinoid X receptor alpha; Tbp, TATA box binding protein.

and inflammation [36]. In mice, supplementation of a HFD (35% kcal fat) with fish oil, resulting in a 2:1 ratio, has been shown to reduce plasma lipid concentrations and liver lipid content [31].

In adipocytes, n-3 LC-PUFAs and their metabolites have been shown to increase mitochondrial biogenesis, fatty acid β-oxidation function, and adiponectin secretion, while lipogenesis regulation and inflammatory and oxidative stress appear to be suppressed [14]; all effects potentially favoring anti-adipogenic outcomes. Despite large variations in EPA and DHA dosage, a reduction of body fat accumulation, especially of visceral adipose tissue, has been reported as a consequence of n-3 LC-PUFA supplementation in the majority of animal models [13]. To our Fat-mod HC diet, we added a small amount of fish oil containing an EPA/DHA ratio of 1.5:1, similar to rodent experiments that studied EPA/DHA ratios of 1.2:1 to 1.7:1 [32,37]. In those experiments, higher proportions of dietary fatty acids were replaced by n-3 LC-PUFAs (25-35% of total fatty acids) than in our study. However, comparably low amounts of n-3 LC-PUFAs (<10% of total fatty acids) with an EPA/DHA ratio of 1.2:1 in obesogenic diets over similar intervention periods (>12 weeks) also revealed improvements in phenotypic outcomes in mice, including reductions in adipose tissue and liver fat accumulation [38].

Interestingly, relative reductions of body weight and fat mass at conception in dams fed the Fat-mod HC diet compared to HC diet-fed dams were estimated to be similar to the findings from our previous studies comparing dams fed the same HC diet versus a control diet (Supplementary Fig. 2) [5]. In this previously reported model of diet-induced maternal adiposity, we found rather mild metabolic consequences in pregnancy including a moderately impaired glucose tolerance [5]. The fat modification we applied did not improve the maternal glucose tolerance but there seemed to be a downward trend, even if not statistically significant, to lower fasting glucose and insulin concentrations in the Fat-mod HC diet versus the HC diet group, possibly pointing to a metabolic influence. Taken together, the beneficial adipose tissue and hepatic phenotype of dams observed in our study could potentially be the result of an additive effect of both types of functional fat components and/or interrelations between fatty acids.

One important mechanism through which fatty acids can exert cellular effects is via regulation of gene transcription [39]. The reduced hepatic fat accumulation in Fat-mod HC diet-fed dams was accompanied by a downregulation of the expression of important *de novo* lipogenesis-related genes including the lipogenic transcription factor *Pparg2* as compared to the HC diet group. PPARG2 is one of the main factors in hepatic steatosis induced by overnutrition [40] and a sensing system for fatty acids. Following dietary modification, expression of PPARG2 and several of its target genes involved in fatty acid synthesis and transport, *Fasn*, *Acaca*, and *Fabp4*, were reduced on both mRNA and protein levels, likely contributing to the lower fat accumulation in the liver. At least for n-3 LC-PUFAs, similar suppressive results have been obtained previously and were shown to be mediated via inhibition of SREBP1, which involves binding of unsaturated fatty acids to *Nr1h3* [41]. However, the lack of a decrease in SREBP1 protein abundance in Fat-mod HC diet-exposed livers, although mRNA expression was downregulated, and our finding that most of SREBP1 protein was represented by the inactive precursor, points to other factors contributing to the improved hepatic phenotype. Post-transcriptional processes need to be hypothesized to explain the discrepancy between mRNA and protein abundances, which we also found for NR1H3, an important inducer of *Srebf1* gene transcription.

Hepatic *Abca1*, another NR1H3 target gene and major modulator of HDL formation, was found to be transcriptionally repressed by n-3 LC-PUFAs *in vitro* [42], similar to our study. However, decreased *Abca1* mRNA abundance was not reflected on the protein level in livers of Fat-mod HC diet-fed dams, a finding that is in accordance with previous reports on post-transcriptional and post-translational regulation of *Abca1* [43]. Low serum HDL cholesterol concentrations, as observed in our Fat-mod HC diet-fed dams, have been reported

previously in mice that also showed atheroprotective features [44]. Our data point to a high complexity in the control of HDL formation and modulation of lipid homeostasis through fatty acids in the liver that appear to involve multiple levels of regulation and interconnected metabolic pathways.

Similar to anti-lipogenic mechanisms in the liver, the transcriptional downregulation of *Srebf1* in the Fat-mod HC diet-fed dams' adipocytes might presumably be mediated by inhibitory n-3 LC-PUFA binding to *Nr1h3* [45]. SREBP1 protein was predominantly detected as the nuclear active form, in contrast to its expression in liver, and a slight tendency to lowered protein expression might point to a possible impact on active protein by the changed fatty acid pattern of the Fat-mod HC diet. In contrast, knowledge on the regulatory potential of MCFAs on adipocytes is only limited [46]. Whereas many key genes of adipocyte lipid metabolism remained unchanged by the altered dietary fatty acid composition, we found the mRNA expression of the *Mest* and *Lep* genes to be downregulated. Both MEST, a member of the α/β fold hydrolase superfamily thought to promote storage of dietary lipids from the periphery, and LEP, which is secreted by white adipose tissue, show associations with the size of fat mass and cells [27,28], similar to our transcriptional findings, which were consistent with the adipocyte phenotype of the Fat-mod HC diet-fed dams. This also goes along with a strong trend towards reduced leptin concentrations in the bloodstream, which may have protective effects on maternal outcome and placental nutrient support as suggested from obese pregnancies in mice and humans [47,48]. Together, our results suggest that the combined change in fatty acid composition acts through tissue-specific mechanisms involving anti-lipogenic processes primarily in the liver, thereby beneficially modulating the maternal phenotype.

By our approach of fat modification of an obesogenic diet during the earliest phases of life, we did not induce differences in placental and fetal weights at delivery. However, placental mRNA expression of PPARG-dependent fatty acid transport genes was sex-specifically upregulated by the dietary change only in placentae of female offspring. Protein expression of transporters could not be analyzed due to a lack of suitable placental tissue. Nevertheless, a generally stronger susceptibility of female versus male adaptations of the placental transcriptome toward nutritional influences has been observed previously in mice [22]. In humans, PPARG activation in the placenta was found to induce beneficial effects, such as a suppression of nitric oxide levels [49]. While n-3 LC-PUFAs are preferential substrates of PPARG-dependent placental transporters and metabolized in the placenta [50], transport mechanisms for MCFAs are not well established, although their placental oxidation is known to be low in humans [51]. Such fatty acid-induced placental transport alterations may possibly contribute to the longer-term "programming" effects observed in rodent studies of perinatal maternal nutritional supplementation [11,52]. Considering the worldwide increasing prevalence of diet-induced obesity of women at conception and during pregnancy, improving the intake of "healthier" fat components might be a promising nutritional prevention approach to be studied in humans for its consequences on both mother and child.

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Transparency document

The Transparency document associated with this article can be found, in the online version.

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